Case report

Neonatal alloimmune thrombocytopenia due to anti-HPA-1b and anti-HLA antibodies: diagnostic and transfusion management of a clinical case

Donatella Londero, Chiara Sindici, Sonia Merluzzi, Egle Bertozzo, Giovanni Barillari



Department of Transfusion Medicine, ASUFC-Udine, Italy

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially life-threatening pregnancy complication, defined by a fetal or neonatal platelet (PLT) count <100×10^9/L (severe if <50×10^9/L)[1]. It is the most common cause of thrombocytopenia in otherwise healthy neonates, with an estimated incidence of 1 in 1,500 pregnancies. FNAIT can occur in first pregnancies, with a recurrence rate of 85-90% in subsequent siblings. Clinical presentations vary, from asymptomatic thrombocytopenia to petechiae or internal bleeding. Intracranial hemorrhage (ICH) is the most severe complication, occurring in approximately 10 per 100,000 live births. Without antenatal screening, ICH is often diagnosed postnatally and may lead to permanent neurological damage or death[2,3].

FNAIT results from maternal IgG alloantibodies targeting fetal human platelet antigens (HPAs) inherited from the father. These HPAs are epitopes on platelet membrane glycoproteins, mainly GPIIIa, GPIIb, and GPIa[4]. To date, 35 HPA systems comprising 41 antigens have been described[5]. The prevalence of HPA antibodies varies among populations: in Caucasians, 80% of FNAIT cases are due to anti-HPA-1a, followed by anti-HPA-5b (10-15%), HPA-15b, -1b (4%), -3a, -5a (2%), and rare antigens like Naka (0.4%)[6]. In Asian populations, anti-HPA-4b and anti-HPA-5b are more common[7].

Diagnostic tools have evolved over the years. Earlier methods used intact platelets or isolated glycoproteins, while current techniques include ELISAs and Luminex bead-based assays[8]. No single method detects all relevant antibodies due to glycoprotein complexity and antibody diversity. Therefore, the choice of assay should reflect population-specific allele frequencies, clinical context, assay sensitivity/specificity, and cost. Management is divided into antenatal (intravenous immunoglobulin-IVIG ± corticosteroids and, in select cases, intrauterine transfusion) and postnatal phases (HPA-compatible platelet transfusions and sometimes IVIG)[9].

This report presents a clinical case of FNAIT to highlight diagnostic and management strategies in a real-world immunohematology setting.

CASE REPORT

A full-term female neonate (39 weeks + 6 days) was born by spontaneous vaginal delivery. Apgar scores were 9 and 10. No maternal autoimmune or hematological history was recorded. Both mother and daughter are blood group O, RhD positive. Within 24 hours of life, the newborn was found to have severe thrombocytopenia (PLT 8×10^9/L), raising suspicion for FNAIT[1]. Initial treatment included two doses

Arrived: 2 July 2025
Revision accepted: 29 August 2025
Correspondence: Donatella Londero
e-mail: donatella.londero@asufc.sanita.fvg.it

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of IVIG (1g/kg) administration and transfusion of two units of random single-donor platelets, raising PLT to 265×10^9/L. Simultaneously, maternal serologic testing and HPA/HLA genotyping of mother, father, and neonate were initiated. Additional evaluations (autoimmune screening, organ function, abdominal ultrasound) were carried out to exclude congenital metabolic diseases, revealing no significant abnormalities.

Maternal serum was tested for anti-HPA antibodies using ELISA (PakPlus - Immucor GTI Diagnostics Inc., Waukesha, WI, USA [a Werfen Company]) and Luminex (PakLx™, Immucor-Werfen), and for anti-HLA Class I antibodies using LabScreen (One Lambda, Los Angeles, CA, USA). Maternal-paternal platelet crossmatch was performed with Capture-P solid-phase assay (Immucor-Werfen). HPA and HLA typing (including HLA-DRB3*01:01, a marker for HPA-1a alloimmunization) was done via real-time PCR (EryQ HPA, BAG Diagnostics, Lich, Germany) and NGS (AlloSeq Tx, CareDx, Brisbane, CA, USA).

RESULTS

Initial ELISA testing did not detect anti-HPA antibodies, and the platelet count (nadir PLT=8×10^9/L) improved after transfusion, suggesting a favorable outcome. However, HPA typing showed maternal-fetal HPA-1 incompatibility and HLA Class I mismatches. Maternal HLA typing revealed the presence of *DRB3*01:01:01* allele. Crossmatch testing showed a strong maternal reaction against paternal platelets; on day 16, the neonate developed gastrointestinal bleeding. So, further immunohematologic investigation and intensified transfusion support were considered: compatible platelet units were selected using maternal serum for crossmatching, and transfusion support was guided by a target platelet count of >50×10^3/μL.

Further testing with Luminex bead assays identified maternal anti-HPA-1b and anti-HLA Class I antibodies. SAB assay revealed high-titer anti-HLA-A*66:01 antibodies, inherited from the father and present in the neonate. Final diagnosis (**Table I**): FNAIT mediated by both anti-HPA-1b and anti-HLA antibodies. After switching to platelets lacking HPA and HLA antigens targeted by preformed antibodies , the neonate stabilized and became transfusion-independent by two months of age.

DISCUSSION

FNAIT often goes unrecognized due to lack of awareness, underdiagnosis, and technical limitations in antibody detection. Most clinical guidelines focus on anti-HPA-1a, leaving other antibodies under investigated. There are no standardized screening or treatment protocols, delaying diagnosis and increasing risk of complications. Antenatal management focuses on preventing ICH through screening for HPA-1a-negative and HLA-DRB3*01:01-positive women, and monitoring for anti-HPA-1a antibodies. IVIG is preferred over invasive approaches due to reduced risk.

Postnataly, early HPA/HLA typing and alloantibody screening are essential. Platelet transfusions –ideally using HPA/HLA-matched units– should not be delayed.

This case illustrates two key issues: a diagnostic delay and prolonged thrombocytopenia. The initial ELISA failed to detect antibodies early due to its lower sensitivity compared to the bead-based multiplex assay

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Table I - *Serological and molecular findings*

Investigation	Method	Sample	Result
HPA Genotyping	Real-time PCR	Mother	**1aa**, 2aa, 3aa, 4aa, 5aa, 6aa, 9aa, 15ab
		Father	**1bb**, 2aa, 3ab, 4aa, 5aa, 6aa, 9aa, 15bb
		Patient	**1ab**, 2aa, 3aa, 4aa, 5aa, 6aa, 9aa, 15ab
HLA Genotyping	NGS	Mother	A*02:01; B*08:01, B*39:24; C*07:01
		Father	A*29:01, A*66:01; B*18:01, B*41:02; C*12:03, C*17:03
		Patient	A*02:01, A***66:01**; B*08:01, B*41:02; C*07:01, C*12:03
Anti-HPA antibody	PakLX	Mother	Positive: **anti-HPA-1b**
Anti-HLA antibody	SA-LX	Mother	Positive (28% PRA): **anti-A*66:01** (MFI=9950)
Crossmatch against paternal platelets	SPRCA	Mother	Positive

Real-time PCR: real-time polymerase chain reaction kit; NGS: next-generation sequencing; PakLx: Pak luminex kit; SA-LX: single-antigen luminex kit; SPRCA: solid phase red cell adherence test.

Blood Transfus. 2026; 24(1): 31-34 doi: 10.2450/BloodTransfus.1131

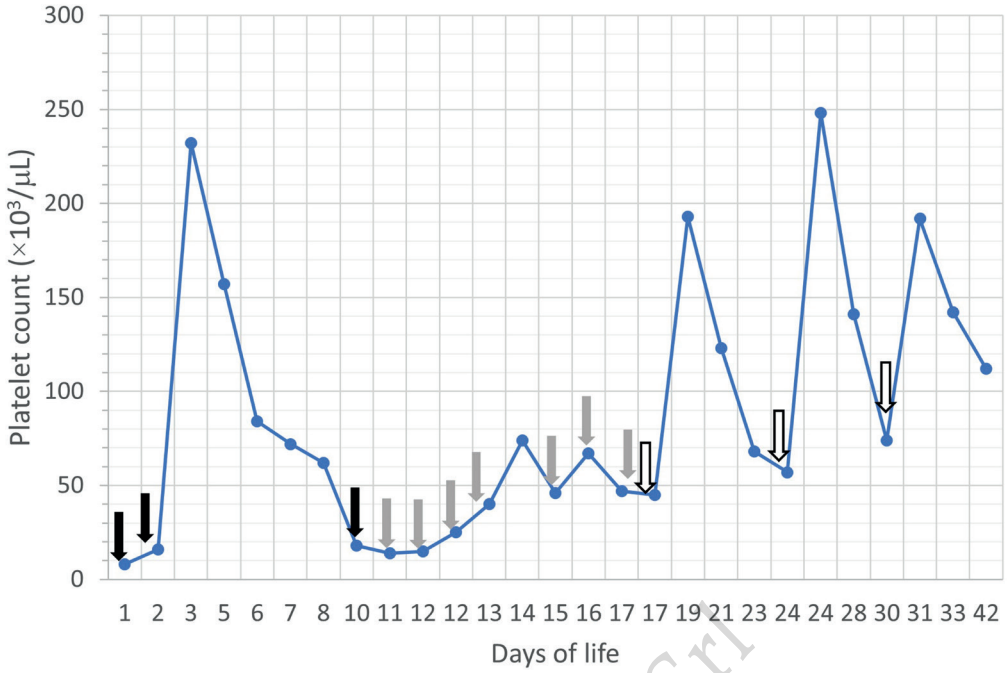


Figure 1 - Platelet dynamics following different types of platelet support
Black arrows indicate transfusion with unselected platelet units; grey arrows indicate transfusion with crossmatch-selected platelet units; white arrows indicate transfusion with HPA/HLA selected platelets. The first transfused random-platelet unit, later typed, was found to be from an HPA-HLA compatible donor.

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(PakLx, Immucor-Werfen), which results from a limited antigen-binding surface and reduced signal amplification. Access to multiple, complementary methods is vital, including platelet crossmatch testing to identify low-titer or rare alloantibodies. The prolonged thrombocytopenia raised the question of additional antibody sources. Literature suggests that maternal breast milk may contain alloantibodies contributing to persistent thrombocytopenia[10,11]. Although we attempted to test breast milk, our methods lacked sufficient sensitivity. Future development of more sensitive tools for such matrices is needed.

Another key factor may have been high titers of anti-HLA antibodies. These are found in up to 40% of pregnancies, though they are usually neutralized by placental expression of HLA. However, strong and persistent antibody titers –as in this case– may contribute to prolonged thrombocytopenia[12].

To date, no direct experimental or clinical evidence has demonstrated suppression of megakaryopoiesis by anti-HPA-1b antibodies as a potential cause of prolonged thrombocytopenia, in contrast to the well-documented effects of anti-HPA-1a antibodies [13].

Platelet count responses in this case (**Figure 1**) clearly showed that HPA/HLA-matched units were more effective than random units, offering better increments and longer-lasting effects. While anti-HPA-1b FNAIT cases have been documented[14], this case emphasizes the added value of using selected donor units –even from on-call typed donors– in clinical practice.

FNAIT is a serious disorder requiring rapid diagnosis and targeted management to avoid adverse outcomes. Postnatal care depends heavily on the availability of advanced diagnostic technologies and a responsive transfusion service. To improve outcomes, transfusion medicine should expand and standardize diagnostic tools, support antenatal screening protocols, investigate alternative biological samples (e.g., breast milk) and ensure access to typed donor platelets.

Establishing reference laboratories and donor registries can significantly reduce intervention time and improve care for affected neonates.

AUTHORSHIP CONTRIBUTIONS

DL designed the research, interpreted data and wrote the manuscript; CS and SM took care to data collection and analysis; EB performed laboratory investigations; GB revised the manuscript. All the Authors approved the final version of the manuscript.

The Authors declare no conflicts of interest.

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