Case report

A rare case of congenital amegakaryocytic thrombocytopenia associated with possible neonatal alloimmune thrombocytopenia and neutropenia by anti-HLA antibodies

Luca Collodel[1], Michela Chirico[2], Paola Cavicchioli[2], Gianluca Gessoni[1]

[1]*Transfusion Medicine Department, dell'Angelo General Hospital, Mestre, Italy*
[2]*Pediatrics Ward and Neonatal Critical Care Unit, dell'Angelo General Hospital, Mestre, Italy*

INTRODUCTION

We report the case of a neonate with thrombocytopenia and neutropenia at birth. Initially a diagnosis of alloimmune thrombocytopenia and alloimmune neutropenia was proposed. Due to the persistence of severe thrombocytopenia, further investigations were performed which led to the identification of an underlying congenital amegakaryocytic thrombocytopenia.

Fetal and neonatal alloimmune thrombocytopenia (FNAIT-NAIT) and neutropenia (FNAIN-NAIN) develop in pregnancy due to maternal alloimmunization and may be considered analogous to hemolytic disease of the fetus and newborn[1]. The clinical presentation of NAIT can be extremely variable, ranging from asymptomatic cases in which the finding of thrombocytopenia is a random event, to patients who have petechiae or hematomas, or with mucous or cutaneous bleeding, up to subjects who develop severe internal hemorrhage[2]. Symptoms of NAIN vary from none to mild skin infections, omphalitis or more severe infections such as pneumonia, sepsis, and meningitis[3].

Congenital amegakaryocytic thrombocytopenia, caused by homozygous or compound heterozygous mutations in *MPL* (CAMT-MPL)[4], the gene encoding for the receptor of thrombopoietin, is a rare inherited bone marrow failure syndrome presenting as an isolated thrombocytopenia at birth, progressing to pancytopenia due to exhaustion of hematopoietic progenitors[5]. The prevalence of CAMT-MPL is unknown, and fewer than 100 cases have been reported in the literature[6]. The reported incidence of NAIT is one case in 1,000–1,500 live births[7]. A limited number of prospective screening studies showed that granulocyte-specific antibodies were detectable in 0.35-1.1% of random, postnatal maternal samples and that the incidence of NAIN was below 0.1%[3].

The rarity of these diseases, to be considered independent and therefore with a very low probability of association, led us to present this case report.

CASE REPORT

Laboratory methods

A blood count was performed using a Sysmex XN1000 instrument (Sysmex Corporation, Kobe, Japan). Immunohematological investigations were conducted using a gel-test method with Grifols Erytra Eflexis automatic instrumentation (Grifols SA, Barcelona, Spain). For the study of human leukocyte antigens (HLA), we used two methods: one involved both high and low resolution single-strand oligonucleotides, supplied by Immucor (Immucor

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Correspondence: Gianluca Gessoni
e-mail: gianluca.gessoni@aulss3.veneto.it

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Italia, Milan, Italy), on Luminex instrumentation, the other was based on sequence-specific primers and agarose gel electrophoresis, supplied by the Olerup company (West Chester, PA, USA).

Assays for antibodies to human platelet antigens (HPA) and anti-HLA were performed using the Pak-LX kit, with a Luminex platform, supplied by Immucor. Assays for anti-human neutrophil antigens (anti-HNA) were performed at the Venetian Regional Reference Center of Padua University using a mixed passive hemagglutination assay.

Clinical and laboratory history

TP, a female, was born on May 16, 2021, at 37 + 1 weeks of gestation by urgent Cesarean section. At birth her Apgar index was 9/10/10; she weighed 2,990 g, was 45.5 cm long, and had a head circumference of 33 cm. Neonatal blood typing showed A CcDee kk; the direct antiglobin test was negative. Antenatal ultrasound performed at 30 and 32 weeks of pregnancy showed normal fetal growth (abdominal circumference 95th percentile at 30 weeks, 80th percentile at 32 weeks). Villocentesis demonstrated a 46 XX karyotype with pericentric inversion of chromosome 4 (also present in the mother).

The mother (CG) was a 36-year-old, para 3003, from Bangladesh, with β-thalassemia trait. No complications were reported in the infants born from her previous pregnancies. With regard to serological status, the mother was negative for hepatitis B and C virus, human immunodeficiency virus and syphilis, was immune to toxoplasma and rubella, and had had a previous cytomegalovirus infection. She had gestational diabetes. The mother's blood type was B CcDee kk and an indirect antiglobulin test was negative.

The father (MP) was a 42-year-old from Bangladesh. His blood type was A CcDee kk. The parents are first cousins.

In the first hours of life, TP showed diffuse cutaneous petechiae and widespread ecchymoses, for which she was transferred to the neonatal pathology unit in our hospital. A blood count revealed thrombocytopenia (platelet count: 18×10^9/L), moderate anemia for age (red blood cell count: 3.34×10^{12}/L; hemoglobin: 123 g/L), and mild neutropenia (white blood cell count: 9×10^9/L; granulocytes 5.3×10^9/L). With the suspicion of neonatal sepsis, the C-reactive protein level was measured and resulted within normal values. A pediatric unit of irradiated platelets was promptly transfused. In the following weeks TP remained symptomatic, with muco-cutaneous bleeding. As illustrated in **Figure 1A**, blood count monitoring showed persistent severe thrombocytopenia, and in the first 3 months of her life, TP received 19 platelet transfusions. The patient showed progressive anemia with reduced reticulocyte count requiring transfusion support (one 100 mL pediatric unit) on a monthly basis. Moreover, as shown in **Figure 1B**, she had intermittent mild neutropenia (minimum value, 0.9×10^9/L), without severe bacterial infection. Somatic growth and neurological development were normal. On August 9, 2021, TP was hospitalized in order to receive a new platelet transfusion, on which occasion a SARS CoV-2 infection was discovered. In the meantime, further diagnostic tests were performed: an assay for anti-HPA, carried out in maternal serum, gave negative results, while an anti-HLA antibody towards the HLA-B*44 antigen was detected. HLA typing in TP, GC and MP was performed and the results are reported in **Table I**. An assay for specific antigens of neutrophil granulocytes (HNA) also gave negative results. Because of the negativity for anti-HPA and anti-HNA antibodies, genotyping for HPA and HNA was not performed. Moreover, and unfortunately, a cross-match between maternal serum and paternal granulocytes was also not performed.

With the presumption that the antibody to the HLA-B*44 antigen could explain both thrombocytopenia and neutropenia, on the basis of the available data, a diagnosis of NAIT and NAIN, attributable to an allo-antibody directed towards the HLA B*44 antigen, was made. However, due to the persistence of severe thrombocytopenia associated with mild neutropenia and slight anemia with a reduced reticulocyte count, the presence of an underlying pathology

Table I - *HLA type of the patient and her parents*

	Mother	Father	Neonate
HLA-A	*03, *11	*11, *33	*11
HLA-B	*37	*44	*37, *44
HLA-C	*06, *07	*06, *07	*06, *07
HLA-DRB1	*10, *15	*15	*10, *15
HLA-DQB1	*05, *06	*06	*06

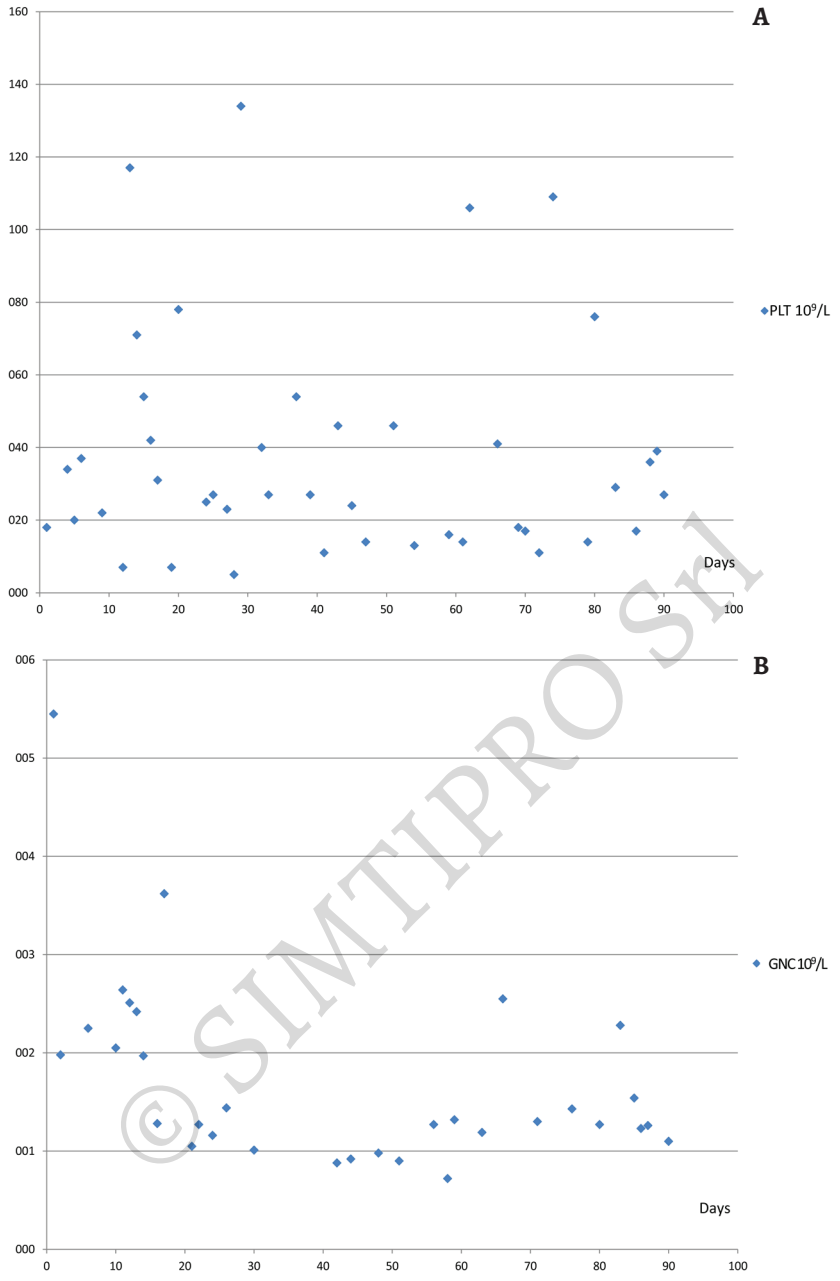

Figure 1 - Trends of platelet and neutrophil granulocyte counts in the first 3 months of the patient's life
(**A**) Trend of platelet number. (**B**) Trend of neutrophil granulocyte number. PLT: platelet count; GNC: granulocyte count.

with trilinear involvement was hypothesized. So, on September 30, 2022, the baby's bone marrow was harvested in Padua: the cellularity was reported to be ++--/+++- and there were rare megakaryocytes (+---), with the presence of immature forms. The myeloid series was well represented (26%) with a preserved maturation pyramid. There were 3% eosinophils with precursors, 45% lymphocytes with immature forms, and 5% monocytes with precursors. The erythroid series was well represented (24%) with a preserved maturation pyramid. There were no atypical cells. The data collected suggested a congenital amegakaryocyte

thrombocytopenia. A genetic study highlighted the variants NM_005373.3 c1069C>T and NM_005373.3 c378 on the *MPL* gene, confirming the clinical suspicion.

DISCUSSION

The case report of this unlucky baby girl indicates a very rare combination of congenital and acquired hematological pathologies. Two of these (NAIT and CAMT-MPL) usually require a differential diagnosis and are not considered in a possible association. Moreover, we had to face other worrying conditions: a moderate neutropenia and a slight anemia. In Caucasians, the great majority of NAIT are due to maternal antibodies directed against paternally inherited HPA, usually HPA-1a, expressed on fetal platelets[8]. Collectively, anti-HLA class I antibodies may be considered as a potential cause of NAIT, especially in cases with a very high titer of antibodies[9]. Rare cases of NAIN have been associated with the presence of anti-HLA calss I antibodies alone, and the ability of anti-HLA to cause neutropenia is still debated, since HLA antibodies can be absorbed by placental cells[10-12]. The simultaneous occurrence of thrombocytopenia and neutropenia in the neonate is rare (0.03%).

We initially made a diagnosis of NAIT and NAIN. Based on this diagnosis, we would have expected, as the weeks went by, a progressive improvement in the clinical picture and blood count as maternal antibodies were gradually cleared from the neonate's circulation. Since we did not observe the expected evolution, we considered alternative diagnoses that could justify a clinical picture characterized by a bone marrow insufficiency involving the myeloid, erythrocyte and megakaryocytic lines. So the bone marrow was analyzed and, together with a genetic study, confirmed the diagnosis of CAMT-MPL.

To our knowledge, no cases of association between these three diseases have been reported in literature, while the problem of the differential diagnosis between NAIN and CAMT has sometimes arisen[10,11]. In our opinion this case report confirms the importance of early diagnosis of FNAIT, NAIN and CAMT, but also highlights the need to consider their possible coexistence, to avoid delays in diagnosis.

The Authors declare no conflicts of interest.

REFERENCES

1. Winkelhorst D, Kamphuis MM, de Kloet LC, Zwaginga JJ, Oepkes D, Lopriore E Severe bleeding complications other than intracranial hemorrhage in neonatal alloimmune thrombocytopenia: a case series and review of the literature. Transfusion 2016; 56: 1230-1235. doi: 10.1182/blood-2016-10-739656 .
2. de Vos TW, Winkelhorst D, de Haas M, Lopriore E, Oepkes D. Epidemiology and management of fetal and neonatal alloimmune thrombocytopenia. Transfus Apher Sci 2020; 59: 102704. doi: 10.1016/j.transci.2019.102704.
3. Porcelijn L, de Haas M. Neonatal alloimmune neutropenia. Transfus Med Hemother 2018; 45: 311-316. doi: 10.1159/000492949.
4. Germeshausen M, Ballmaier M. CAMT-MPL: congenital amegakaryocytic thrombocytopenia caused by MPL mutations - heterogeneity of a monogenic disorder - a comprehensive analysis of 56 patients. Haematologica 2021; 106: 2439-2448. doi: 10.3324/haematol.2020.257972.
5. Ballmaier M, Germeshausen M. Congenital amegakaryocytic thrombocytopenia: clinical presentation, diagnosis and treatment. Semin Thromb Hemost 2011; 37: 673-681. doi: 10.1055/s-0031-1291377.
6. Germeshausen M, Ballmaier M. Congenital amegakaryocytic thrombocytopenia - not a single disease. Best Pract Res Clin Haematol 2021; 34: 101286. doi: 10.1016/j.beha.2021.101286.
7. Mella MT, Eddleman K. Neonatal alloimmune thrombocytopenia. Int J Clin Transfus Med 2015; 3: 29-40. doi: 10.2147/IJCTM.S51926.
8. Sachs UJ, Wienzek-Lischka S, Duong Y, Qiu D, Hinrichs W, Cooper N, et al. Maternal antibodies against paternal class I human leukocyte antigens are not associated with foetal and neonatal alloimmune thrombocytopenia. Br J Haematol 2020; 189: 751-759. doi: 10.1111/bjh.16419.
9. Bonstein L, Haddad N. Taking a wider view on fetal/neonatal alloimmune thrombocytopenia. Thromb Res 2017; 151(Suppl 1): S100-S102. doi: 10.1016/S0049-3848(17)30078-6.
10. Doan J, Kottayam R, Krishnamurthy MB, Malhotra A. Neonatal alloimmune neutropaenia: experience from an Australian paediatric health service. J Paediatr Child Health 2020; 56: 757-763. doi: 10.1111/jpc.14735.
11. Murray NA. Evaluation and treatment of thrombocytopenia in the neonatal intensive care unit. Acta Paediatr Suppl 2002; 91: 74-81. doi: 10.1111/j.1651-2227.2002.tb02908.x.
12. Abbas SA, Lopes LB, Moritz E, Martins JO, Chiba AK, Kunioshi AM, et al. Serologic and molecular studies to identify neonatal alloimmune neutropenia in a cohort of 10,000 neonates. Br J Haematol 2021; 192: 778-84. doi: 10.1111/bjh.17295.