

## Case Report

# Neonatal Alloimmune Thrombocytopenia Associated with HLA-A11 Alloantibody

Pimlak Charoenkwan<sup>1</sup>, Rungrote Natesirinilkul<sup>1</sup>, Nipapan Leetrakool<sup>2</sup>,

Sucheela Chomsook<sup>2</sup>, Pawinee Kupatawintu<sup>3</sup> and Somporn Chotinaruemol<sup>1</sup>

<sup>1</sup>Department of Pediatrics; <sup>2</sup>Blood Bank Section, Faculty of Medicine, Chiang Mai University, Chiang Mai; <sup>3</sup>National Blood Centre, Thai Red Cross Society, Bangkok

**Abstract:** Neonatal alloimmune thrombocytopenia (NAIT) is a result of platelet destruction by maternal antibodies directed against paternally-inherited antigens on neonatal platelets. NAIT is mostly caused by antibodies to human platelet antigens (HPA). This is a report of a term newborn with NAIT associated with an antibody to the human leukocyte antigens (HLA). The newborn presented with cutaneous bleeding at 5 days of age. The lowest platelet count was  $36.8 \times 10^9/L$ . An antibody to HLA-A11 was present in maternal serum while HPA antibodies were not detected. The HLA-A11 antigen was identified in the patient, sister, and father, but not the mother. The newborn received intravenous immunoglobulin and had a favorable outcome.

**Keywords :** ● HLA antibody ● Human leukocyte antigen ● NAIT ● Neonatal alloimmune thrombocytopenia  
*J Hematol Transfus Med* 2015;25:149-54.

### Introduction

Neonatal alloimmune thrombocytopenia (NAIT) is a result of platelet destruction by maternal antibodies directed against paternally-inherited antigens on fetal platelets. The incidence of NAIT is 1:1,000-1:2,000 pregnancies.<sup>1,2</sup> The most serious complication of NAIT is intracranial hemorrhage, which occurs in 10-30% of patients.<sup>1,3-5</sup> NAIT can occur in the first pregnancy, and recur in approximately 90% of subsequent pregnancies.<sup>5-7</sup> The most common cause of NAIT is antibodies to one of the human platelet antigens (HPA). In the western population, antibodies to HPA-1a and HPA-5b are the most common causes, while in the Japanese population antibody to HPA-4b is the most common.<sup>1,2</sup>

HLA antibodies frequently occur in pregnant women, although their role in NAIT has been controversial.<sup>8</sup> We

herein report a case of NAIT associated with HLA-A11 antibody. The antibody reacting to paternal HLA phenotype specific cells was detected in maternal serum. The patient was treated with intravenous immunoglobulin (IVIg) and had a favorable outcome.

### Case report

A five-day-old female newborn presented with petechiae scattering over her face and body. She was the second child of the family, born by Caesarean section at 39 weeks of gestation. Her birth weight was 3,344 g. The Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. The 31-year-old mother was healthy and had not received any blood transfusion. Her 2-year-old sister was healthy with no history of NAIT. Physical examination showed an active newborn with generalized petechiae. She was otherwise well, without hepatosplenomegaly, dysmorphic features or signs of infection.

A complete blood count showed a hemoglobin (Hb) of 15.3 g/dL, hematocrit (Hct) 45.4%, white blood cell

Received 11 March 2014 Accepted 7 April 2015

Requests for reprints should be addressed to Assoc. Prof. Pimlak Charoenkwan, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand E-mail: pimlak.c@cmu.ac.th

(WBC) count  $7.4 \times 10^9/L$  (neutrophil 71%, eosinophil 3.4%, basophil 1.3%, lymphocyte 15.3%, monocyte 9.2%), and a platelet count of  $63.5 \times 10^9/L$ . Maternal platelet count was  $298 \times 10^9/L$ . Later at 6 days of age, the platelet count decreased to  $36.8 \times 10^9/L$ . The patient was then treated with 5 g (1.5 g/kg) of IVIg. Platelet transfusion was not given. Her platelet count increased to  $155.1 \times 10^9/L$  on the next day. After a discharge from the hospital, the patient was clinically well without further bleeding. A cranial ultrasound showed no evidence of intracranial hemorrhage. The platelet count was  $529 \times 10^9/L$  and  $434 \times 10^9/L$  at 14 and 51 days of age, respectively.

## Materials and Methods

### Screening for platelet antibodies

The maternal and the patient's sera were collected at 5 days after delivery. The sera were screened for platelet antibodies by a qualitative solid-phase enzyme-linked immunosorbent assay (ELISA) (Pakplus, Gen-Probe Incorporated, Waukesha, WI, USA). The test screened for antibodies to HLA class I antigens and to epitopes on platelet glycoproteins IIb/IIIa, Ib/IX, Ia/IIa, and IV. The maternal serum was also screened for platelet-associated immunoglobulin G (IgG) by a solid phase red cell adherence (SPRCA) assay.

### Screening and identification of HLA antibodies

The maternal serum was screened for HLA antibodies by microlymphocytotoxicity test (LCT), with known panels of T and B lymphocytes which covered the common HLA antigens in Thai populations, and was subsequently tested by the flow-based bead assays (Luminex, One Lambda Inc., Canoga Park, CA, USA) to determine the HLA class I specificity. The patient's serum was also tested by the flow-based bead assays.

### HPA Typing

Genotyping of HPA-1 to HPA-7 and HPA-15 of the patient, her parents and sister were performed by using a multiplex polymerase chain reaction as previously described.<sup>9</sup>

### HLA typing and crossmatching

HLA typing was performed in the patient, her parents and sister. HLA-A and HLA-B were serologically typed by micro LCT with qualified anti-HLA sera (Department of Transfusion Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand). HLA-A, B, and HLA-C alleles were determined by the polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSO) method (Innogenetics, Gent, Belgium). The crossmatching between maternal serum and the patient's, sister's, and paternal lymphocytes was performed by micro LCT. The crossmatching between patient's serum and the sister's, and paternal lymphocytes was performed by the same method. The crossmatching between maternal serum and paternal platelets was also performed by a SPRCA assay.

## Results

The results of platelet HPA and HLA genotyping and antibody identification from the patient and her family were summarized in Tables 1 and 2. The ELISA assay and platelet-associated IgG screening showed no detectable antibodies. There were HPA-3 and HPA-15 incompatibilities between the newborn and her mother. HLA antibody screening and identification showed anti-HLA antibodies to HLA class I (HLA-A11) in the mother. HLA crossmatching in the newborn initially showed positive result, but after the serum was treated with dithiothreitol (DTT), the antibody was not detected. This may be explained by a low level of antibody.

Similarly, HLA-A11 antigen was found in the patient (HLA-A\*11:01/31:01), sister (HLA-A\*11:02/31:01) and father (HLA-A\*11:01/11:02), but not in the mother. The crossmatching between maternal serum and the patient's, sister's, and paternal lymphocytes by micro LCT showed strongly positive results. The crossmatching between maternal serum and paternal platelets by SPRCA assay also showed positive result. The mother's blood group was O, Rh(D) positive. The patient's, sister's, and paternal blood group were B, Rh(D) positive. To exclude a

**Table 1** Platelet HPA and HLA genotypes

Platelet HPA and HLA genotypes	Mother	Father	Sister	Newborn
HPA-1	aa	aa	aa	aa
HPA-2	aa	aa	aa	aa
HPA-3	bb	aa	ab	ab
HPA-4	aa	aa	aa	aa
HPA-5	aa	aa	aa	aa
HPA-6	aa	aa	aa	aa
HPA-7	aa	aa	aa	aa
HPA-8	aa	aa	aa	aa
HPA-9	aa	aa	aa	aa
HPA-10	aa	aa	aa	aa
HPA-11	aa	aa	aa	aa
HPA-13	aa	aa	aa	aa
HPA-14	aa	aa	aa	aa
HPA-15	aa	bb	ab	ab
HPA-17	aa	aa	aa	aa
HLA-A	02:01/31:01	11:01/11:02	11:02/31:01	11:01/31:01
HLA-B	15:02/51:01	46/44:03	46/51:01	44:03/51:01
HLA-C	08:01/14:02	01:02/07:01	01:02/14:02	07:01/14:02

**Table 2** Results of HPA and HLA antibodies studies

Platelet HPA and HLA antibodies	Mother	Newborn
Platelet antibody screening by ELISA	Negative	Negative
Platelet associated IgG screening by SPRCA	Negative	Not tested
Crossmatch serum with paternal lymphocytes by microlymphocytotoxicity test	Positive	Negative
Crossmatch serum with paternal platelets by SPRCA assay	Positive	Not done
HLA class I antibody screening by Luminex method	Positive 65%	Negative
HLA class I antibody identification by Luminex method	HLA-A11 antibody	Not tested

ELISA, enzyme-linked immunosorbent assay; HLA, human leukocyte antigen; HPA, human platelet antigen; IgG, immunoglobulin G; SPRCA, solid phase red cell adherence

false positive crossmatching result from anti-B, a cross-matching between O-type serum from a male control was negative.

### Discussion

This is a report of NAIT associated with an HLA antibody. The newborn presented with early-onset thrombocytopenia with cutaneous bleeding at five days of age. The diagnosis was made by the presence of HLA antibody directed to the newborn HLA-A11 antigen. An anti-HLA-A11 antibody detected in maternal but

not in the patient's serum may be explained by a delay in serum collection, at five days of age. The father had HLA-A11 (HLA-A\*11:02/11:01), and the elder daughter and the patient had different alleles of HLA-A11. The HLA-A11 antigen presented in both daughters likely caused repeated sensitization and alloimmunization in the mother. The crossmatching between maternal serum and the patient's, sister's, and paternal lymphocytes showed positive results. The patient did not have infections or hepatosplenomegaly that could explain thrombocytopenia. She responded well to IVIg.

Of note, our case had HPA-3 and HPA-15 incompatibilities. Although HPA antibodies were not detected, the HPA incompatibilities may weaken our conclusion that the NAIT in this case was caused solely by the HLA antibody. Antibody to HPA-3a was reported to react with labile component of HPA-3a, and only with fresh, unfixed platelets by SPRCA, immunofluorescence test and mixed passive hemagglutination test. The MAIPA assay showed negative result.<sup>10</sup> An ELISA test performed in our case may have failed to detect an antibody to labile component of HPA-3a.

Antibodies to human leukocyte antigen (HLA) are seen in 7-39% of pregnancies.<sup>11</sup> As platelets express class I HLA on the surface<sup>12</sup>, it is conceivable that HLA antibodies are a cause of NAIT. Nevertheless, several large studies have shown that the antibodies are not associated with NAIT.<sup>13-15</sup> In a prospective study by King *et al*, HLA-antibodies were seen in 31% of 447 maternal sera at delivery, but the presence of antibodies was not associated with the newborns' platelet count.<sup>13</sup> Sharon *et al* showed that HLA antibodies were detected in 33% of 1,507 maternal sera. Although the antibodies reacted with paternal lymphocytes, none of the newborns had thrombocytopenia.<sup>14</sup> Reasons refuting the role of HLA antibodies include the presence of HLA antigens on fetal placental tissue and all nucleated cells that can adsorb the antibodies, and the reduced HLA expression on neonatal platelets.<sup>8</sup>

On the other hand, Mueller-Eckhardt *et al* reported that in 6.9% of 144 HPA-1A negative mothers of newborns with presumed NAIT, only HLA antibodies were present.<sup>1</sup> This implicated the role of HLA antibodies in NAIT. There are also several case reports of NAIT caused by HLA antibodies.<sup>11,16-22</sup> The evidences are especially strong for the antibody to HLA-A2 and NAIT.<sup>11,16</sup> HLA-A2 is the common HLA antigen in most populations. In this case, the antibody is directed to HLA-A11, which is the common HLA antigen in Thai population (frequency of 27.7%).<sup>23</sup>

In NAIT associated with HLA antibodies, the onset is usually shortly after birth, but may be later to within a few days after birth.<sup>16-22</sup> The platelet counts are moderately to severely decreased. Cases with concurrent neutropenia were reported.<sup>21,22</sup> Treatments of choice for those with severe thrombocytopenia or bleeding are IVIg and maternal or cross-matched platelet transfusion.

In our case, the patient responded well to IVIg without platelet transfusion. There have been several reports of mild cases of NAIT associated with antibodies to HLA who resolved spontaneously or responded to IVIg alone and not needing platelet transfusion.<sup>18-20,22</sup> Although the mother and patient were not tested for autoimmune diseases, neonatal autoimmune thrombocytopenia was less likely as her mother had no history of autoimmune disease and had a normal platelet count.

Anti-HLA antibody was reported to cause a refractoriness to random platelet transfusion in a newborn who had NAIT from antibody to HPA-1a.<sup>24</sup> Transfusion of maternal platelets was effective in increasing the platelet count. It was suggested that anti-HLA antibodies, although not associated with NAIT, may complicate the treatment by causing refractoriness, and transfusion of maternal platelet was a treatment of choice.

Our case supports the role of HLA antibodies as a cause of NAIT. The condition should be considered in newborns with isolated thrombocytopenia, who have HLA antibodies directed to paternal antigens, no HPA incompatibilities, and other causes of thrombocytopenia excluded.

## References

1. Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, et al. 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989;1(8634):363-6.
2. Murphy MF, Williamson LM. Antenatal screening for fetomaternal alloimmune thrombocytopenia: an evaluation using the criteria of the UK National Screening Committee. *Br J Haematol* 2000;111:726-32.
3. Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Decary F. Alloimmunization to the PlA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990;74:209-15.

4. Lipitz S, Ryan G, Murphy MF, Robson SC, Haeusler MC, Metcalfe P, et al. Neonatal alloimmune thrombocytopenia due to anti-P1A1 (anti-HPA-1a): importance of paternal and fetal platelet typing for assessment of fetal risk. *Prenat Diagn* 1992;12:955-8.
5. Bussel JB, Zabusky MR, Berkowitz RL, Robson SC, Haeusler MC, Metcalfe P, et al. Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997;337:22-6.
6. Kaplan C, Forestier F, Daffos F, Tchernia G, Waters A. Management of fetal and neonatal alloimmune thrombocytopenia. *Transfus Med Rev* 1996;10:233-40.
7. Letsky EA, Greaves M. Guidelines on the investigation and management of thrombocytopenia in pregnancy and neonatal alloimmune thrombocytopenia. Maternal and Neonatal Haemostasis Working Party of the Haemostasis and Thrombosis Task Force of the British Society for Haematology. *Br J Haematol* 1996;95:21-6.
8. Taaning E. HLA antibodies and fetomaternal alloimmune thrombocytopenia: myth or meaningful? *Transfus Med Rev* 2000;14:275-80.
9. Kengkate M, Butthep P, Kupatawintu P, Kanunthong S, Chantratita W, Nathalang O. Genotyping of HPA-1 to -7 and -15 in the Thai population using multiplex PCR. *Transfus Med* 2012;22:272-6.
10. Lin M, Shieh SH, Liang DC, Yang TF, Shibata Y. Neonatal alloimmune thrombocytopenia in Taiwan due to an antibody against a labile component of HPA-3a (Baka). *Vox Sang* 1995;69:336-40.
11. Chow MP, Sun KJ, Yung CH, Hu HY, Tzeng JL, Lee TD. Neonatal alloimmune thrombocytopenia due to HLA-A2 antibody. *Acta Haematol* 1992;87:153-5.
12. Mueller-Eckhardt C, Santoso S, Kiefel V. Platelet alloantigens--molecular, genetic, and clinical aspects. *Vox Sang* 1994;67(Suppl 3):89-93.
13. King KE, Kao KJ, Bray PF, Casella JF, Blakemore K, Callan NA, et al. The role of HLA antibodies in neonatal thrombocytopenia: a prospective study. *Tissue Antigens* 1996;47:206-11.
14. Sharon R, Amar A. Maternal anti-HLA antibodies and neonatal thrombocytopenia. *Lancet* 1981;1(8233):1313.
15. Marshall LR, Brogden FE, Roper TS, Barr AL. Antenatal platelet antibody testing by flow cytometry--results of a pilot study. *Transfusion* 1994;34:961-5.
16. Evans, DI. Immune amegakaryocytic thrombocytopenia of the newborn: association with anti-HLA-A2. *J Clin Pathol* 1987;40:258-61.
17. del Rosario ML, Fox ER, Kickler TS, Kao KJ. Neonatal alloimmune thrombocytopenia associated with maternal anti-HLA antibody: a case report. *J Pediatr Hematol Oncol* 1998;20:252-6.
18. Saito S, Ota M, Komatsu Y, Ota S, Aoki S, Koike K, et al. Serologic analysis of three cases of neonatal alloimmune thrombocytopenia associated with HLA antibodies. *Transfusion* 2003;43:908-17.
19. Thude H, Schomer U, Helfricht C, Loth M, Maak B, Barz D. Neonatal alloimmune thrombocytopenia caused by human leukocyte antigen-B27 antibody. *Transfus Med* 2006;16:143-9.
20. Monchamont P, Dubois V, Obegi C, Vignal M, Merieux Y, Gebuhrer L, et al. HLA antibodies and neonatal alloimmune thrombocytopenia. *Acta Haematol* 2004;111:215-20.
21. Gramatges MM, Fani P, Nadeau K, Pereira S, Jeng MR. Neonatal alloimmune thrombocytopenia and neutropenia associated with maternal human leukocyte antigen antibodies. *Pediatr Blood Cancer* 2009;53:97-9.
22. Starcevic M, Tomicic M, Malenica M, Zah-Matakovic V. Neonatal alloimmune thrombocytopenia caused by anti-HLA-A24 alloantibodies. *Acta Paediatr* 2010;99:630-2.
23. Kupatawintu P, Pheancharoen S, Srisuddee A, Tanaka H, Tadokoro K, Nathalang O. HLA-A,-B, -DR haplotype frequencies in the Thai Stem Cell Donor Registry. *Tissue Antigens* 2010;75:730-6.
24. Grainger JD, Morrell G, Yates J, Deleacy D. Neonatal alloimmune thrombocytopenia with significant HLA antibodies. *Arch Dis Child Fetal Neonatal Ed* 2002;86:F200-1.

## ภาวะเกล็ดเลือดต่ำจากการทำลายด้วยอัลโลอิมมูนในทารกแรกเกิด ที่เกี่ยวข้องกับแอนติบอดีชนิด HLA-A11

พิมพ์ลักษณ์ เจริญขวัญ<sup>1</sup> รุ่งโรจน์ เนตรศิริณกุล<sup>1</sup> นิภาพรณ ลีตระกูล<sup>2</sup> สุชีลา ชมสุข<sup>2</sup>

ภาวิณี คุปตวินท์<sup>3</sup> และ สมพร โชตินฤมล<sup>1</sup>

<sup>1</sup>ภาควิชากุมารเวชศาสตร์ <sup>2</sup>งานธนาคารเลือด คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ <sup>3</sup>ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

**บทคัดย่อ** ภาวะเกล็ดเลือดต่ำจากการทำลายด้วยอัลโลอิมมูนในทารกแรกเกิด (neonatal alloimmune thrombocytopenia, NAIT) เป็นผลจากการทำลายเกล็ดเลือดโดยแอนติบอดีจากมารดาซึ่งจับกับแอนติเจนบนผิวเกล็ดเลือดของทารกที่รับถ่ายทอดจากบิดา NAIT ส่วนใหญ่เกิดจากแอนติบอดีต่อแอนติเจนชนิด human platelet antigen (HPA) งานวิจัยนี้ได้รายงานผู้ป่วยทารกแรกเกิดที่เป็น NAIT ที่เกี่ยวข้องกับแอนติบอดีต่อแอนติเจนชนิด human leukocyte antigen (HLA)-A11 โดยทารกมีอาการเลือดออกที่ผิวหนังเมื่ออายุ 5 วัน จำนวนเกล็ดเลือดต่ำที่สุด  $36.8 \times 10^9$  ต่อลิตร พบแอนติบอดีต่อ HLA-A11 ในซีรัมของมารดาโดยไม่พบแอนติบอดีต่อ HPA พบแอนติเจนชนิด HLA-A11 ในทารก พี่สาว และบิดา แต่ไม่พบในมารดา ทารกได้รับอิมมูโนโกลบูลินทางหลอดเลือดดำ และตอบสนองต่อการรักษาดี

**Keywords :** ● แอนติบอดีต่อ HLA ● Human leukocyte antigen ● NAIT

● ภาวะเกล็ดเลือดต่ำจากการทำลายด้วยอัลโลอิมมูนในทารกแรกเกิด

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2558;25:149-54.