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FOETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA AS A RARE EXAMPLE OF THROMBOCYTOPENIA IN NEWBORN

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Key words: FNAIT, Thrombocytopenia, Newborn, HPA-1a, Prevfnait, autoimmunization

Abstract

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by antigenic incompatibility of platelets between a pregnant woman and her child, resulting in producing antibodies by a mother against specific antigens (HPA- Human Platelet Antigens) located on foetal platelets inherited from his father.

The aim of this study is to present a 29-year-old patient in the 40th week of the second pregnancy admitted to the Department after rupturing of membranes. A male infant was born vaginally. After 2 hours numerous petechiae were observed on the skin. Due to severe thrombocytopenia 1 unit of leucocyte-reduced, irradiated, reconstituted platelet concentrate (LRIRPC) of blood group O RhD (+), suspended in plasma type AB was ordered. Human immunoglobulin (Kiovig preparation) was transfused. A check exam of complete blood count of the newborn revealed 5 x 10^3/µL of platelets count 4 hours after the transfusion. Following transfusions of LRIRPC and Kiovig were ordered. Again, with no therapeutic effect. The newborn's HPA antigens were identified as: 1a/b; 2a/a; 3a/a; 5a/a; 4a/a; 15b/b, platelet antibodies derived from the mother were found in his serum. After transfusion of 1 unit of HPA-1b/b LRIRPC at 37 hours of the newborn's life the platelet count increased to 67 x 10^3/µL. The treatment with dexamethasone and Kiovig was continued. The infant was discharged in good condition in the 33rd day of life.

The FNAIT diagnostics is usually carried out only as a result of clinical manifestations of thrombocytopenia in the newborn. There was a possibility for all pregnant women from 8 weeks of pregnancy to have their blood tested for the presence of HPA-1a antigen in the period between October of 2013 to January of 2017 in Poland. It made it possible to nominate HPA-1a negative women.

Introduction

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by antigenic incompatibility of platelets between a pregnant woman and her child, resulting in producing antibodies by a mother against specific antigens, i.e. human platelet antigens (HPA) located on foetal platelets inherited from his father [1,2]. The FNAIT is a potentially devastating disease that can lead to intracranial haemorrhage in the foetus or neonate, neurologic damage in the future life or even foetal death. It is estimated that in Poland FNAIT occurs 1:2000 pregnancies [3-5]. The FNAIT diagnostics is usually carried out only as a result of clinical manifestations of thrombocytopenia in the newborn. It would be very useful to conduct diagnostics of FNAIT by all pregnant women and to nominate HPA-1a negative women, whose offspring could be at risk of severe immunological thrombocytopenia. The aim of this study is to present a 29-year-old patient in the 40th week of the second pregnancy admitted to the Chair and Department of Obstetrics and Perinatology, Medical University of Lublin.

Case study

A 29-year old patient admitted in the 40th week of the second pregnancy to the Chair and Department of Obstetrics and Perinatology, Medical University of Lublin due to the premature rupture of membranes (PROM).

The patient was under the perinatal care since the 8th week of the pregnancy, held 9 medical visits in total every 4 weeks. The course of pregnancy was physiological and she denied any medical conditions. During the pregnancy the patient took folic acid at a dose of 0.4 mg, Aspirin Protect 100 mg and vitamins for pregnant women. The laboratory tests, including complete blood count, urinalysis, the venereal disease research laboratory (VDRL) test, hepatitis B antigen, antibodies anti-human immunodeficiency virus (HIV), antibodies anti-hepatitis C virus (HCV), antibodies towards toxoplasmosis (immunoglobulin (Ig) G and IgM), rubella IgG, thyroid-stimulating hormone (TSH), Pap smear and oral glucose tolerance test remained normal.

Procedures connected with admission a delivering patient were performed, i.e. the general medical history (healthy) and obstetric history (one miscarriage in the 5th week of pregnancy) were taken, the physical examination, including internal obstetric examination (passing out of the amniotic fluid and cervical dilation - 2 cm was confirmed) and external obstetric examination was performed. A cardiotocography tracing was reassuring. In view of the above considerations, the patient was admitted to the Department.

Due to the slow progress of cervical ripening, the delivery was stimulated by the drip infusion of oxytocin. Considering the PROM, the patient received prophylactic antibiotic.

The first stage of labor lasted 11 hours and 10 minutes, the second stage – 50 minutes and the third – 15 minutes.

The course of early postpartum period without complications. The laboratory tests managed after the labor:

- Blood serum revealed the presence of lymphocytotoxic antibodies - serological test (LCT by Terasaki)
- the test detected the presence of platelet antigens – HPA: 1b/b, 2a/a, 3a/a, 4a/a, 5a/a, 15a/b – test made by the polymerase chain reaction (PCR)-SSP method

- the test detected the presence of antibodies against platelets targeting platelet membrane glycoprotein (GP) IIb/IIIa most likely specificity of anti-HPA 1a and detected antibodies anti-HLA - study was performed using enzyme-linked immunosorbent assay (ELISA) using a diagnostic kit PakPlus manual Gen-Probe
- blood count – white blood cells (WBC) 21,54 x 10^3/µL, red blood cells (RBC) 4,08 x 10^6/µL, haemoglobin (Hgb) 12,5 g/dL, haematocrit (Hct) 37,6%, platelet count (PLT) 220,0 x 10^3/µl.
- Cytomegalovirus (CMV): antibodies IgG - 114.800 IU/mL (positive), IgM - 1,420 COI (positive)

Male infant born in good general condition, body weight 3550g, evaluation according to Apgar score in the 1' - 9 points. Skin pink and clean, the newborn was respiratory and circulatory efficient. The first feeding in the delivery room. Admitted to the neonatology ward after 2 hours and 40 minutes since labor. On the skin had many petechiae and bruising around the limbs, trunk, and single ones on face. The little yellowish of skin was noticed. The complete blood count done urgently found WBC - 23,6 x 10^3/µL, RBC - 5,37 x 10^6/µL, PLT – 8,0 x 10^3/µL, Hgb - 20,6 g/dL, Hct - 55,5%. Simultaneously the level of bilirubin was measured - 5,3 mg/dL, and blood clotting tests – activated partial thromboplastin time (APTT) - 26,7 sec, international normalized ratio (INR) - 1,37, prothrombin time (PT) - 15,2 sec, fibrinogen - 0,88 g/L, antithrombin III (AT III) - 52%. The drew blood sample on citrate - PLT 16,0 x 10^3/µL. The blood type of a child – B RhD (+), direct antiglobulin test (DAT) lightly positive, in the newborn's serum were found anti-B IgG antibodies derived from the mother. Blood type of the mother - O RhD (+).

Due to severe thrombocytopenia transfusion of 1 unit of leuko-reduced, irradiated packed platelets of 0 RhD (+) blood type was ordered. Due to the presence of numerous ecchymosis on the skin and reduced level of fibrinogen, it was decided to suspend the platelets in the

plasma of the AB blood type. While waiting for packed platelets, the newborn was transfused human immunoglobulin (preparation Kiovig) at a dose of 300 mg/kg b.w. Simultaneously tests towards TORCH, including PCR for the presence of CMV DNA and the antiplatelet antibodies in the mother's blood were used. Due to the risk of intrauterine infection (PROM 13 hours before birth) and the clinical condition of the child, the patient received antibiotics (Unasyn, Biodacyna). The control blood counts performed 4 hours after the transfusion of blood platelets found PLT - 5.0 x 10^3/μL, and therefore lack of the expected post-transfusion platelet growth. Once again there was a transfusion of 2 units of leuko-reduced, irradiated packed platelets of 0 RhD (+) blood type. At the same time, human immunoglobulin (Kiovig) at a dose of 1g/kg b.w was ordered. Again, despite transfusion of 2 units of platelets, there was a noticeable lack of the therapeutic effect, which might suggest consumption or destruction of platelets; platelet count after the transfusion - 37.0 x 10^3/μL, 4 hours later - 15.0 x 10^3/μL.

In 21 hour of life, test results of maternal antiplatelet antibodies using ELISA was obtained - in the patient serum there were antibodies to GP IIb / IIIa platelet membrane, most likely of specificity of anti HPA-1a. Furthermore, the lack of antigen HPA-1a on mothers platelets was stated and also, using this test, the antibodies against HLA was discovered. In the LCT test by Terasaki there were found lymphocytotoxic antibodies in maternal serum. At the same time a negative PCR result for the presence of CMV DNA was received. Newborns' antigens stating: 1a / b; 2a / a; 3a / 5a / a; 4a / a; 15b / b was defined and in his serum the presence of antibodies against platelets from his mother were found. This way immunological background of the neonatal thrombocytopenia was confirmed. The search for platelet concentrate HPA-1a negative began but this preparation was not available in the Regional Center of Blood Donation and Blood Therapy (RCKiK) in Lublin. Suitable preparation of platelets (HPA-1b / b) had to be imported from RCKiK in Warsaw. There was no possibility of collecting

platelets from his mother at that exact moment. Due to the critical value of neonatal blood platelets, during the search of the preparation of platelet HPA-1a negative, the treatment with dexamethasone 0.15 mg/kg b.w. was extended, in two divided doses, simultaneously with high-dose transfusions of human immunoglobulin.

After the transfusion of 1 unit of the preparation of HPA-1b/b platelet concentrate, the blood platelet count increased to 67.0 x 10^3/µL in the 37th hour of life. The treatment with dexamethasone and human immunoglobulin was continued. Kiovig was given twice at a dose of 1 g/kg b.w. obtaining stabilization of the platelet count - PLT consecutive: 78.0 x 10^3/µL, 74.0 x 10^3/µL, 70.0 x 10^3/µL, 75.0 x 10^3/µL, 79.0 x 10^3/µL, 90,0 x 10^3/µL, 96.0 x 10^3/µL 98.0 x 10^3/µL.

In the sixth day of life the doses of dexamethasone to 0.1 mg/kg b.w. per day were reduced. After two days of treatment with dexamethasone in reduced dose we found a decrease in the number of platelets to 50.0 x 10^3/µL. Returned to the dose of 0.15 mg/kg b.w./day of dexamethasone we gave human immunoglobulin at 1 g/kg b.w. again. Platelet count remained in a downward trend (50,0x 10^3/µL, 49.0 x 10^3/µL, 57.0 x 10^3/µL, 51.0 x 10^3/µL, 54.0 x 10^3/µL, 50.0 x 10^3/µL, 38.0 x 10^3/µL) despite treatment with dexamethasone in higher dose, repeated transfusion of the human immunoglobulin and early course of human immunoglobulin at a dose of 1 g/kg b.w. during 2 days. As a consequence of that, the infant was transferred to the Department of Neonatal Pathology, Prof. Antoni Gębala Children's Hospital of Lublin (USzD) in Lublin, in order to continue the treatment under the supervision of a pediatric haematologist.

Until the transfer of the newborn to the USzD, ultrasonographic examinations of central nervous system and abdominal cavity were performed three times. There was no evidence of bleeding in any of the tests performed. Newborn was transferred in good general condition,

efficient respiratory and circulatory, fed orally with modified milk. In the USzD, PCR tests for the search of CMV DNA were repeated – results were negative and the infant was continued to be treated with dexamethasone. The child was discharged from hospital in good general condition in the 33rd day of life.

Discussion:

The FNAIT is a relatively rare disease and occurs in Poland with a frequency of 1: 2000 births. For this reason, screening is not routinely performed, which is justified, apart from the low incidence of the disease, at a high cost of testing. It creates that alloimmunological thrombocytopenia in the foetus can be diagnosed only after the occurrence of complications. It is estimated that FNAIT is the most common cause of deep thrombocytopenia in foetal life, and at the same time, the most common cause of intracranial haemorrhages in foetuses and newborns, and consequently, may cause foetal death or disability throughout their later life [6]. Currently, the best diagnostic method seems to be an obstetric interview, and most of all, autoimmune thrombocytopenia or intracranial haemorrhage in older siblings [7].

In October 2013 in Poland started a three-year program "Prevention of foetal / neonatal alloimmune thrombocytopenia (FNAIT) in Polish foetuses and new-borns" called PREVFNAIT which was dedicated to maternal-foetal conflict in platelet HPA-1 antigens. The project consisted in providing the optimal medical care of 600 HPA - 1a of negative Polish women whose foetuses are potentially exposed to the occurrence of thrombocytopenia.

Calculating for FNAIT only in the presence of clinical symptom can cause the omission of the diagnosis in more than 80% of cases. Studies show that even in neonates born with severe thrombocytopenia, timely diagnostic testing for FNAIT was not performed in 15% of the cases, with severe consequences for the subsequent pregnancies [6].

In the Caucasian race (including Poland) the most common cause of FNAIT is the HPA-1a antigen located on the IIIa GP. From the screening tests carried out by the Institute of Haematology and Transfusion in Warsaw, it appears that 1.9% of women in Poland does not have the HPA-1a antigen. These women constitute a group of high risk of platelet conflict in the newborn, therefore it seems reasonable to conduct a diagnosis for the prevention of FNAIT in women from this group. Diagnosis should be confirmed by testing of the presence of antibodies in the mother's blood serum reacting in the father's blood platelets. The most effective test is considered to be the monoclonal antibody immobilization of platelet antigen (MAIPA) test [7].

Dębska et al. revealed that in the studied group of 15 204 pregnant women typed for HPA-1a: 373 (2.5%) females were HPA-1a negative and 32 (8.6%) tested positively for anti-HPA-1a. Antibodies were detected in 22 women during pregnancy [8].

Early detection of the presence of antibodies and therefore placement of appropriate treatment before complications appears to be crucial and extremely important when it comes to health and life of a newborn.

Foetal anti-HPA-1a can be removed by intrauterine platelet transfusions. Alloimmunized HPA-1a negative women can be donors of platelet concentrates for their offspring, that were subjected to foetal blood sampling [8].

Recently a new non-invasive, rapid test has been offered to detect HPA-1ab foetal antigen after the detection of an HPA-1-homozygous mother by using plasma cell-free DNA (cfDNA) [9].

The other new method to predict the foetal HPA-1a positive genotype is Real-time PCR. Unfortunately the number of false positive results is relatively high and is around 4% [10].

Conclusions:

Indications for the use of concentrated platelets in neonates are based on the number of platelets and the presence or risk of bleeding. In this case, indications for this were clear, hence the order of the platelet concentrate. Mother's blood type – 0 Rh (+), infant's blood type – B Rh (+). Direct antiglobulin test (DAT) lightly positive, in the infant's serum were found anti-B IgG of maternal origin. Due to this fact and irregularities in the plasma coagulation system we ordered and transfused 1 unit of leuko-reduced, irradiated packed platelets of 0 RhD (+) blood, suspended in 50 mL of plasma AB. There were no expected, post-transfusion platelet growth. Another 2 units of platelets from the same donor suspended in 50 mL of AB serum were transfused. Although much higher doses - still without a therapeutic effect, indicating consumption or destruction of platelets were used. In RCKiK in Lublin there were performed diagnostics of the suspected neonatal platelet conflict. On mothers' platelets was found a lack of HPA-1a antigen and in her blood serum were found antibodies against platelets targeting platelet membrane GP IIb / IIIa most likely specificity of anti-HPA 1a antibodies anti-HLA. The newborns' antigens were: 1a / b; 2a / a; 3a / 5a;, a / a; 4a / a; 15b / b and in his serum we found the presence of antibodies against platelets from his mother which resulted in neonatal thrombocytopenia. The therapeutic effect can only be observed after transfusion of platelets phenotypically compatible with the mothers, which were not available in Lublin, hence blood platelets HPA1b / b were searched and imported from RCKiK in Warsaw. This transfusion caused the therapeutic effect and the infant did not require further transfusions.

The FNAIT diagnostics is usually carried out only as a result of clinical manifestations of thrombocytopenia in the newborn. There was a possibility for all pregnant women from 8 weeks of pregnancy to have their blood tested for the presence of HPA-1a antigen in the period between October of 2013 to January of 2017 in Poland. It made it possible to nominate

HPA-1a negative mothers, whose offspring could be at risk of severe immunological thrombocytopenia.

Recently the use of the PCR method contributed to the occuring of new non-invasive tests for the detection of negative HPA-1a women.

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