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LETTER TO THE EDITOR

HLA class I and HPA9b related fetal-neonatal alloimmune thrombocytopenia

Dear Editor,

A 33-year-old Caucasian woman was referred at 32^{+5} weeks of gestation after the finding of severe fetal intracranial haemorrhage (ICH) at routine ultrasound. The woman had two previous uneventful pregnancies (singleton and twins). Fetal ultrasound and magnetic resonance imaging confirmed multiple ICHs especially located in the left hemisphere, wide areas of periventricular leukomalacia, obstructive hydrocephalus and macrocrania. Fetal-neonatal alloimmune thrombocytopenia (FNAIT) was investigated. Maternal blood group was A RhD positive, paternal O RhD positive. The maternal sample was screened for platelet-reactive antibodies using solid phase technology for the detection of IgG anti-HLA class I and anti-HPA antibodies (SPRCA Capture P Ready Screen, Immucor, Italy) with and without chloroquine treatment to remove HLA antigen interference. The results were positive and negative respectively. No antibodies attached to maternal platelets were found (Capture-P, Immucor, Italy). ELISA and Luminex based platforms were used to identify the specificity of the detected antibodies (Pak-Lx Luminex and ELISA Pak plus, Immucor, Italy and Luminex MoAb, Lagitre, Italy). The assays only recognised the presence of anti-HLA A02 and anti-HLA B51 at high titre, greater than 8000 and 20 000 MFI (average fluorescence intensity) respectively, in association with different cross-reactions. Cross-match testing (Capture-P, Immucor, Italy) using maternal serum against paternal platelets tested reactive with both chloroquine-untreated and treated platelets. Additional cross-match testing was performed using maternal serum against 14 random donor platelet samples. Eight donors were compatible and six were not. Two non-compatible donors were HLA class I A*02 and A*02 B*51 respectively. The remaining four non-compatible donors were not typed for HLA I antigen. All compatible donors were typed for the main HPA antigens but comparison of the typings did not allow to quickly exclude HPA 4b, 6b, 7b, 8b, 9b, and 11b antigen immunisation. A male newborn was delivered by caesarean section at 36 weeks of gestation after spontaneous onset of labour. At birth, platelet count was $4x10^3/\mu l$ with normal white and red blood cell count. An urgent transfusion with a platelet blood component not tested with maternal serum increased platelets to 116 x $10^3/\mu l$; intravenous immunoglobulins were also infused. Another two transfusions were administered on days 4 and 13 due to a drop in the number of platelets (28 x $10^3/\mu l$ and 48 x $10^3/\mu l$ respectively): the platelet pools were obtained from cross-match between maternal serum and sample platelets of random donors. Normal values were reached on day 17.

The newborn blood group was 0 Rh D positive. Capture-P Ready Screen aimed to detect anti-platelet antibodies was non-reactive. Cross-match testing using newborn blood was performed twice. At birth, the neonatal sample was cross-matched against paternal and maternal platelet samples: results were positive and negative, respectively. After 14 days, cross-matching against paternal platelets was repeated with and without chloroquine treatment; both resulted non-reactive.

Results of parental and neonatal HLA I and HPA genotyping performed using polymerase chain reaction (PCR) with sequence-specific oligonucleotides (PCR-SSO) and HPA BeadChip (Immucor, Italy), are shown in Table 1. The mismatches identified prompted further testing in the mother. Cross-match testing against 6 HPA-9b antigen negative donors resulted in two non-compatible and four compatible donors. The same two non-compatible donors were all compatible when cross-matching was performed with chloroquine. Cross-match testing against two donors expressing the HPA9b antigen was reactive with and without chloroquine. Cross-match testing against paternal and neonatal platelets with and without chloroquine was equally reactive. Cross-match testing was performed between maternal serum and 107 different donors in order to have available and compatible blood components available for any neonatal transfusions.

TABLE 1 Trio HLA and platelet genotyping. Newborn genes not present in the mother are in bold

	Mother	Father	Newborn
HLA class I genotype	A*01*69 B*35*37 C*06*12	A***02** B*35***51** C*04*16	A*01***02** B*37***51**
HPA genotype	1a/a, 2a/a, 3a/b, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/a**, 11a/a, 15a/b	1a/a, 2a/a, 3b/b, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/b**, 11a/a, 15a/b	1a/a, 2a/a, 3a/b, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/b**, 11a/a, 15b/b

Abbreviations: HLA, human leukocyte antigen; HPA, human platelet antigen.

FNAIT is a cause of severe thrombocytopenia and ICH in both the fetus and newborn.[1] FNAIT-related ICH is estimated to occur in at least 10:100 000 neonates. It mainly occurs in the third trimester of pregnancy and is associated with severe neurological sequelae and mortality. In most cases, FNAIT is caused by an alloimmune response against human platelet antigens (HPAs). In the Caucasian population HPA-1a antigen accounts for up to 80% cases,[2] followed by HPA-5b (8–15% of cases) and to a lesser extent HPA-3a/5a/15b.[3] More rarely, FNAIT is associated with low-frequency human platelet antigens (LFHPAs) or to HLA class I antigens, especially when related to locus A and B and with a highly expressed titre.[4] Among LFHPAs, HPA-9b is emerging as a significative trigger for FNAIT.[5] Almost two-thirds of apparent cases of FNAIT are not resolved by laboratory confirmation of maternal immunisation against HPA antigens. When other causes of thrombocytopenia are not identified, a possible explanation may be involvement of HLA antibodies or limitations of laboratory studies.

In the current case, parental ABO compatibility excluded ABO-mediated thrombocytopenia. The detection of maternal HLA class I antibodies, identified as HLA A02 and HLA B51 antibodies, and paternal HLA I genotype (HLA A*02; B*35*51), were consistent with the clinical suspicion of FNAIT. However, an additional factor was likely to be involved, presumably related to the HPA system: genotyping showed a parental mismatch in the HPA 9 locus (mother HPA- 9a/a, father HPA 9a/b) and neonatal inheritance of the HPA-9b antigen from the father. Search for HPA antibodies (Pak-Lx Luminex and ELISA Pak plus) was inconclusive because no reactivity was detected against the glycoproteins GPIIb/IIIa, GPIa/IIa, GPIb/IX and GPIV. This can be explained by the limitations of the GP assay used which was not able to recognise the rare specificity HPA-9b. Moreover, it was not possible to find readily available source platelets from local donors carrying the target antigen because only a limited number of them had been typed for HPA and the expected frequency of HPA 4b, 6b, 7b, 8b, 9b, 11b in the population is extremely low (<1%).[3] Cross-match testing between maternal serum and both paternal and neonatal platelets was reactive after chloroquine treatment. This finding was supported by cross-match performed against HPA9b positive donors. This allowed to attribute the FNAIT to the presence of the paternally inherited HPA9b antigen on the son's platelets. It was not possible to identify any antibody specificity in neonatal serum (Capture—P Ready Screening method) presumably due to the extremely low platelet count as a result of the adhesion of the maternal alloantibodies to the neonatal platelets with consequent uptake and elimination.

Since the first report of a HPA-9b related FNAIT in 1995,[6] a total of 15 cases have been reported[5,7,8] and increasing evidence suggests that its prevalence in the population and among fathers of unresolved cases of FNAIT might be greater than previously reported.[6,9] The severity of thrombocytopenia and clinical presentation in our case is consistent with the argument that HPA-9b might be more immunogenic than others HPAs.[5] Our case supports the need to investigate alloimmunisation to HPA-9b and other rare specificities when routine screening for the most common antigens is negative or inconsistent with the laboratory and clinical findings.[3,5] We experienced diagnostic limitations mainly due to the fact that the Ag panel used (Pak-Lx Luminex Immunocor and Elisa Pak Plus Immunocor) does not identify HPA-9b. Difficulties with the detection of antibodies against HPA-9b antibodies have been described by some authors who urged further studies to fully understand the issue.[5,7] Nonetheless, even if FNAIT was strongly suspected both on clinical grounds and after the finding of anti-HLA I antibodies, the mother could not be offered intrauterine therapy of proven efficacy to begin at that gestational age.[1,9]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not openly available due to sensitivity reasons and are available from the corresponding author upon reasonable request.

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