CASE REPORT

Neonatal alloimmune thrombocytopaenia associated with maternal HLA antibodies

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SUMMARY

Neonatal alloimmune thrombocytopaenia (NAIT) generally results from platelet opsonisation by maternal antibodies against fetal platelet antigens inherited from the infant's father. Newborn monochorionic twins presented with petechial haemorrhages at 10 hours of life, along with severe thrombocytopaenia. Despite the initial treatment with platelet transfusions and intravenous immunoglobulin, they both had persistent thrombocytopaenia during their first 45 days of life. Class I human leucocyte antigen (HLA) antibodies with broad specificity against several HLA-B antigens were detected in the maternal serum. Weak antibodies against HLA-B57 and HLA-B58 in sera from both twins supported NAIT as the most likely diagnosis. Platelet transfusion requirements of the twins lasted for 7 weeks. Transfusion of HLA-matched platelet concentrates was more efficacious to manage thrombocytopaenia compared with platelet concentrates from random donors. Platelet genotyping and determination of HLA antibody specificity are needed to select compatible platelet units to expedite safe recovery from thrombocytopaenia in NAIT.

BACKGROUND

Fetal and neonatal alloimmune thrombocytopaenia (FNAIT) frequently involves maternal alloantibodies directed against human platelet antigen (HPA)-1a and HPA-5b.¹ Human leucocyte antigen (HLA) antibody binding to platelets is less specific and has rarely been implicated as a cause of FNAIT.^{2–4} Sound data on the duration of thrombocytopaenia due to maternal HLA antibodies currently do not exist.

Management of neonatal alloimmune thrombocytopaenia (NAIT) includes treatment with platelet transfusions and adjunct intravenous immunoglobulin (IVIg).² Rapid diagnosis and effective treatment strategies must be implemented to prevent and reduce the risk of intracranial haemorrhage.⁵

CASE PRESENTATION

Monochorionic female twins with birth weights of 2050 and 2300 g were delivered at 37 weeks' gestational age by uncomplicated caesarean section due to protracted labour after an uneventful pregnancy. A single dose of 1 mg vitamin K was given intramuscularly to both infants immediately after birth. Breast feeding was progressively introduced from day 1. At 10 hours of life, they exhibited petechial haemorrhages and thrombocytopaenia with platelet counts of $28 \times 10^9/L$ and $20 \times 10^9/L$, respectively.

Haemoglobin and other blood cell counts were in the normal range. Serological tests for HIV, toxoplasma, cytomegalovirus, rubella and parvovirus B19 IgM were negative. No coagulation disorders were found. The twins were transfused immediately with random buffy coat platelet concentrates. We suspected the involvement of HLA antibodies when thrombocytopaenia persisted for 1 week.

The mother was a primipara, non-consanguineous woman from Pakistan with no history of miscarriages, pre-eclampsia, autoimmune or haematological disorders, previous transfusion, any regular medication, drug ingestion or infections. She was blood group B RhD-positive, with no history of thrombocytopaenia. No anti-HPA antibodies were detected by flow cytometry. Her genotype was HPA-1aa, HPA-3aa, while the father was HPA-1ab, HPA-3bb. No platelet-specific antibodies against HPA-1–5 or HPA-15 were found; however, there was a mismatch between the mother and the father in HPA-3 type.

An asymptomatic septic episode in twin 2 one week after birth due to *Staphylococcus aureus* infection was unlikely to explain the persistent thrombocytopaenia. The infant recovered quickly after antibiotic treatment had been initiated.

Cerebral ultrasounds scan of the infants within the first week of life showed no evidence of intracranial haemorrhage. A cerebral MRI on day 10 revealed an 8 mm cerebellar bleeding event in twin 2, whereas twin 1 had a normal MRI scan. We cannot definitely correlate this finding with the mother's HLA antibodies. However, the increased risk of intracranial haemorrhage with alloimmunisation suggests a link with an immune response involving HLA antibodies.

During the first week the twins were transfused with two platelet transfusions from random donors. IVIg 0.8 g/kg body weight was administered twice during the same period. After day 10, apheresis platelet concentrates from compatible HLA platelet donors were transfused whenever the platelet count was less than $30 \times 10^9/L$. As reviewed by Cremer *et al*,⁶ the current threshold for platelet transfusion varied among centres. The selected management at our institution of 15 mL/kg body weight with a trigger $<30 \times 10^9/L$ platelet count was based on expert opinion. All platelet concentrates used were irradiated.

A Luminex single antigen test performed at day 8 demonstrated strong HLA antibodies of broad spectrum against several HLA-B antigens in the mother, and NAIT caused by HLA antibodies was

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Figure 1 Platelet counts in monozygotic twins with neonatal alloimmune thrombocytopenia due to HLA antibodies during their first 63 and 105 days of life. The initial treatment consisted of platelet concentrates from random donors and adjunct IVIG. Transfusion of HLA compatible thrombocytes produced more sustainable responses. T1, twin 1 (blue line); T2, twin 2 (red line). Green circles, treatment with IVIG. HLA, human leucocyte antigen; IVIG, intravenous immunoglobulin; Plts, platelets.

then suspected. Based on the mother's Luminex results, apheresis donors with HLA antigens against which the mother did not have antibodies were chosen. On day 10, twin 2 was transfused with HLA-matched platelets for the first time. A prompt rise in platelet count to $263 \times 10^9/L$ was measured the following day; however, the platelet count gradually decreased over the following 10 days (figure 1), and therefore a Luminex assay was performed at 24 days of age. This demonstrated low reactivity to HLA-B57 and HLA-B58 in both infants (<2000 mean fluorescence intensity (MFI) in twin 1 and <1000 MFI in twin 2).

OUTCOME AND FOLLOW-UP

Both twins were discharged from the hospital at 25 days post-natal age. The hospital team subsequently carried out home visits twice weekly over a period of 15 weeks to assess their platelet counts. During this period, the twins were twice readmitted to the hospital for platelet transfusions. Twin 1 required a total of six and twin 2 a total of ten platelet transfusions (figure 1). The clinical follow-up at 2 years of age indicated a normal neurodevelopmental pattern in both twins.

DISCUSSION

Basically, we did not expect to find HLA antibodies because the mother was primipara. However, based on the knowledge that even non-transfused men may present with 'naturally occurring' HLA antibodies,⁷ we considered the possibility. We used an exclusion strategy since the twins otherwise appeared well; they did not have severe sepsis, the mother did not have HPA antibodies or idiopathic thrombocytopenia purpura (ITP), and tests for several infectious agents were negative.

Both twins received non-HLA-matched apheresis platelet concentrates and twin 2 received one buffy coat-derived platelet transfusion. Transfusion of HLA-matched maternal platelets would have been the preferred treatment strategy in the initial

phase; however, waiting for collection of maternal platelets would have caused an unacceptable delay in treatment, since the risk of intracerebral haemorrhage in the first 2 days of life is especially high. Platelet concentrates from random donors can be used initially until compatible platelets are available. However, non-optimally matched platelets have shorter in vivo survival times. Although some neonatal intensive care units advocate the use of IVIG combined with either random donor or matched platelets, the efficacy of this strategy has not been evaluated prospectively. Transfusion of HLA-matched platelets resulted in a better improvement in platelet counts, but transfused platelets have limited survival. As long as the cause of thrombocytopenia is not eradicated, it is expected that thrombocytopenia will persist.

Despite the low antibody levels against HLA-B57 and HLA-B58 at 3 weeks of age (<2000 MFI in twin 1 and <1000 MFI in twin 2), the twins still suffered from thrombocytopenia. These antibodies were regarded as passively transferred. Luminex antigen test was not performed in the first couple of weeks of life because prolonged thrombocytopenia beyond 2 weeks is rare.

The onset of thrombocytopenia in the case of the twins was detected after 10 hours. It is therefore unlikely that IgA anti-platelet antibodies in breast milk will cause thrombocytopenia in this short timescale. However, IgA antiplatelet antibodies might have contributed to persistent thrombocytopenia during the 7 weeks of observation period.

The mother presented a number of HLA antibody specificities with higher MFI values than those against HLA-B57 and HLA-B58. However, we were not able to explain why other maternal HLA antibodies with higher MFI values were not detected in the twins. It would have been useful to identify and follow the course of passively transferred HLA antibodies from the early days of life, and to correlate the MFIs of the mother's HLA antibodies with those of the twins.

Maternal and paternal serum should have been tested in an antigen capture assay that covers all platelet glycoproteins to rule in/out the production of maternal antibodies against a low-frequency paternal HPA, that is, HPA-6b to HPA-29b or a new HPA. HLA-typing the twins would have confirmed whether the mother's antibodies were directed against the twins' HLA antigens.

IVIG is human plasma-derived IgG pooled from thousands of donors. Consequently, it may contain low concentrations of HLA antibodies either naturally occurring or from alloimmunised donors. Since we performed Luminex single antigen assay on day 24, we cannot exclude that the detected HLA antibodies may have originated from IVIG. The half-life of IVIG being usually 26–41 days may have clouded the assay. However, it is unlikely that the low concentrations of HLA antibodies were the reason for thrombocytopenia because thrombocytopenia was evident before administration of IVIG.

IVIG is accepted as adjunct therapy to random platelets if no compatible platelets are available. Small and non-randomised studies have shown that the infusion of IVIG may produce a rise in platelet count in 75% of cases.⁸ A dosage of 1 g/kg body weight for 2 days or 400 mg/kg for 5 days instead of 3×0.8 g/kg in 1 week might have been more efficient.

We suspected the mother's strong and broad-spectrum anti-HLA antibodies as the most plausible explanation for the twin's thrombocytopenia. However, the twins had only weak passively transferred HLA-B57 and HLA-B58 antibodies at 24 days of age and yet thrombocytopenia still persisted.

The role of HLA antibodies as a reason for neonatal thrombocytopenia is debatable.^{3,4} HLA antigens are expressed on many fetal cells and tissues; therefore, HLA antibodies have much more targets in addition to platelets. This may explain the milder course of thrombocytopenia due to HLA antibodies. NAIT is a worrying complication of pregnancy that can offer diagnostic and therapeutic challenges. Daily platelet counts should be performed until the platelet count has been demonstrated not to be falling. Rapid diagnosis and effective treatment strategies must be implemented to prevent severe bleeding complications associated with NAIT.

Our treatment strategy for neonatal thrombocytopenia in the case of the twins was based on our early speculation about possible HLA antibodies involvement, which was supported subsequently by their detection using immunoassay. Given the presence of HLA antibodies in the mother and a better

transfusion response on HLA-matched platelet units, having enrolled both twins in this case study reduces the likelihood that the improved platelet transfusion response was chance as could have been the case in a singleton pregnancy.

We feel that the successful treatment of the twins provides clear evidence of the role of HLA antibodies in neonatal thrombocytopenia. The better response to HLA-matched platelet transfusion adequately supports the association of NAIT and HLA antibodies. If HLA antibodies are suspected as the most likely reason for NAIT, HLA genotyping should be performed to expedite individualised treatment.

Contributors KW participated in the design of the case report, the data collection and interpretation of results. She participated in drafting and contributed to critically review the article, and approved the final manuscript. She agreed to be accountable for the article and to ensure that all questions regarding the accuracy or integrity of the article are investigated and resolved. CAA participated in the design of the report, data collection and interpretation of results. She participated in drafting and contributed to critically review the article, and approved the final version of the manuscript. She agreed to be accountable for the article and to ensure that all questions regarding the accuracy or integrity of the article are investigated and resolved. SK conceptualised the article and participated in the design of the report, data collection and interpretation of results. He participated in drafting the case report, made critical review of the article and approved the final manuscript for submission to BMJ. He agreed to be accountable for the article and to ensure that all questions regarding the accuracy or integrity of the article are investigated and resolved.

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Learning points

- ▶ Neonatal alloimmune thrombocytopenia (NAIT) is commonly associated with antibody to human platelet antigen (HPA). However, human leucocyte antigen (HLA) antibody-caused NAIT should be suspected in a thrombocytopenic newborn infant with or without signs of bleeding within the first 3 days of life, especially when the newborn does not present with illness and/or inadequate post-transfusion platelet increment is evident, no HPA mismatch is shown and has no antiplatelet antibodies detected.
- ▶ Ensure urgent correction of the thrombocytopenia with random platelet transfusion. Use intravenous immunoglobulin as adjunct therapy. Perform rigorous monitoring of platelet count until sustainable numbers $>100 \times 10^9/L$ are reached.
- ▶ Analyse maternal serum for HLA antibodies by flow cytometry and/or Luminex for diagnosis of HLA antibody-caused NAIT and select HLA-matched platelet donors.
- ▶ Once NAIT caused by HLA antibodies is confirmed, transfuse HLA-matched thrombocytes instead of platelets from random donors.
- ▶ In later pregnancies, plan for an antenatal follow-up programme to detect HLA antibody levels and perform ultrasound examinations of the fetus.

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