Int J Hematol (2018) 107:251–256
https://doi.org/10.1007/s12185-017-2310-5

CASE REPORT

Successful management of a hydropic fetus with severe anemia and thrombocytopenia caused by anti-CD36 antibody

Xiuzhang Xu[1,2] · Lin Li[3] · Wenjie Xia[1] · Haoqiang Ding[1] · Dawei Chen[1] · Jing Liu[1] · Jing Deng[1] · Yangkai Chen[1] · Zhiming He[3] · Jiali Wang[1] · Yuan Shao[1] · Sentot Santoso[1,2] · Xin Ye[1] · Qun Fang[3]

Received: 28 March 2017 / Revised: 2 August 2017 / Accepted: 3 August 2017 / Published online: 16 August 2017

Abstract Cases of CD36 deficiency are not rare in Asian populations, foetal and neonatal alloimmune thrombocytopenia (FNAIT) caused by anti-CD36 *iso*antibodies appears more frequent than other HPA alloantibodies. However, little is known about the treatment of anti-CD36 mediated FNAIT in this region. A Chinese male foetus, whose mother had a history of multiple intrauterine foetal demise and/or hydrops, was diagnosed with severe FNAIT at 27 weeks of gestational age. Immunological analysis revealed total absence of CD36 on platelets and monocytes from mother, caused by a 329–330*del*AC mutation of the CD36 gene. Anti-CD36 and anti-HLA class I antibodies were detected in the maternal serum, whereas only anti-CD36 *iso*antibodies were detectable in the foetal blood sample. Serial intrauterine transfusions with red blood cells (RBC) and platelets from a CD36*null* donor were performed to improve the severe anaemia and thrombocytopenia. The baby (2250 g; Apgar scores 10) was delivered vaginally at 32 weeks of gestation with normal haemoglobin (186 g/L) but low platelet count (48×10^9/L). After 2 days the platelet count rose to 121×10^9/L. This report suggests that intrauterine transfusions with compatible RBC and CD36*null* platelets are useful in preventing the deleterious clinical effects of anti-CD36-mediated severe FNAIT.

Keywords Foetal and neonatal alloimmune thrombocytopenia · Anti-CD36 antibody · Foetal anaemia and hydrops · Intrauterine transfusion

Introduction

Foetal and neonatal alloimmune thrombocytopenia (FNAIT), occurring in 1/800–1/1000 live births, is the most common cause of severe thrombocytopenia and intracranial haemorrhage (ICH) in foetus and term newborns [1, 2]. FNAIT is caused by maternal antibodies which recognize paternal-derived antigen on foetal platelets leading to platelet destruction. In Caucasians, more than 75% of FNAIT cases are induced by alloantibodies against human platelet antigen (HPA)-1a [3, 4], whereas the most common antibodies related to FNAIT for Japanese are anti-HPA-4b alloantibodies [5]. Both alloantibody specificities, however, have not been reported in Chinese population so far. In contrast, anti-CD36 *iso*antibodies, developed in type I CD36 deficient mothers are frequently reported as the cause of FNAIT in China [6, 7].

CD36 (also known as GPIV), is a highly glycosylated 88-kDa protein and is expressed widely on human cells including platelets, monocytes, macrophages, erythroid precursors and endothelial cells [8]. There are two types of CD36 deficiency; type I with total absence of CD36 expression on platelets and monocytes and type II lacking of CD36 on platelets but not on monocytes [9]. CD36 deficiency on

Xiuzhang Xu and Lin Li contributed equally to this work.

✉ Xin Ye
huang_yexin@qq.com

✉ Qun Fang
fang_qun@163.com

[1] Institute of Blood Transfusion, Guangzhou Blood Centre, 31 LuYuan Road, Guangzhou 510095, Guangdong, People's Republic of China

[2] Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig University, Giessen, Germany

[3] Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-sen University, 58 ZhongShan 2nd Road, Guangzhou 510080, Guangdong, People's Republic of China

platelets is more frequent in Asians (about 3–11%) and Africans (about 8%) than in white people (<0.4%) [7].

Anti-CD36 antibodies (originally named anti-Nak[a]) were first described in a case of platelet transfusion refractoriness [10, 11]. Meanwhile, the clinical relevance of anti-CD36 antibodies has been reported in other immune mediated disorders including thrombotic thrombocytopenic purpura, adverse transfusion reactions and FNAIT [12–14].

The clinical pictures of anti-CD36 mediated FNAIT are heterogeneous ranging from widespread petechial haemorrhages, gastrointestinal bleeding, severe anaemia and thrombocytopenia and hydrops fetalis [14–17]. Recently, we reported two cases of FNAIT with recurrent abortions and hydrops [6, 18].

In comparison to anti-HPA alloantibodies, little is known about the management of FNAIT caused by anti-CD36 *iso*antibodies. Here, we present a successful management of a life-threatening case of FNAIT case with hydrops fetalis, serious anaemia and thrombocytopenia caused by anti-CD36 *iso*antibodies.

Case report

A Chinese male foetus was found with ascites, pericardial effusion, and cardiomegaly and hydropic placenta by ultrasound at 26 weeks of gestational age, and these clinical symptoms became severe at 27 weeks of gestational age (Fig. 1a). Doppler assessment of the middle cerebral artery revealed a peak systolic velocity (MCA-PSV) is 2.1 multiples of median (MOM). Umbilical cord centesis showed foetal anaemia (haemoglobin 84 g/L, haematocrit 25.1%) at 23 weeks of gestational age, whereas the reticulocytes (0.0385×10^{12}/L) and platelet count (192×10^{9}/L) were normal. However, at 27 weeks of gestational age foetal anaemia (haemoglobin 48 g/L, haematocrit 16.6%) became severe; the reticulocytes (0.1417×10^{12}/L) showed reactive proliferation and thrombocytopenia (platelet 16×10^{9}/L) was found. Parvovirus and cytomegalovirus infections, maternal syphilis, thalassemia, fetomaternal haemorrhage had been ruled out. Furthermore, immunization against red blood cells (RBC) was excluded by negative direct antiglobulin test (DAT) and indirect antiglobulin test (IAT) using DG Gel Coombs (GRIFOLS).

His mother, a 36-year-old Chinese woman, gravida 3 para 1, had a history of abortion at the first pregnancy and intrauterine foetal deaths with foetal hydrops occurred during 6–8 months in the following five pregnancies. Blood tests of his mother for cytomegalovirus, parvovirus, irregular and autoantibody were negative. Interestingly, anti-CD36 *iso*antibodies were detectable in the maternal serum indicating a severe case of FNAIT associated with anti-CD36 *iso*antibodies.

Materials and methods

The platelet antibodies in sera collected from the mother and umbilical cord were detected using a commercial ELISA Kit (PAKPLUS, Immucor GTI Diagnostics Inc., Waukesha, USA). Aliquots of 50 µL sera (1:3 dilutions) were added to microtiter wells coated with different platelet glycoproteins or HLA class I antigens. Bound antibodies were detected with alkaline-phosphatase-conjugated anti-human IgG and the appropriate substrate. The characterization of CD36 on protein as well as on DNA level was performed as previously described [18].

Results

Figure 2 shows the results of antibody containing in sera of maternal and umbilical cord at 14, 23 and 27 weeks of gestation. Increasing amounts of HLA antibodies against B*27:08, B*45:01, B*27:05, B*49:01, B*15:12, B*07:02, B*15:01, B*41:01, B*44:03, B*73:01, B*44:02, B*35:01

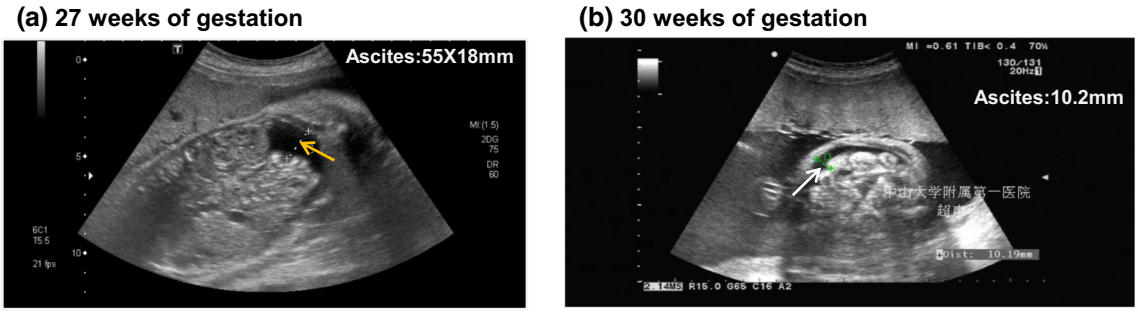

Fig. 1 Ascites was measured in diameter of fluid in the abdominal cavity by ultrasound. **a** 55 mm diameter of ascites was found at 27 weeks of gestation (*arrow*). **b** The diameter of fluid (10.2 mm) at 30 weeks of gestation shows significant reduction of ascites in the transversal view (*arrow*)

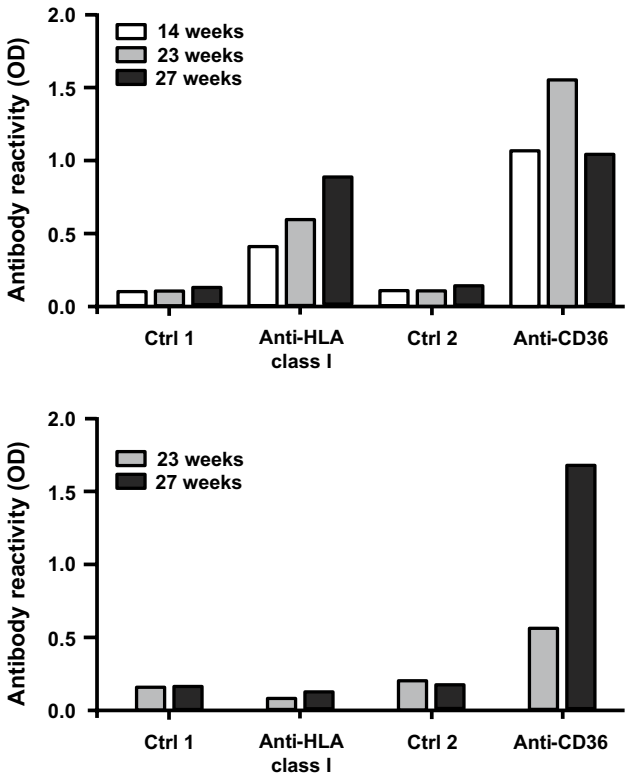

Fig. 2 Detection of anti-CD36 antibodies and anti-HLA class I by ELISA. Platelet reactive antibodies were screened by a commercial ELISA Kit (PAKPLUS). In maternal serum (*upper panel*), antibodies against CD36 and HLA class I were found at 14, 23 and 27 weeks of gestation age, respectively as indicated. In serum of umbilical cord (*bottom panel*) only anti-CD36 antibodies was identified at 23 and 27 weeks of gestation age. Controls (Ctrl) 1 and 2 represent the negative control of anti-HLA class I and anti-CD36, respectively

and B*07:03 were found in maternal serum using LIFECODES LSA (Gen-Probe). However, these antibody specificities could not be found in umbilical cord blood sample, and inconsistent with the HLA-genotype of foetus (A*02:03, A*24:02; B*40:01, B*54:01; C*01:02, C*07:02; DRB1*08:03, DRB1*16:02). This observation indicated that these HLA class I antibodies did not play a role in the pathomechanism of thrombocytopenia in this case. Interestingly, although the amount of anti-CD36 *iso*antibodies in maternal sera decreased from 23 to 27 weeks of gestation, significant increase of anti-CD36 *iso*antibodies was detected in the umbilical cord blood sample. Flow cytometry analysis showed the normal CD36 expression of father and foetus on platelets (MFI 240; MFI 20.0) as well as on monocytes (MFI 480; MFI 60.1). In contrast, total absence of CD36 was observed on the maternal platelets (MFI 2.87) and monocytes (MFI 1.14) (Fig. 3), demonstrating type I CD36 deficiency. This is in accordance with the molecular biological analysis. Two nucleotides deletion AC at position 329–330 located in exon 5 of the CD36 gene of the mother was found. As expected, normal CD36 gene (wild-type) was found in the father. Accordingly, the foetus is heterozygous carrying both the mutant and the wild-type allele (Fig. 4). All together, these results strongly indicated that anti-CD36 *iso*antibodies developed by CD36*null* phenotype mother, passed through placenta and induced severe FNAIT conditions with hydrops fetalis and serious anaemia.

From 27 until 29 weeks of gestation, a total of three intrauterine transfusions with RBC were performed to improve the severe anaemia. The haemoglobin value increased rapidly (113 g/L), and the ultrasound assessment showed the diameter of fluid in the abdominal cavity of fetus was decreased markedly (Fig. 1B). However, the platelet count (11×10^9/L) remained low (Table 1). At 30 weeks of gestation, 4.81×10^{10} CD36*null* leuko-depleted, non-irradiated platelets were transfused to the foetus and the platelet count rose to 177×10^9/L. In addition, the mother (body weight, 50 kg) received prednisone for 11 days (10 mg, bid). Due to the persistent elevation of MCA-PSV (1.8–2.5 MOM), RBC and platelets from a CD36*null* donor were given 3 days later. The post-transfusion platelet count and haemoglobin were 105×10^9/L and 114 g/L, respectively. Foetal hydrops could not be detected by the subsequent ultrasound (data not shown). During this time the mother refused the prenatal treatment with intravenous immunoglobulin (IVIG).

At 32 weeks of gestation, the male baby (2250 g) was delivered vaginally due to premature rupture of membranes with Apgar scores of 10 (after 5 min). At birth, the haemoglobin was normal (186 g/L), but the platelet count was low (48×10^9/L). Although diffuse petechial haemorrhages in lower limbs and chest were observed shortly after birth, intracranial haemorrhage (ICH) was not detected by ultrasounds as well as by computed tomography (CT) scan. After 2 days the platelet count rose to 121×10^9/L. The child was discharged from the hospital on day 30. No neurologic abnormality was observed until today (9 months old).

Discussion

We report a severe case of FNAIT complicated with foetal anaemia and hydrops due to anti-CD36 *iso*antibodies, which represents a rare clinical condition in this disorder. Usually the clinical features of FNAIT are associated with the occurrence of petechiae, hematomas and ICH [19]. Only anti-CD36 *iso*antibodies, but not specific anti-HLA class I antibodies could be found in the foetal blood sample indicating that anti-CD36 *iso*antibodies were directly responsible for the clinical complications observed in this FNAIT case.

Curtis et al. described five FNAIT cases caused by anti-CD36, but neither prenatal nor postnatal anaemia was observed [15]. In two of these infants, the platelet counts rose to the normal range within 2 weeks without any

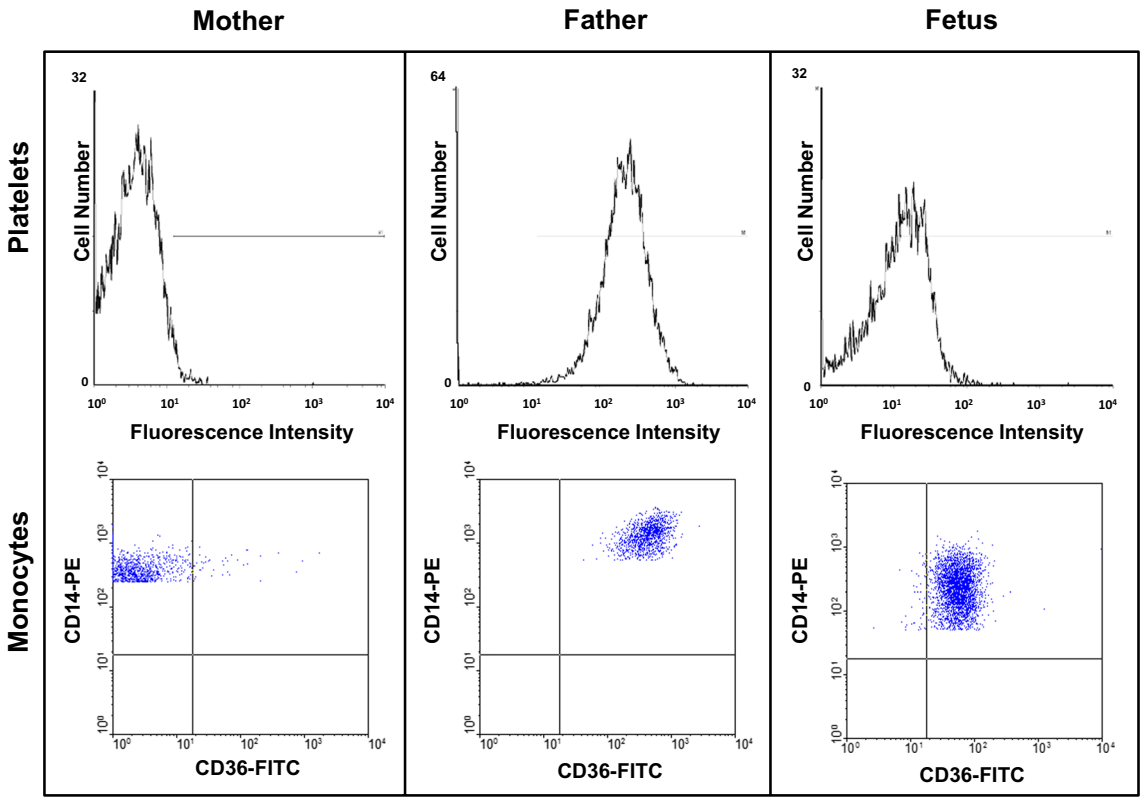

Fig. 3 Flow cytometry analysis of CD36 expression on platelets and monocytes. Platelets and monocytes of mother, father and foetus were measured with FITC labelled mab against CD36 and PE-labelled mab against CD14. Anti-CD36 reacted with platelets (*top panel*) from the father and the foetus, but none with the mother. Similarly, only monocytes (CD14 positive) of the father and the foetus reacted with anti-CD36 antibodies (*bottom panel*)

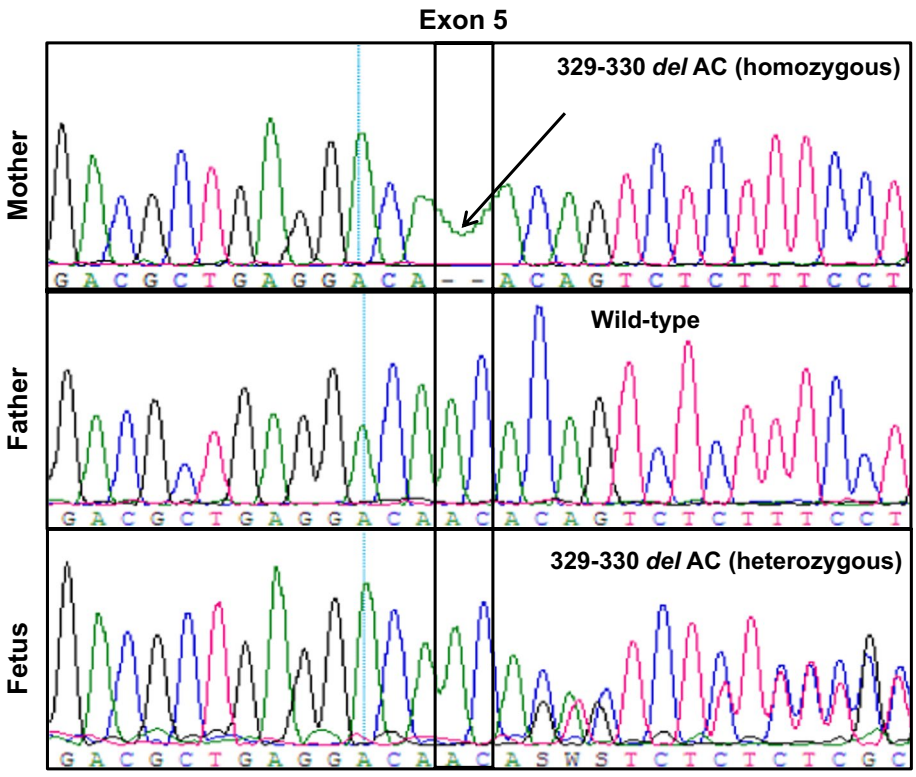

Fig. 4 Sequencing analysis of exon 5 of CD36 gene. The two nucleotides deletions AC at position 329–330 (*arrow*) in mother (homozygous state) and in foetus (heterozygous state) in comparison to the father (wild-type) are presented

Table 1 Clinical characteristics before and after five intrauterine interventions

No.	GA (weeks + days)	FBS	Intrauterine transfusion		Before intrauterine transfusion			After intrauterine transfusion		
			RBC (mL)	PLT (mL)	Hb (g/L)	Hct (%)	PLT (10^9/L)	Hb (g/L)	Hct (%)	PLT (10^9/L)
1	23 + 3	√	–	–	84	25.1	192	–	–	–
2	27 + 1	√	–	–	48	16.6	16	–	–	–
3	28 + 1	√	21	–	39	12.8	12	51	16.2	10
4	28 + 3	√	60	–	34	10.9	18	75	23.8	14
5	29 + 1	√	92	–	61	18.4	7	113	33.5	11
6	30	√	–	67	85	26.2	20	75	24	177
7	30 + 3	√	85	34	71	21.4	57	114	33.4	105

No number of cordocentesis or intrauterine transfusion, *GA* gestational age, *FBS* fetal blood sample by cordocentesis, *Hb* hemoglobin, *Hct* hematocrit, *RBC* red blood cells, *PLT* platelets

specific treatment. Recently, Okajima et al. [17] described a case of two siblings with hydrops fetalis due to anti-CD36. Foetal anaemia was observed in the elder sister. She was managed by intraperitoneal transfusion with RBC and delivered at 30 weeks of gestation. The younger sister was delivered at 29 weeks of gestation without intrauterine intervention.

In our patient, intrauterine transfusions with compatible RBC seem to be of benefit to manage severe foetal anaemia and the development of hydrop fetalis. Actually, platelet transfusion prior to RBC administration is recommended in severe thrombocytopenia (platelet count $<50 \times 10^9$/L) to prevent haemorrhage [20]. In our case, transfusion with CD36*null* platelets did not be administered until 30 weeks of gestation for two reasons. First, compatible platelet donor was not formerly available. Second, comparing to foetal thrombocytopenia, hydrops fetalis and anaemia threatened the foetal life and may result in foetal demise so that immediate red cell transfusion should be performed as the first priority. Transfusion with CD36*null* platelets after 30 weeks of gestation resulted in significance increase of foetal platelet counts. Fortunately, signs of ICH, intrauterine growth restriction or other structural abnormalities were not found by prenatal ultrasound or postnatal computer tomography.

Although ICH is regarded as the most severe complication of FNAIT [21], the mechanism has not been fully understood. Recently, Yougbaré et al. [22] demonstrated that anti-β3 antibodies developed in immunized β3 knock-out mice mothers could induce ICH in pups in a murine model of FNAIT. More recently, Santoso and co-workers found significant association between certain types of anti-HPA-1a alloantibodies with the development of ICH. This antibody type bound strongly to αvβ3 integrin expressed on endothelial cells and inhibited thereby the interaction between αvβ3 and its ligand vitronectin leading to endothelial cell apoptosis (anoikis) and consequently impaired angiogenesis [23]. These observations underline the important of anti-endothelial antibodies as important trigger of ICH. The question whether anti-CD36 *iso*antibodies may impair endothelial function(s) leading to foetal hydrops is intriguing.

Nowadays, high risk cases of FNAIT cases caused by anti-HPA-1a alloantibodies are commonly treated with intravenous immunoglobulin (IVIG) and corticosteroid [24]. Currently, little is known about the benefit of IVIG for the treatment of FNAIT caused by anti-CD36 *iso*antibodies. In our case, this clinical approach could not be approved, because no compliance could be achieved.

Taking together, although anti-CD36 *iso*antibodies turn out to be the most frequent platelet antibodies found in Asia and can cause life-threatening FNAIT, this serious disorder is still precious little considered. In this study, we present the careful elaboration of the case, the laboratory diagnostic as well as the successful management of severe case of FNAIT caused by anti-CD36 antibodies. Since hydrops fetalis and anaemia rather than thrombocytopenia seem to threatening for the foetal life, immediate RBC transfusion should be recommended as the first priority.

Acknowledgements This work was supported by the grant from National Natural Science Foundation of China (Ref. number: 81601451), by the Natural Science Foundation of Guangdong Province of China (no. 2016A030313124/no. 2016A030313123), by the German Research Foundation, Excellence Cluster Cardiopulmonary System (to S. S.), and by Science and Technology and Innovative Commission of Guangzhou City (no. 201607010007/no. 201509010009/no. 201707010021).

Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

References

1. Kamphuis MM, Paridaans NP, Porcelijn L, Lopriore E, Oepkes D. Incidence and consequences of neonatal alloimmune thrombocytopenia: a systematic review. Pediatrics. 2014;133:715–21.

2. Turner ML, Bessos H, Fagge T, Harkness M, Rentoul F, Seymour J, et al. Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. Transfusion. 2005;45:1945–56.
3. Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, et al. 348 cases of suspected neonatal alloimmune thrombocytopenia. Lancet. 1989;1:363–6.
4. Peterson JA, McFarland JG, Curtis BR, Aster RH. Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management. Br J Haematol. 2013;161:3–14.
5. Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S, et al. The natural history of maternal immunization against foetal platelet alloantigens. Transfus Med. 2004;14:399–408.
6. Xia W, Ye X, Xu X, Ding H, Liu J, Deng J, et al. Two cases of platelet transfusion refractoriness and one case of possible FNAIT caused by antibodies against CD36 in China. Transfus Med. 2014;24:254–6.
7. Wu G, Zhou Y, Li L, Zhong Z, Li H, Li H, et al. Platelet immunology in China: research and clinical applications. Transfus Med Rev. 2017;31:118–25.
8. Silverstein RL, Febbraio M. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. Sci Signal. 2009;2:re3.
9. Yamamoto N, Akamatsu N, Sakuraba H, Yamazaki H, Tanoue K. Platelet glycoprotein IV (CD36) deficiency is associated with the absence (type I) or the presence (type II) of glycoprotein IV on monocytes. Blood. 1994;83:392–7.
10. Ikeda H, Mitani T, Ohnuma M, Haga H, Ohtzuka S, Kato T, et al. A new platelet-specific antigen, Naka, involved in the refractoriness of HLA-matched platelet transfusion. Vox Sang. 1989;57:213–7.
11. Tomiyama Y, Take H, Ikeda H, Mitani T, Furubayashi T, Mizutani H, et al. Identification of the platelet-specific alloantigen, Naka, on platelet membrane glycoprotein IV. Blood. 1990;75:684–7.
12. Porta C, Caporali R, Montecucco C. Thrombotic thrombocytopenic purpura and autoimmunity: a tale of shadows and suspects. Haematologica. 1999;84:260–9.
13. Morishita K, Wakamoto S, Miyazaki T, Sato S, Fujihara M, Kaneko S, et al. Life-threatening adverse reaction followed by thrombocytopenia after passive transfusion of fresh frozen plasma containing anti-CD36 (Nak) isoantibody. Transfusion. 2005;45:803–6.
14. Kankirawatana S, Kupatawintu P, Juji T, Veerakul G, Ngerncham S, Chongkolwatana V, et al. Neonatal alloimmune thrombocytopenia due to anti-Nak(a). Transfusion. 2001;41:375–7.
15. Curtis BR, Ali S, Glazier AM, Ebert DD, Aitman TJ, Aster RH. Isoimmunization against CD36 (glycoprotein IV): description of four cases of neonatal isoimmune thrombocytopenia and brief review of the literature. Transfusion. 2002;42:1173–9.
16. Taketani T, Ito K, Mishima S, Kanai R, Uchiyama A, Hirata Y. Neonatal Isoimmune thrombocytopenia caused by type I CD36 deficiency having novel splicing isoforms of the CD36 gene. Eur J Haematol. 2008;81:70–4.
17. Okajima S, Cho K, Chiba H, Azuma H, Mochizuki T, Yamaguchi M, et al. Two sibling cases of hydrops fetalis due to alloimmune anti-CD36 (Naka) antibody. Thromb Haemost. 2006;95:267–71.
18. Xu X, Ye X, Xia W, Liu J, Ding J, Chen Y, et al. Studies on CD36 deficiency in South China: two cases demonstrating the clinical impact of anti-CD36 antibodies. Thromb Haemost. 2013;110:1199–206.
19. Brojer E, Husebekk A, Debska M, Uhrynowska M, Guz K, Orzińska A, et al. Fetal/neonatal alloimmune thrombocytopenia: pathogenesis, diagnostics and prevention. Arch Immunol Ther Exp. 2016;64:279–90.
20. Segata M, Chaoui R, Khalek N, Bahado-Singh R, Paidas MJ, Mari G. Fetal thrombocytopenia secondary to parvovirus infection. Am J Obstet Gynecol. 2007;196:61.e1–4.
21. Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. N Engl J Med. 1993;329:1463–6.
22. Yougbaré I, Lang S, Yang H, Chen P, Zhao X, Tai WS, et al. Maternal anti-platelet β3 integrins impair angiogenesis and cause intracranial hemorrhage. J Clin Investig. 2015;125:1545–56.
23. Santoso S, Wihadmadyatami H, Bakchoul T, Werth S, Al-Fakhri N, Bein G, et al. Antiendothelial αvβ3 antibodies are a major cause of intracranial bleeding in fetal/neonatal alloimmune thrombocytopenia. Arterioscler Thromb Vasc Biol. 2016;36:1517–24.
24. Bussel JB, Primiani A. Fetal and neonatal alloimmune thrombocytopenia: progress and ongoing debates. Blood Rev. 2008;22:33–52.