DONORS, COLLECTION AND PRODUCTION OF BLOOD COMPONENTS AND PLASMA DERIVATIVES

Original Article

Establishment of the first platelet-donor registry in Argentina

Sebastián Blanco[1,2], Maria C. Frutos[2], Luis H. Carrizo[1], Nuria Nogués[3,4], Sandra V. Gallego[1,2]

[1]*Central Blood Bank Foundation, Córdoba, Argentina;*
[2]*"Dr. J. M. Vanella" Virology Institute, Faculty of Medical Sciences, Córdoba National University, Córdoba, Argentina:*
[3]*Blood and Tissue Bank, Barcelona, Spain;*
[4]*Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain.*

Background - Platelet transfusions are necessary to prevent and treat haemorrhages in thrombocytopenic patients or those with severe platelet dysfunction. In Latin American countries, including Argentina, blood supplies from voluntary non-remunerated blood donors remain dependent on family replacement donors, since altruistic repeat donors are exceptional and platelet donors are very scarce. The aim of this study was to recruit a group of frequent, voluntary, altruistic blood donors and determine their human platelet antigen (HPA)-genotype in order to establish the first registry of HPA-typed voluntary platelet donors in Argentina.

Material and methods - In this study, we invited and recruited voluntary blood donors who attended the *Fundación Banco Central de Sangre* between July 2016 and July 2017. DNA was extracted from K2EDTA anticoagulated whole blood and genotyping was performed by polymerase chain reaction, using sequence-specific primers to type the HPA-1 to -6, -9 and -15 systems. A subset of samples was also tested using a commercial HPA-TYPE kit. Donors were invited to join the National Register of Haematopoietic Stem Cell Donors of Argentina.

Results - A cohort of 500 platelet donors was recruited and characterised and a database with their personal information, including their genotype for the most relevant HPA alloantigens, was created. Eight of the 500 donors (1.6%) were HPA-1a negative. HPA allelic variants -4b, -6b and -9b were detected for the first time in our population. There was 100% concordance between our in-house assay and the commercial kits in the subset of 150 donor samples assayed in parallel.

Discussion - The efforts made to recruit, characterise and register voluntary platelet donors will provide the first sustainable source of HPA and human leukocyte antigen-typed platelets for compatible transfusions in the country. Remarkably, we identified a higher percentage of HPA-1a-negative donors than previously detected in the Argentinean population.

Keywords: *human platelet antigens, genotyping, platelet-donor registry, platelet transfusion.*

Arrived: 17 January 2020
Revision accepted: 9 April 2020
Correspondence: Sebastián Blanco
e-mail: seba_36@hotmail.com

INTRODUCTION

Platelets are essential for haemostasis and constitute key components in pathogenic thrombosis[1]. Transfusion of these blood components is used in clinical practice to prevent and treat haemorrhages in thrombocytopenic patients or those with severe

platelet dysfunction[2]. Over the past years, the demand for platelets has augmented[3-5]. The increasing age of the general population, a rise in the incidence and prevalence of haematological malignancies, and changes in the management of patients with blood-related diseases are key factors for understanding the increase of this demand[3]. Platelet availability is usually limited because of the scarcity of donors. This is especially critical in countries in which blood supplies do not come mainly from altruistic repeat donors. Although more than half of people are eligible to donate blood, it is estimated that only 5% of the population donates, and despite several awareness-raising campaigns, only 1% donates frequently[6]. According to a report of the Pan American Health Organization in June 2017, less than half of the blood donors in Latin America and the Caribbean are voluntary donors[7]. This report also reveals that only 33.4% of the volunteer donors in Latin America donate blood or blood components at least twice a year, and in Mexico this percentage is only 2.7%. In Latin American countries, including Argentina, the supplies of blood from voluntary non-remunerated blood donors remain dependent on family replacement donors since altruistic repeat donors are the exception instead of the rule and platelet donors are very scarce. In this context, we consider that the implementation of a platelet-donor recruitment programme is essential in these countries, in order to count on a permanent source of platelets to meet their needs.

One of the essential aspects to consider when creating a platelet-donor registry is the need for adequate systems to characterise the immunogenic antigens expressed on the platelet surface. Some of these antigens, including those of the human leucocyte antigen (HLA) and ABO systems, are shared with other cell types (e.g., HLA, ABO), whereas others, such as human platelet antigens (HPA), are specific to platelets. HPA are expressed in a genetically polymorphic form due to single nucleotide variant in the genes that encode platelet glycoproteins. Clinical guidelines do not differentiate between the application of pool platelet concentrates or apheresis platelet concentrates, except for patients with immune-mediated transfusion refractoriness and foetal/neonatal alloimmune thrombocytopenia (FNAIT), for whom a single-donor apheresis platelet concentrate match is recommended[8]. In this context, it is important to count on a group of voluntary platelet donors who have been genotyped for their HPA and, ideally, determine their HLA class I genotype as well. The availability of this group (panel) of typed donors would allow immunised patients to be treated with compatible platelets.

In the context of a platelet-donor registry, it is also important to determine the allelic frequency of different HPA polymorphisms within the regional population to infer the incidence and potential implications of low frequency HPA genotypes in clinical cases of alloimmune thrombocytopenia occurring in our region. A previous analysis of HPA frequency published by De la Vega Elena *et al*. demonstrated that HPA allele frequencies of an Argentinean cohort were similar to those reported for European populations[9]. In that study, HPA-4b and HPA-6b alleles were not detected and HPA-9 was not included in the analysis. Since the publication of the mentioned study, demographic changes have occurred around the world with great impact on the Argentinean population. In this sense, we considered it important to analyse a different and more heterogeneous population from a distinct region to compare the frequencies previously reported and investigate the frequency of HPA-4, HPA-6 and HPA-9.

The importance of creating a database with a large number of donors typed for the clinically most relevant HPA alloantigens has already been discussed[10,11]. In the context of replacement blood donations, the aim of this study was to recruit a group of frequent, voluntary and altruistic donors from Córdoba, Argentina, and characterise their HPA-genotype in order to establish the first Argentinean registry of voluntary platelet donors. These individuals constitute a "captive pool of donors", becoming a valuable source of characterised platelets for compatible transfusion when these blood components are required, thus, contributing to improving the quality and safety of transfusion medicine in Argentina.

MATERIALS AND METHODS

This study was developed in accordance with local and national regulations; it was approved by the Training and Teaching Committee of the *Fundación Banco Central de Sangre* and by the Ethics Committee on Human Research (CIEIS) Oulton Romagosa on March 2016 and the Ethical Evaluation Council of Health Research (COEIS) of the Ministry of Health of the province of Córdoba, Argentina on May 2016.

Five hundred voluntary blood donors who attended the Fundación Banco Central de Sangre from July 2016 to July 2017 were included in the registry. The only inclusion criterion was to have made at least two voluntary donations of either whole blood or apheresis product during the year prior to the recruitment date. Every donor who participated in the study signed an informed consent document that explained the molecular studies that would be performed for HPA genotyping and had a separate section in which donors expressed their agreement to be contacted to donate platelets when necessary.

Donors included in the HPA registry were invited to join the National Register of Haematopoietic Stem Cell (HSC) Donors of Argentina as well. The HSC registry operates within the Central National Institute for Coordination of Ablation and Implantation (INCUCAI). Before being included in the HSC registry, donors were informed about the procedure following protocols established by INCUCAI.

Epidemiological information was obtained from blood donors through a questionnaire and when additional information was needed, for example, from donors carrying low frequency HPA alleles, further specific questions were asked.

DNA was extracted from K2EDTA-anticoagulated whole blood samples with the commercially available High Pure PCR Template preparation kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Genotyping for HPA-1 to -6, -9 and -15 systems was performed by polymerase chain reaction (PCR) amplifications using sequence-specific primers (SSP), in accordance with protocols described by Klüter *et al.*[12], Cavanagh *et al.*[13], Skogen *et al.*[14], and Schuh *et al*[15]. These protocols were transferred from the Immunohaematology Laboratory, *Banc de Sang* I Teixits, Barcelona, Spain and optimised in our laboratory (*Online Supplementary Data*).

In parallel, a subset of 150 of the total 500 recruited donors (including all donors carrying low frequency HPA genotypes) was tested with a commercial kit, BAGene SSP HPA-TYPE (BAG Health Care, Lich, Germany) using an aliquot from the same DNA isolation tested using the in-house methodology. A second blood sample was only obtained for those donors carrying low frequency HPA genotypes in order to re-confirm the genotypes.

Amplification products were analysed by electrophoresis in 2% agarose gel (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) with Sybr safe DNA Gel Stain (Invitrogen, Molecular Probes Inc, Eugene, OR, USA) and visualised and recorded by BioDoc-It Imaging System™ (Analytik Jena US LLC, Upland, CA, USA).

The HPA genotype profiles of all recruited donors were recorded in the Blood Bank informatics system and other data bases. Data were analysed with Stata Statistical Software: Release 14. Stata Corp 2015, StataCorp LP (College Station, TX, USA).

RESULTS

Five hundred voluntary, altruistic blood donors who donate regularly at the *Fundación Banco Central de Sangre* were HPA-genotyped and included in the platelet-donor registry. All of them also agreed to be enrolled in the national HSC registry. Of the 500 components of the panel, 315 (63%) were male and 185 (37%) were female. With regards to their age, the mean and median were 36 years, the mode was 24 years and the range was 16 to 69 years. Among these donors, the ABO composition of the HPA panel was 31% A, 8% B, 3% AB and 58% O. The HPA genotype frequencies of our blood donor population are shown in **Table I** and allele frequencies in **Table II**. For all of the 150 donor samples studied in parallel by in-house and commercial kits, there was 100% concordance between the results of the assays. The study of this subset of samples corroborated the robustness of the in-house technique and confirmed the identification of the low frequency HPA genotypes found in blood donors.

All HPA alleles tested with the SSP molecular technique have been detected in our blood donor population, although the homozygote HPA-4b/4b, HPA-6b/6b and HPA-9b/9b status has not been detected in this cohort.

Among the 500 donors studied, we identified eight (1.6%) who were HPA-1a negative, one of whom had been a regular apheresis donor since 2008. The ABO type of these eight donors was O in four cases, B in two cases and A in the other two cases.

In order to investigate the ethnic background of blood donors carrying the low frequency HPA-4b, HPA-6b and HPA-9b alleles, data from family members were collected.

Table I - Genotype frequencies in Argentinean blood donors

Blood donors (n=500)		
HPA genotype	**Mean**	**Binomial exact (95% CI)**
1a1a	78.0	(74.00-81.56)
1b1b	1.60	(0.67-3.03)
1a1b	20.4	(17.00-24.13)
2a2a	73.40	(69.36-77.17)
2b2b	2.52	(1.35-4.28)
2a2b	24.08	(20.45-28.01)
3a3a	40.58	(36.31-44.96)
3b3b	14.95	(11.98-18.32)
3a3b	44.47	(40.11-48.87)
4a4a	99.61	(98.60-99.95)
4b4b	0.00	(0.00 -0.01)*
4a4b	0.39	(0.05-1.40)
5a5a	81.17	(77.52-84.45)
5b5b	1.36	(0.54-2.78)
5a5b	17.47	(14.29-21.04)
6a6a	99.81	(98.92-99.99)
6b6b	0.00	(0.00-0.71)*
6a6b	0.19	(0.00-1.08)
9a9a	99.42	(98.31-99.88)
9b9b	0.00	(0.00-0.71)*
9a9b	0.58	(0.12-1.69)
15a15a	26.40	(22.65-30.44)
15b15b	22.14	(18.62-25.97)
15a15b	51.46	(47.05-55.85)

CI: confidence interval. *One-sided, 97.5% confidence interval.

Table II - Allele frequencies in Argentinean blood donors

Blood donors (n=500)		
Alelle	**Mean**	**Binomial exact (95% CI)**
HPA-1a	0.8805825	(0.8591986-0.8997621)
HPA-1b	0.1174757	(0.0984416-0.138726)
HPA-2a	0.8533981	(0.8303039-0.8744505)
HPA-2b	0.1446602	(0.1237322-0.1676414)
HPA-3a	0.6262136	(0.5958549-0.6558487)
HPA-3b	0.3718447	(0.3422463-0.4021778)
HPA-4a	0.9961165	(0.9900868-0.9989409)
HPA-4b	0.0019417	(0.0002352-0.0069965)
HPA-5a	0.8970874	(0.876897-0.9149731)
HPA-5b	0.1009709	(0.0832466-0.1210118)
HPA-6a	0.9970874	(0.9915119-0.9993989)
HPA-6b	0.0009709	(0.0000246-0.0053974)
HPA-9a	0.9951456	(0.9887082-0.998422)
HPA-9b	0.0029126	(0.0006011-0.0084881)
HPA-15a	0.5194175	(0.4884015-0.5503222)
HPA-15b	0.4786408	(0.4477464-0.5096575)

CI: confidence interval.

DISCUSSION

Here we report on the first registry of HPA-typed platelet donors in Argentina, achieved through the recruitment of voluntary donors with the aim of setting the basis for programmes to ensure the availability of platelets when and where they are needed. Even though Central and South American countries have made great progress to ensure the adequacy and safety of blood supplies, we still face great challenges. In this context, it is important to work towards the promotion of unpaid, regular voluntary donors. Strategies implemented in countries that have already reached these goals through the establishment of policies and systems to achieve self-sufficiency should be taken as models. The current study is based on these models and with the intention to follow their example.

Blood Transfus 2020; DOI 10.2450/2020.0018-20

Our blood bank centralised different blood transfusion services working all over the 165,321 km^2 of Córdoba province. The blood bank also increases blood supply through outdoor blood donation campaigns and providing different blood-collection points in the province. Thus, the blood bank donor population is not circumscribed to a particular area but distributed in a large region of the centre of the country, since Córdoba province is the second most populated region in Argentina.

Although our aim was to create the first platelet-donor registry in the country, we understand that our efforts need to be complemented by others that focus on education and promotion of blood donation, in order to achieve access to a safe, sufficient and sustainable blood supply by altruistic donors in our region. Given that our blood bank has worked in cooperation with the National Register of HSC Donors of Argentina since 2005, we also invite all donors who joined the platelet-donor registry to be part of the HSC registry as well. The HSC registry operates within the INCUCAI and is part of the International Network of Bone Marrow Donors Worldwide. The registry has signed a cooperative agreement with the National Marrow Donor Program from the USA and with European registries through the European Marrow Donor Information System. In this context, INCUCAI performed the HLA typing of all donors included in the registry and made this information available to our platelet-donor registry. This information adds important value to our platelet-donor registry since it will allow us to provide HLA-compatible platelets to those patients who are refractory to platelet transfusion due to HLA class I alloimmunisation.

In our study, in-house PCR analyses were performed to determine the HPA allele frequencies in the recruited population and a subset of this group, including carriers of low-frequency HPA alleles, were also tested using the HPA-TYPE kit (BAG Health Care). The results obtained with the commercial kit confirmed the reliability of the results obtained with the in-house method and the robustness of the method, as well as validating the results of the low frequency alleles found. Concordance between the two techniques showed that the in-house PCR SSP approach implemented in our laboratory is a useful and reliable tool for determining the HPA genotype in our population.

The results from our current study corroborate that the genotype distribution of HPA-1, HPA-2, HPA-3, HPA-5 and HPA-15 systems in Argentina is similar to that described in European populations, as reported by De la Vega Elena *et al.*[9]. However, we detected HPA-4b, HPA-6b and HPA-9bw carriers for the first time in a blood donor population from Argentina.

Investigation of the ethnic background of the donor carrying HPA-6b revealed that this donor has Asian ascendance. The HPA-6b allele has been described in Chinese, Japanese, Korean, French Polynesian, Thai, Taiwanese, Indonesian and Finnish populations[16-19]. Current demographic movements are introducing Asian immigrants into Latin American countries and for this reason it is important to include this ethnic population in the platelet-donor-typed panel in order for this to be sufficiently representative of the genetic background of the current population.

The donors with the HPA-4b allele have a mixed ethnic background since both of them were born in Argentina and have ancestors from Europe (Italy and Spain) and America. HPA-4b alloantigen has been detected in Asian populations, but also in Switzerland[20], Mexico[9] and the South American native population of Mapuches[21]. Three donors who carried genotype HPA-9a/9b also have a mixed ethnic background: they were born in Argentina and have ancestors from Europe (Italy and Spain) and America (the father of one of them was Brazilian). Allele HPA-9bw had been detected in Brazilian[22] and Caucasian populations[23-25].

The HPA frequencies in each population have a direct impact on the prevalence of HPA-alloimmunisation and associated clinical entities: FNAIT, platelet transfusion refractoriness, post-transfusion purpura and transplantation-associated alloimmune thrombocytopenia.

The clinical relevance of different anti-HPA antibodies varies among different ethnic groups. In Caucasian populations, antibodies against HPA-1a carried on β3 integrin are the most common cause of FNAIT, but antibodies against HPA-5b, -15b, -1b, -3a and -5a have been involved in FNAIT cases as well[26]. In African and Asian populations, the risk of alloimmunisation has been reported to be highest for HPA-2 and HPA-5 antigens[27]. Since low frequency HPA can trigger FNAIT, it has been suggested that in neonates with Asian ancestries typing HPA-4bw, HPA-6bw and HPA-21bw needs to be considered[26]. Despite being rare in the general

population, maternal alloimmunisation against HPA-9bw has been described as an important cause of FNAIT[24,25]. The detection of HPA-4bw, HPA-6bw and HPA-9bw in our population is concordant with the fact that the genetic background in Argentine is formed of a mix of several ethnic groups, as already pointed out in other studies on blood group alleles in this country[28]. This fact highlights the importance of assessing the potential risk of alloimmune sensitisation associated with these genotypes.

We found that the prevalence of HPA-1b/1b homozygous platelet donors among the population studied was 1.6%, which is a higher percentage than previously detected in the Argentinean population[9]. This is one of the main contributions of the HPA donor registry, since HPA-1a-negative platelets are not routinely available for transfusion practice. The identification of these donors will allow us to coordinate apheresis donations so that HPA-1a-negative platelets are available when necessary. Besides, this group of platelet donors constitutes a sustainable source to support not only our blood bank's demands but also requests from other blood transfusion services of the region and even other Latin-American countries.

CONCLUSIONS

In conclusion, the efforts made by our team to recruit, characterise and register voluntary, altruistic platelet donors have contributed to creating the first sustainable source of platelets for compatible transfusions in the country, thereby improving the quality and safety of transfusion medicine in Argentina. This study has also had an impact on platelet alloimmunisation diagnostics since platelet samples from the panel of HPA-typed donors are useful as a source of reagent platelets to identify anti-platelet antibodies.

ACKNOWLEDGEMENTS

We are grateful to Dr. Eduardo Muñiz-Díaz, from the Immunohaematology Laboratory, Blood and Tissue Bank, Barcelona, Spain for his generosity hosting Sebastián Blanco in his laboratory for a training/educational stay and to Dr. Graciela Panzetta Dutari, Researcher of the National Council of Scientific and Technical Research (CONICET) and Associated Professor, Department of Clinical Biochemistry, Faculty of Chemical Sciences, National University of Córdoba, for her support during the implementation of the techniques in our laboratory.

FINANCIAL SUPPORT

This work was supported by the Central Blood Bank Foundation, Córdoba, Argentina. Funding was also provided by Felsan S.A, Argentina, and the Faculty of Medical Sciences, National University of Córdoba, Argentina.

AUTHORSHIP CONTRIBUTIONS

SB conceived and designed the study, carried out the molecular assays, analysed/interpreted the data and drafted the manuscript. MCF helped with the molecular assays and revised the final version of manuscript. LHC conceived the study, recruited platelet donors and edited the final version of the manuscript. NN transferred the genotyping protocols, analysed and interpreted the data and revised and edited the final version of manuscript. SVG conceived and designed the study, analysed and interpreted the data and revised/edited the final version of paper.

The Authors declare no conflicts of interest.

REFERENCES

1. Handigund M, Cho YG: Insights into platelet storage and the need for multiple approaches. Ann Clin Lab Sci 2015; **45**: 713-9.
2. Blumberg N, Heal JM, Phillips GL: Platelet transfusion: trigger, dose, benefits and risks. F1000 Med Rep 2010; **2**: 5.
3. Estcourt LJ: Why has demand for platelet components increased? A review. Transfus Med 2014; **24**: 260-8.
4. Wood EM, Crighton GL, Estcourt LJ, et al. An update on indication for platelet transfusion. ISBT Science Series 2016; **11**: 170-6.
5. Aubron C, Flint AWJ, Ozier Y, McQuilten Z. Platelet storage duration and its clinical and transfusion outcomes: a systematic review. Crit Care 2018; **22**: 185.
6. Martín-Santana JD, Beerli Palacio A. [Achieving donor repetition and motivation among current blood donors.] Revista Europea de Dirección y Economía de la Empresa 2012: **21**: 283-90. [In Spanish.]
7. Organización Panamericana de la Salud – OPS [Internet]. [World Blood Donor Day 2016: Latin America and the Caribbean is almost halfway to reaching 100% of voluntary blood donors.] Available at https: //www.paho.org/hq/index.php?option=com_content&view=article&id=12143:lac-approaching-half-way-100-voluntary-blood-donation&Itemid=1926&lang=es. Accessed on: 14/06/2019. [In Spanish.]
8. Berger K, Schopohl D, Wittmann G, et al. Blood product supply in Germany: the impact of apheresis and pooled platelet concentrates. Transfus Med Hemother 2016; **43**: 389-94.
9. De la Vega Elena CD, Nogués N, Fernández Montoya A, et al. Human platelet specific antigens frequencies in the Argentinean population. Transfus Med 2008; **18**: 83-90.
10. Mangerona CMB, Garcia FB, Moraes-Souza H. Frequency of human platelet antigens (HPA)-1,-2,-5 and -15 in Brazilian blood donors and establishment of a panel of HPA-typed donors. Transfus Med 2015; **25**: 189-94.
11. Fernandez Silva-Malta MC, Tavares do Oliveira LG, Barreiros LF, et al. Human platelet antigens in Brazilian multiethnic populations: occurrence of regional variation and frequency in a large urban center (Belo Horizonte). Transfus Med Hemother 2018; **45**: 388-96.
12. Klüter H, Fehlau K, Panzer S, et al. Rapid typing for human platelet antigen systems -1, -2, -3 and –5 by PCR amplification with sequence-specific primers. Vox Sang 1996; **71**: 121-5.

13. Cavanagh G, Dunn AN, Chapman CE, Metcalfe P. HPA genotyping by PCR sequence-specific priming (PCR-SSP): a streamlined method for rapid routine investigations. Transfus Med 1997; **7**: 41-5.
14. Skogen B, Bellissimo DB, Hessner MJ, et al. Rapid determination of platelet alloantigen genotypes by polymerase chain reaction using allele-specific primers. Transfusion 1994; **34**: 955-60.
15. Schuh AC, Watkins NA, Nguyen Q, et al. A tyrosine703serine polymorphism of CD109 defines the Gov platelet alloantigens. Blood 2002; **99**: 1692-8.
16. [16] Kekomäki S, Partanen J, Kekomäki R. Platelet alloantigens HPA-1,-2,-3,-5 and -6b in Finns. Transfus Med 1995; **5**: 193-8.
17. Seo DH, Park SS, Kim DW, et al. Gene frequencies of eight human platelet-specific antigens in Koreans. Transfus Med 1998: 8: 129-32.
18. Liu TC, Shih MC, Lin CL, et al. Gene frequencies of the HPA-1 to HPA-8w platelet antigen alleles in Taiwanese, Indonesian, and Thai. Ann Hematol 2002; **81**: 244-8.
19. Halle L, Bach KH, Martageix C, et al. Eleven human platelet systems studied in the Vietnamese and Ma´ohis Polynesian population. Tissue Antigens 2004; **63**: 34-40.
20. Boehlen F, Bulla O, Michel M, et al. HPA-genotyping and antiplatelet antibodies in female blood donors. Hematol J 2003; **4**: 441-4.
21. Inostroza J, Kiefel V, Mueller-Eckhardt C. Frequency of platelet-specific antigens PIA1, Baka, Yuka, Yukb, and Bra in South American (Mapuches) Indians. Transfusion 1988; **28**: 586-7.
22. Carmo JCD, Klippel PS, Cordeiro SDC, et al. Molecular typing of human platelet antigens in immune thrombocytopenia patients in northern Brazil. Rev Bras Hematol Hemoter 2017; **39**: 122-6.
23. Noris P, Simsek S, de Bruijne-Admiraal LG, et al. Max(a), a new low-frequency platelet-specific antigen localised on glycoprotein IIb, is associated with neonatal alloimmune thrombocytopenia. Blood 1995; **86**: 1019-26.
24. Peterson JA, Balthazor SM, Curtis BR, et al. Maternal alloimmunization against the rare platelet-specific antigen HPA-9b (Max a) is an important cause of neonatal alloimmune thrombocytopenia. Transfusion 2005; **45**: 1487-95.
25. Kaplan C, Porcelijn L, Vanlieferinghen P, et al. Anti-HPA-9bw (Maxa) fetomaternal alloimmunization, a clinically severe neonatal thrombocytopenia: difficulties in diagnosis and therapy and report on eight families. Transfusion 2005; **45**: 1799-803.
26. Peterson JA, Gitter M, Bougie DW, et al. Low-frequency human platelet antigens as triggers for neonatal alloimmune thrombocytopenia. Transfusion 2014; **54**: 1286-93.
27. Tiller H, Husebekk A, Ahlen MT, et al. Current perspectives on fetal and neonatal alloimmune thrombocytopenia - increasing clinical concerns and new treatment opportunities. Int J Womens Health 2017: **9**: 223-34.
28. Boggione CT, Luján Brajovich ME, Mattaloni SM, et al. Genotyping approach for non-invasive foetal RHD detection in an admixed population. Blood Transfus 2017; **15**: 66-73.

ONLINE SUPPLEMENTARY CONTENT

Primers

Genotyping for HPA-1 to -6, -9 and -15 systems was performed by polymerase chain reaction (PCR) amplifications using sequence-specific primers (SSP). Primer sequences and amplicon sizes for each of the HPA-systems and the internal control are detailed in Supplementary **Table SI**.

Master Mixes

The assays for HPA-1,-2 and -3 systems were modified from those described by Klüter *et al.* and performed as multiplex PCR SSP. The assays for HPA-4, -5, -6, -9, and -15 systems were performed in uniplex PCR SSP with some modifications to protocols described by Skogen *et al.*, Cavanagh *et al.* and Schuh *et al.*

Two different amplification reaction mixtures were prepared for each HPA system, mix a for amplification of allele a (containing primer forward a and the common reverse primer) and mix b for amplification of allele b (containing primer forward b and a common reverse primer). All PCR were carried out using PCR Reagent Buffer 10x, 25 mM $MgCl_2$ stock solution, 10 mM stock PCR nucleotide mix (dNTP solution), and Taq Polymerase 5 U/µL from the FastStart Taq DNA Polymerase dNTPack kit (Roche Diagnostics, Mannheim, Germany). The mixtures were prepared and added in 0.2 mL PCR tubes in cold blocks. DNA was also added to the mix in cold blocks. The concentration of DNA was between 10-50 ng/µL. The temperature condition was maintained until the tubes were placed in the thermocycler.

To genotype HPA-1, -2 and -3 systems, 2 µL of DNA were added to each 0.2 mL PCR tube containing 23 µL of amplification reaction mixture a or b. The following reagents were added to each mixture: 10X PCR reaction buffer, 2 mM $MgCl_2$, 0.4 mM dNTP, 0.148 µM of each internal control primer (HGH-1/-2), 0.375 µM of each allele-specific primer HPA 1a or HPA 1b and HPA 1II, 0.25 µM of HPA 2a or HPA 2b and HPA 2 II, 0.2 µM of HPA3aorHPA3bandHPA3IIand1.5UofTaqDNAPolymerase 5 U/µL.

For HPA 4 system genotyping, 1 µL of DNA was added to 9 µL of the amplification mixtures containing: 10X PCR reaction buffer, 3 mM MgCl2, 0.075 mM dNTP, 0.16 µM of each internal control primer, 1.05 µM of each allele-specific primer, HPA 4a or HPA 4b and 1.01 µM of HPA 4c and 0.5 U of Taq DNA Polymerase.

For HPA 5 system genotyping, 0.5 µL of DNA were added to 12.5 µL of each reaction mixture containing: 10X PCR reaction buffer, 3.5 mM MgCl2, 0.2 mM dNTP , 0.48 µM of forward control primer and 0.55 µM of reverse control primer, 0.94 µM of HPA 5a and 0.8 µM of HPA 5c to mix "a"

***Table SI** - Primers*

HPA System	Primer	Amplicon size
HPA 1a	5'-ACT TAC AGG CCC TGC CTC T-3'	189 bp
HPA 1b	5'-ACT TAC AGG CCC TGC CTC C-3'	
HPA 1II	5'-GTG CAA TCC TCT GGG GAC T-3'	
HPA 2a	5'-CCC CCA GGG CTC CTG AC-3'	241 bp
HPA 2b	5'-CCC CCA GGG CTC CTG AT-3'	
HPA 2II	5'-GCC AGC GAC GAA AAT AGA GG-3'	
HPA 3a	5'-GGG GGA GGG GCT GGG GA-3'	293 bp
HPA 3b	5'-GGG GGA GGG GCT GGG GC-3'	
HPA 3II	5'-GGC CCT GGG ACT GTG AAT G-3'	
HPA 4a	5'-GCT GGC CAC CCA GAT GCG-3'	120 bp
HPA 4b	5'-GCT GGC CAC CCA GAT GCA-3'	
HPA 4c	5'-CAG GGG TTT TCG AGG GCC T-3'	
HPA 5a	5'-AGG AAG AGT CTA CCT GTT TAC TAT CAA AG-3'	252 bp
HPA 5b	5'-AGG AAG AGT CTA CCT GTT TAC TAT CAA AA-3'	
HPA 5c	5'-CTC TCA TGG AAA ATG GCA GTA CAC T-3'	
HPA 6a	5'-GAC GAG TGC AGC CCC CG-3'	238/239 bp
HPA 6b	5'-GGA CGA GTG CAG CCC CCA-3'	
HPA 6c	5'-CCT ATG TTT CCC AGT GGT TGC A-3'	
HPA 9a	5'-CTC CTT TGC CCC CCC AGG-3'	185 bp
HPA 9bw	5'-CTC CTT TGC CCC CCC AGA-3'	
HPA 9c	5'-GAG AGC CTG CTC ACT ACG AG-3'	
HPA 15a	5'-TTC AAA TTC TTG GTA AAT CCT GG -3'	225 bp
HPA 15b	5'-TTC AAA TTC TTG GTA AAT CCT GT -3'	
HPA 15c	5'-ATG ACC TTA TGA TGA CCT ATT -3'	
HGH-1	5'-CAG TGC CTT CCC AAC CAT TCC CTT A-3'	429 bp
HGH-2	5'-ATC CAC TCA CGG ATT TCT GTT GTG TTT C-3'	

and 1.88 µM of HPA 5b and 1.62 µM of HPA 5c allele specific primers to mix "b", and 0.5 U of Taq DNA Polymerase.

For HPA 6 genotyping, 1 µL of DNA was added to 24 µL of the amplification mixtures containing: 10X PCR reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.1 µM of each internal control primer, 0.2 µM of each allele-specific primer: HPA 6a or HPA 6b and HPA 6c and 0.5 U of Taq DNA Polymerase.

For HPA 9 genotyping, 0.5 µL of DNA were added to 9.5 µL of the amplification reaction mixture containing: 10X PCR reaction buffer, 4.5 mM MgCl2, 0.075 mM dNTP, 0.15 µM of each internal control primer, 1 µM of each allele-specific primer HPA 9a or HPA 9bw and HPA 9c and 0.5 U of Taq DNA Polymerase.

For genotyping HPA 15, 2 µL of DNA were added to 8 µL of each reaction mixture containing: 10X PCR reaction buffer, 2 mM MgCl2, 0.2 mM dNTP, 0.072 µM of each internal control primer, 0.6 µM of each allele-specific primer, HPA 15a or HPA 15b primer and HPA 15c and 0.5 U of Taq DNA Polymerase.

Amplification conditions

Amplification of the HPA systems was carried out in a Biometra UNO II thermocycler. The conditions are detailed in Supplementary **Table SII**

***Table SII** - Amplification conditions*

	HPA-1-2-3			HPA-4 and HPA-5			HPA-6	
	1 Cycle	**10 Cycles**	**22 Cycles**	**1 Cycle**	**10 Cycles**	**22 Cycles**	**1 Cycle**	**30 Cycles**
Denaturalisation	95 ° 10´	95° 30´´	95° 30´´	95 ° 5´	95° 25´´	95° 25´´	95 ° 10´	95° 30´´
Annealing		65° 60´´	58° 60´´		68° 45´´	61° 45´´		58° 60´´
Extension		72° 30´´	72° 30´´		72° 30´´	72° 30´´		72° 30´´
Final extension	72° 4´			72° 10´			72° 10´	
	HPA-9			**HPA-15**				
	1 Cycle	**10 Cycles**	**22 Cycles**	**1 Cycle**	**5 Cycles**	**20 Cycles**	**8 Cycles**	
Denaturalisation	95 ° 5´	95° 25´´	95° 25´´	95 ° 5´	95° 25´´	95° 25´´	95° 25´´	
Annealing		63° 45´´	58° 45´´		70° 45´´	58° 45´´	51° 45´´	
Extension		72° 30´´	72° 30´´		72° 30´´	72° 30´´	72° 30´´	
Final extension	72° 7´			72° 7´´				

°: degrees centigrade; ´ minutes; ´´ seconds