Foetal and neonatal alloimmune thrombocytopenia: a review article and retrospective analysis of clinical and laboratory characteristics of patients in Slovenia between 1996 and 2016

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Abstract

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) results from the transplacental transmission and binding of alloimmune antibodies on the child's platelet antigens which were inherited from the father. Alloimmunisation of the mother against platelet antigen can occur during present or previous pregnancies or platelet transfusions. FNAIT is a rare disease whose course may be insignificant or may present with signs of haemorrhagic diathesis. Its most serious complication is intracranial bleeding, therefore early diagnosis and, in the case of indications, appropriate treatment are very important. The estimated incidence of FNAIT is 1 in 1,000–2,000 live births. According to the Slovenia's national vital statistics data, we estimate that there should be between 10 and 20 serologically confirmed cases of FNAIT annually. The incidence of FNAIT in Slovenia is not known. The aim of the present retrospective study was to assess the incidence and aetiology of FNAIT in Slovenia and clinical characteristics of the disease. The results of a retrospective study in which we analysed the results of blood tests for the detection of platelet antibodies in infants or mothers have shown a much lower incidence. In the period from 1996 to 2016, there were on average 9 requests for FNAIT diagnostic tests per year and 39 cases of FNAIT were confirmed, resulting in an incidence rate of 1 in 10,000 live births in Slovenia. We are aware that the obtained incidence may be underestimated due to retrospective analysis of the data; nevertheless, our results confirm our clinical observations that FNAIT is underdiagnosed in our area. In the present article, in addition to the results of a retrospective study on the incidence, aetiology and clinical picture of FNAIT in Slovenia, we review current knowledge of FNAIT. This contribution is aimed at increasing awareness about FNAIT, which can be life-threatening, and its prompt diagnosis may be very important for the child as well as for the mother's following pregnancies.

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1 Introduction

Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare disease occurring as a result of foeto-maternal incompatibility and immune destruction of the foetal or newborn's platelets. FNAIT is among the most common causes of severe thrombocytopenia of the foetus and the newborn (1). Based on the results of prospective studies of HPA–1a negative pregnant women the incidence of FNAIT is estimated to be 1 in 1,000 to 2,000 live births (2,3,4). According to the data of Norwegian and English researchers, however, the estimate of the incidence of FNAIT on the basis of clinical diagnosis is 1 in 7,700 to 8,000 live births (5,6).

2 Etiopathogenesis

FNAIT occurs as a result of maternal sensitisation against foetal platelet antigens that the foetus inherited from the father. The pathogenesis of the disease is similar to a haemolytic foetal and neonatal disease due to maternal sensitisation against RhD erythrocyte antigens. Unlike in foetal and neonatal haemolytic disease, the sensitisation to platelet alloantigens is often present already during the first pregnancy, so that in sensitised primiparae thrombocytopenia occurs in 20–60 % of first newborns (7,8). The period and mechanism of sensitisation have not been fully explained yet.

In FNAIT, sensitisation is caused by specific human platelet antigens (HPA) that are present in platelets from week 16 on and pass through the placenta into the maternal circulation where they may give rise to alloantibodies (9). At the onset of sensitisation the level of foetal platelets that pass into the placenta is probably too low to trigger an immune response (10). The researchers therefore believe that some other HPA-expressing cells, such as placental trophoblasts, are also involved in the pathogenesis of FNAIT (11).

Following sensitisation, the maternal immune system starts to produce alloantibodies. IgG antibodies enter the foetal blood circulation across the placenta where they bind to the foetal platelets; these are then broken down by the reticuloendothelial system, which leads to thrombocytopenia. On the other hand, IgM alloantibodies do not pass across the placenta and are not involved in the pathogenesis of FNAIT (9,12,13).

HPA antigens are found on platelet membrane glycoprotein receptors. Currently, there are 35 different platelet antigens, 12 of them belong in 6 biallelic systems (HPA–1, -2, -3, -4, -5, and -15; Table 1) (14). In Caucasians, 80–90 % of FNAIT cases are caused by antibodies to HPA–1a (7,12,15). HPA antigen is found on β3 integrin and is associated with Leu33Pro polymorphism. Leu33 allele carriers are HPA–1a positive, while Pro33 allele homozygotes are HPA–1a negative and marked as HPA–1b/b (1,4). The share of HPA–1a negative persons in Caucasian (our) population is 2.5 %, however, only 10 % of HPA–1a negative pregnant women carrying a HPA–1a positive foetus will develop anti-HPA–1a antibodies (10). The presence of HLA-DR B3*0101 antigen significantly increases the possibility of sensitisation during pregnancy with a HPA–1a positive foetus. Namely, this combination of antigens is responsible for sensitisation in as many as 35 % of pregnant women, which means that the molecule of haplotype HLA-DR B3*0101 represents HPA–1a antigen to HPA–1a immune cells of a HPA–1a negative mother very successfully (8). The next most common antibodies responsible for the onset of FNAIT are anti-HPA-5b (in 10–15 % of cases). The remaining

Table 1: HPA alloantigens/proteins. Resumed after http://www.ebi.ac.uk/ipd/hpa/table1.html, julij 2017.

System	Antigen	Original name	Glycoprotein	CD
HPA-1	HPA-1a HPA-1b	Zw^a, Pl^{A1} Zw^b, Pl^{A2}	GPIIIa	CD61
HPA-2	HPA-2a HPA-2b	Ko^b Ko^a, Sib^a	GPIb*alpha*	CD42b
HPA-3	HPA-3a HPA-3b	Bak^a, Lek^a Bak^b	GPIIb	CD41
HPA-4	HPA-4a HPA-4b	Yuk^b, Pen^a Yuk^a, Pen^b	GPIIIa	CD61
HPA-5	HPA-5a HPA-5b	Br^b, Zav^b Br^a, Zav^a, Hc^a	GPIa	CD49b
	HPA-6bw	Ca^a, Tu^a	GPIIIa	CD61
	HPA-7bw	Mo^a	GPIIIa	CD61
	HPA-8bw	Sr^a	GPIIIa	CD61
	HPA-9bw	Max^a	GPIIb	CD41
	HPA10bw	La^a	GPIIIa	CD61
	HPA11bw	Gro^a	GPIIIa	CD61
	HPA12bw	Iy^a	GPIb*beta*	CD42c
	HPA13bw	Sit^a	GPIa	CD49b
	HPA14bw	Oe^a	GPIIIa	CD61
HPA-15	HPA-15a HPA-15b	Gov^b Gov^a	CD109	CD109
	HPA-16bw	Duv^a	GPIIIa	CD61
	HPA-17bw	Va^a	GPIIb/IIIa	CD61
	HPA-18bw	Cab^a	GPIa	CD49b
	HPA-19bw	Sta	GPIIIa	CD61
	HPA-20bw	Kno	GPIIb	CD41
	HPA-21bw	Nos	GPIIIa	CD61
	HPA-22bw	Sey	GPIIb	CD41
	HPA-23bw	Hug	GPIIIa	CD61
	HPA-24bw	$Cab2^{a+}$	GPIIb	CD41
	HPA-25bw	Swi^a	GPIa	CD49b
	HPA-26bw	Sec^a	GPIIIa	CD61
	HPA-27bw	Cab^{3a+}	GPIIb	CD41
	HPA-28bw	War	GPIIb	CD41
	HPA-29bw	Kha^b	GPIIIa	CD61

Legend: HPA – human platelet antigens; GP – glycoproteins; CD – cluster of differentiation.

most common antibodies also include anti-HOA–1b, anti-HPA–15, anti-HPA–3 and anti-HPA–96 (altogether 5 % of all cases) (7,8,9,15).

Rarely, thrombocytopenia of the newborn is a result of the transplacental transfer of autoantibodies in a mother with primary immune thrombocytopenia (ITP). Maternal autoantibodies generally do not cause thrombocytopenia in the newborn or the latter is present only in a mild form without clinical signs. Only a few cases of severe neonatal thrombocytopenia caused by maternal autoantibodies have been reported (16).

The role of anti-A and anti-B antibodies from ABo blood-group system as well as of anti-HLA-A and HLA-B antibodies in the serum of pregnant women as regards the occurrence of thrombocytopenia has not been fully clarified. Studies have shown that anti-A and –B antibodies as well as anti-HLA-A and HLA-B antibodies are most likely irrelevant for FNAIT pathogenesis and do not cause thrombocytopenia in the newborn. Only a few cases of FNAIT caused by anti-A and –B antibodies and anti-HLA-A and HLA-B have been reported in the literature (8,17).

3 Clinical picture and laboratory findings

A newborn with FNAIT my be free of symptoms, while in 85–90 % of FNAIT cases caused by anti-HPA–1a antibodies there is evidence of haemorrhagic diathesis, most often petechiae, haematomas and mucosal haemorrhage. Gastrointestinal bleeding is found in one third of the patients while bleeding from the urinary tract is rare. Intracranial haemorrhage, though being rare (present in 10–15 % of symptomatic newborns) (8,10,13), is the most common cause of death and irreversible neurological sequels (18). In 80 % of cases, intracranial haemorrhage occurs prenatally (19), while in the postnatal period the greatest risk of its occurrence is within the first 96 hours of life (8). In anti-HPA-5b-induced FNAIT, 2/3 of patients have no signs of disease, but nevertheless they may suffer an intracranial haemorrhage (9,10).

An intracranial haemorrhage due to FNAIT may occur already in the foetus, which may result in cerebral/cerebellar destruction, ventriculomegaly, the formation of porencephalic cyst, foetal hydrops and death (21). The risk of intracranial haemorrhage in the foetus of a pregnant woman who have already had a foetus with intracranial haemorrhage in her previous pregnancy is 72 % (range 46–98 %) (20).

In the newborn with FNAIT, only unspecified thrombocytopenia is present after birth, usually within the first 24–48 hours of life. The platelet count (PC) generally ranges between 10 and 50×10^9/L. In most cases, thrombocytopenia aggravates over the first 48 hours and PC falls below 10×10^9/L (12). Thrombocytopenia induced by anti-HPA–1a antibodies is generally more severe compared to anti-HPA–5b-induced one, which is slightly milder (13). In a study by Bussel et al. the average PC in a foetus with foetal alloimmune thrombocytopenia due to anti-HPA–1a antibodies was found to be 18×10^9/L, while with other anti-HPA antibodies it was 60×10^9/L (21). Thrombocytopenia generally lasts 2–6 weeks and is self-limiting, so that most newborns do not require any treatment (8). In rare cases, however, FNAIT is associated with anaemia and hyperbilirubinaemia due to haemorrhage (22). In each consequent sibling with inconsistency in platelet antigens, thrombocytopenia is expressed with equal or higher intensity (8,13).

4 Diagnosis

FNAIT is suspected in a newborn with isolated thrombocytopenia with or without haemorrhagic diathesis and the absence of other more frequent causes of thrombocytopenia. The maternal platelet count is normal, the course of pregnancy and delivery are uneventful, there is no history of autoimmune disease of the mother or intake of any medication that may cause thrombocytopenia in the newborn (10,12,13). By clinical examination and investigations, other causes of neonatal thrombocytopenia, such as e.g. intrauterine infection, polycythaemia, thrombosis, congenital abnormalities characterised by thrombocytopenia, disseminated intravascular coagulation as a result of infection or severe bleeding, are excluded (10,23).

In case of suspected FNAIT, laboratory tests should be used to establish whether platelet antibodies are actually present in the mother's serum and then determine their specificity. It is sensible to also determine the mother's and father's HPA genotype. Incompatibility in HPA antigens between the mother and the father is important for predicting the risk associated with subsequent pregnancies as well as for pregnancy management and appropriate treatment when necessary (24).

Serological FNAIT diagnosis is based on proving the presence of platelet antibodies in the mother's and newborn's serum by an indirect immunofluorescence test and demonstrating the presence of platelet antibodies bound to the newborn's platelets by a direct immunofluorescence test (25). As the newborn's serum and platelets are often not available, the mother's serum is used instead to demonstrate the presence of platelet antibodies. If platelet antibodies are found in the mother's serum, these need to be further specified.

Enzyme linked immunosorbent assay (ELISA) is used for the specification of HPA antibodies. ELISA test allows differentiation of anti-HPA–1a, –1b, –3a, –3b, –4a, –5a, –5b as well as anti-gpIa/IIa and anti-HLA antibodies (26).

Serological confirmation of FNAIT and the specification of maternal platelet antibodies are very important for the management of further pregnancies since it is known that not all the platelet antibodies are equally aggressive and that thrombocytopenia is expressed with the same or even greater intensity in each subsequent child with platelet-antigen incompatibility (8,13).

Serological and molecular-biological tests are used for determining HPA platelet antigens. Polymerase chain reaction with sequence specific primers (PCR-SSP) is used most frequently. In the case that the mother is HPA–1a negative (HPA–1b/b) while the father is a homozygote HPA–1a/a, the foetus will always be HPA–1a/a positive and thus at risk of developing FNAIT. If, however, the mother is HPA–1b/b and the father is heterozygote HPA–1a/b, there is a 50 % chance that the foetus is HPA–1a positive. In this case, the determination of foetal platelet antigens from amniotic fluid cells or biopsy of the chorionic villi by PCR-SSR method is of particular importance as it allows early treatment of the endangered foetus. So far, tests for non-invasive prenatal determination of foetal HPA–1a genotype from free cell fragments of foetal DNK that are present in the mother's peripheral blood are still in a developmental phase, and thus only available in research laboratories (14).

Cordocentesis intended to determine thrombocytopenia and the need for platelet transfusion in a foetus is no

longer recommended due to the risk associated with the intervention (14).

Pre-implantation genetic diagnostics as part of the *in vitro* fertilisation process, which allows the selection of a HPA–1a negative embryo that can be then transferred into a HPA–1a negative woman by embryo transfer procedure, is currently an option with the potential in future, as only one such case has been described in the literature so far (27).

The use of PCS-SSP method for establishing incompatibility between maternal and paternal HPA antigens seems also reasonable in cases when serological tests fail to prove the presence of platelet antibodies in the maternal serum either due to a low titre or low antibody avidity 9.

5 Treatment

5.1 Newborn

The treatment for FNAIT depends on the level of thrombocytopenia and clinical picture. Treatment with platelet transfusion is recommended when the platelet count is below 30×10^9/L in a clinically stable newborn (particularly in the first days of life (28,29), or below 50×10^9/L in a newborn with signs of haemorrhage or concomitant diseases (8,10,16,28).

Whenever possible, the newborn will receive a transfusion of the mother's platelets, since there is no reaction between these and the alloproteins that are present in the child's blood. Prior to use, the mother's platelet unit should be adequately prepared by alloantibody removal. Each platelet unit intended for the newborn is filtered and irradiated to prevent transfusion-induced graft-versus-host disease (GVHD) (15).

If maternal platelets are not available, platelet preparations are obtained by apheresis, preferably from HPA–1a negative donors or by pooling several units (buffycoat) from different donors. Platelet donors should be CMV negative, but even in this case, their platelets should be filtered and irradiated (3).

The newborn may also be treated with intravenous immunoglobulins (IVIg) at a dose of 0.4–1 g/kg body weight/day for 2 to 5 consecutive days (1,8,29). Following IVIg application, a favourable effect is noted in 2/3 patients, with a platelet count increase over 50×10^9/L after 48 hours. The drawback of IVIg treatment is in its delayed effect, and therefore – particularly in the case of haemorrhage or severe thrombocytopenia – it is combined with platelet transfusions (1,8).

5.2 Foetus

Measures to be taken in a HPA allosensitised pregnant woman depend on the outcome of a previous pregnancy and the genotype of foetal or paternal platelets. If the father is a homozygote for a platelet antigen, the foetus will in any case be incompatible with the mother. If the father is a heterozygote, the probability that the foetus will be incompatible with the mother is 50 %. Foetal genotype can be determined by analysis of cells obtained by amniocentesis or biopsy of the chorionic villi.

If there is an incompatibility in platelet antigens between the mother and the foetus for which the mother has proven antibodies against, the delivery should be planned and performed with caesarean section after the completed Week 37. Thus, the risk of intracranial haemorrhage during vaginal delivery is reduced (31).

Indications for prenatal therapy are not clearly defined. In 2011, a group of experts issued recommendations for

the treatment and management of pregnant women with platelet antibodies or a history of thrombocytopenia of the newborn after a previous delivery. The recommendations were based on the results of clinical studies and expert opinion (31). Considering the risk associated with cordocentesis for the determination of foetal platelet count, the current therapeutic indications are based on the results of serological testing, the level of thrombocytopenia and the occurrence of intracranial haemorrhage in a previous pregnancy (31). Although a foetal platelet transfusion is a feasible therapeutic option, current recommendations are in favour of the mother's treatment with immunoglobulins or corticosteroids or both, with the latter being most effective (32).

6 Prevention and screening for pregnant women

Although it is believed that allosensitisation in a HPA–1a negative pregnant woman may occur very early in the course of pregnancy, recent prospective studies show that more than 75 % of HPA–1a negative pregnant women are alloimmunised at delivery (33). These findings confirm the similarity between FNAIT and haemolytic foetal and neonatal disease, so studies are underway to support the assumption that a postnatal application of anti-HPA–1a antibodies in a HPA–1a negative mother that gives birth to a HPA–1a positive child can prevent allosensitisation (14).

There is currently no national screening programme in the world to test all pregnant women in order to identify HPA–1a negative ones who are at risk for allosensitisation (14). Although Norwegian researchers have demonstrated that the screening programme improves the clinical outcome (4) and is economically viable (34), national screening programmes have so far not been approved mainly due to the lack of preventive measures and effective and uniform treatment. Due to the absence of screening, the proposed protocols of prenatal treatment are based on the anamnestic data of intracranial haemorrhage or thrombocytopenia in a previous child, thus usually failing to register the first pregnancy in which FNAIT occurred.

7 Retrospective review of patients with FNAIT in Slovenia in the period between 1996 and 2016

7.1 The aim of the study

The incidence and clinical dimension of FNAIT in Slovenia have not been established. The aim of the presented retrospective study was to establish the incidence and aetiology of FNAIT in Slovenia along with the clinical picture of patients with FNAIT.

7.2 Methods

7.2.1 Research design and study population

Our research was designed as a retrospective cohort study. All the children born between January 1996 and December 2016 who met the criteria for FNAIT and were hospitalised at two wards of the University Medical Centre Ljubljana (Clinical Department of Perinatology of the Division of Gynaecology and Obstetrics, and the Clinical Department of Neonatology of the University Hospital of Paediatrics), and at the Department of Perinatology of the University Medical Centre Maribor were included in the study. Considering

the division of work and organisation of the neonatal service in Slovenia, with the inclusion of the mentioned departments in the study, probably a vas majority of Slovenian patients with FNAIT diagnosis were included.

Diagnostic criteria for FNAIT were thrombocytopenia in the newborn (platelets $< 120 \times 10^9$/L) and positive serological tests to prove platelet antibodies in the mother and/or the newborn, and the absence of other possible causes of thrombocytopenia.

The newborns were recruited in two phases. In the first phase, a list of all pregnant women, neonatal women and newborns from the aforementioned hospital departments, who underwent examination for the presence of platelet antibodies, was obtained for the period from January 1996 to December 2016 from the Institute of the Republic of Slovenia for Transfusion Medicine (ZTM). In the second phase, all mothers with proven platelet antibodies and whose newborns had thrombocytopenia at the absence of other possible causes of thrombocytopenia, as well as all newborns with proven platelet antibodies were identified. Newborns with thrombocytopenia whose mothers did not have evidence of platelet antibodies were excluded from the study. Medical records were then obtained for the final set of patients.

The study was approved by the Medical Ethics Committee of the Republic of Slovenia (Decision No. 0120–635/2016–5).

7.2.2 Data Collection

The basic demographic data and information on the clinical course, treatment and outcome of the disease were collected from the children's medical records. Mild thrombocytopenia was defined as a platelet count between 100–120×10^9/L, moderate as a platelet count between 50–100×10^9/L, and severe as a platelet count $< 50 \times 10^9$/L. The data on the results of serological testing and the specification of platelet antibodies were obtained form the ZTM's information system.

7.2.3 Statistical Analysis

When calculating the incidence of FNAIT, it was taken into account that all the requests for platelet antibodies identification from all Slovenian health institutions are sent to the ZTM. Furthermore, we assumed that Slovenian neonates with unexplained thrombocytopenia are transferred to two tertiary health institutions (KON UKCL and UKCM) or they are born in the maternity hospitals Ljubljana (KOP UKCL) and Maribor (UKCM), where pregnant women at risk of FNAIT or testing positive for platelet antigens are referred to. The so-obtained number of patients with FNAIT is the numerator, and the number of live births in the 21-year period is the denominator in the calculation of FNAIT incidence in Slovenia. The obtained quotient was expressed per 1,000 live births. The data on the number of live births in Slovenia were obtained from the Statistical Yearbook of the Republic of Slovenia for years 1996–2016 (http://www.stat.si).

Statistical data processing was performed using the IBM SPSS Statistics software package, version 21 (IBM Corporation, Armonk, USA). For the acquired data, the proportions, averages, standard deviations, median interquartile range (IQR) and confidence intervals (CI) were presented. CI was calculated for the rates with normal approximation (for $n < 30$ by Agresti Coull) (35). In the case of a negative lower CI value, the lower cut-off limit of CI was set at zero. The Mann-Whitney test was used to compare the asymmetrically distributed

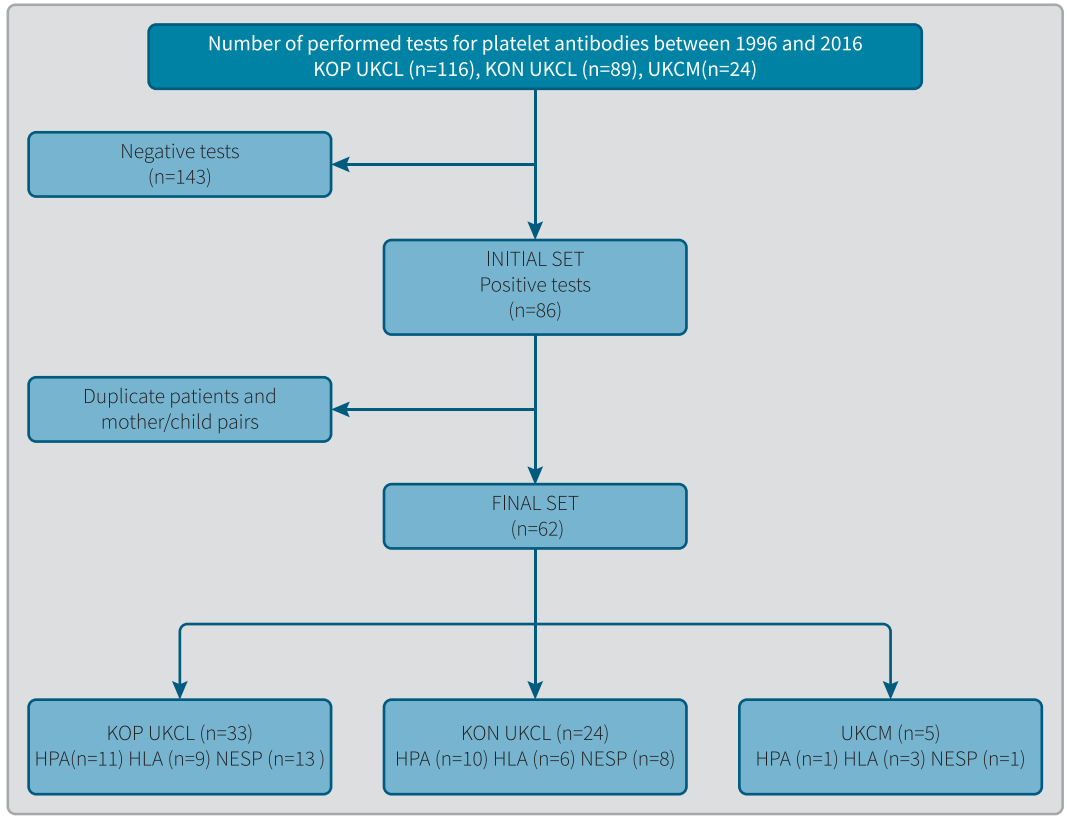

Figure 1: Patient collection scheme.

Legend: KOP UKCL – Clinical Department of Perinatology of the Division of Gynaecology of the University Medical Centre Ljubljana; KON UKCL – Clinical Department of Neonatology of the Division of Paediatrics of the University Medical Centre Ljubljana; UKCM – Department of Perinatology of the University Medical Centre Maribor; HPA – anti HPA antibodies; HLA – anti-HLA antibodies, NESP – antibody specification has not been performed.

numerical variables between groups. To compare the proportions in subgroups, Fisher's exact test was used. The values at $p < 0.05$ were considered statistically significant.

7.3 Results

7.3.1 Patients under study

In the period from January 1996 to December 2016, 229 tests to detect platelet antibodies in newborns, neonatal and pregnant women from three departments participating in the study (KOP UKCL, KON UKCL and UKCM) were performed at the ZTM. Of these, 143 tests for platelet antibodies were negative and 86 positive. Following exclusion of duplicate patients and identification of child/mother pairs, there were 62 positive serological tests available that could be associated with newborns: 24 (39 %) from KON UKCL, 33 (53 %) from KOP UKCL and 5 (8 %) from UKCM; of these, 54 samples were from mothers, 1 sample from a child and 7 paired child-mother samples (Figure 1). The newborns of 10 neonatal women who had proven platelet antibodies (all of them had anti-HLA and none anti-HPA antibodies) did not develop thrombocytopenia. We could not obtain medical records for 16 chil-

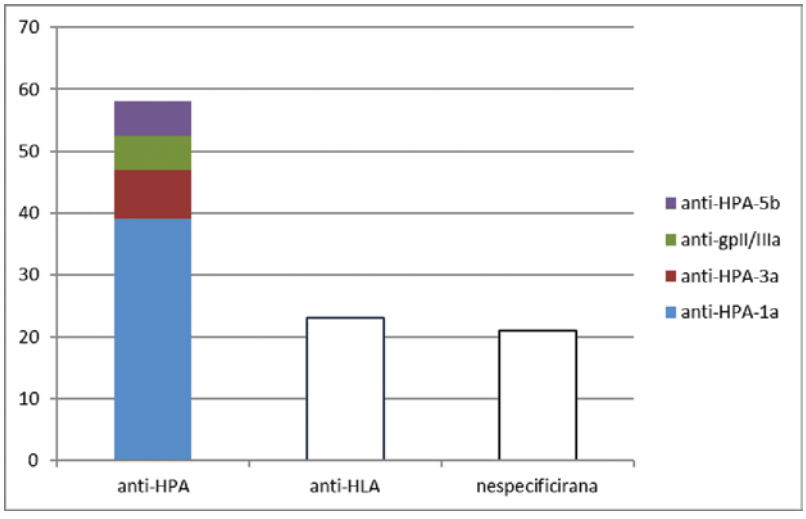

Figure 2: Proportion of children with FNAIT according to the type of antibodies.

Legend: FNAIT – foetal and neonatal alloimmune thrombocytopenia; HPA – human platelet antigens; HLA – human leukocyte antigens.

dren. Thus, data on demographic characteristics and clinical picture of children with FNAIT as well as the findings of platelet testing and treatment of pregnant women and children were obtained for 39 cases.

7.3.2 FNAIT incidence

In the period from 1996 to 2016, 410,310 children were born in Slovenia (http://www.stat.si). According to the data of the ZTM, i.e. the only institution that performs the diagnostics of platelet antibodies, 62 samples of mothers or children tested positive for platelet antibodies. The newborns of 10 neonatal women did not develop thrombocytopenia despite the presence of maternal platelet antibodies. We could not obtain medical records for 16 children, and therefore information on whether they had thrombocytopenia is missing. Three pregnant women had each two children with thrombocytopenia. Thus, in the period from 1996 to 2016, 39 children definitely had FNAIT (perhaps even 55 (39 + 16) children), which represents the numerator and the number of live birth in the appointed period the denominator in the calculation of incidence. Accordingly, the incidence of FNAIT in Slovenia in that period was 1/10,000 live births (95 % CI; [0.6/10,000; 1.3/10,000]), i.e. two children with FNAIT per year.

7.3.3 Clinical features and platelet antibodies

Twelve (30.5 %) girls and 27 (69.5 %) boys were included in the final analysis. The median gestation age of children with FNAIT was 39 weeks (IQR 3), 8 children were born prematurely, and the median birth weight was 3180 g (IQR 760). (Table 2) The majority, 31 children (79 %) were born by vaginal birth. Positive history of thrombocytopenia in neonatal period in a sibling was present in 6 (15 %) (95 % CI [4 %; 26 %]) cases; only two of the mentioned 6 cases were delivered with caesarean section.

In 22 (56 %) (95 % CI [40 %; 72 %]) cases, FNAIT was caused by HPA antibodies, in 9 (23 %) (95 % CI [10 %; 36 %]) only HLA antibodies were found, while in 8 (21 %) (95 % CI [10 %; 36 %]) cases antibody identification was not performed. As to HPA antibodies, anti-HPA–1a antibodies were demonstrated in 15 (68 %) (95 % CI [47 %; 84 %]) cases (in 4 both anti-HPA–1a and anti-HLA were proven), anti-HPA–3a in 3 (14 %) (95 % CI [4 %; 34 %]), antibodies anti-gpIIb/IIIa in 2 (10 %) (95 % CI [1 %; 29 %]), and both anti-HPA-5b and anti-HLA in 2 (10 %) (95 % CI [1 %; 29 %]) cases (Figure 2).

Twenty-seven (69.5 %) (95 % CI [55 %; 84 %]) children had petechiae and/or suffusions (87 % of children with HPA–1a and 55 % with other antibodies), 1 (2.5 %) (95 % CI [0 %; 7 %]) had intracranial haemorrhage, 2 had (5 %) (95 % CI [0 %; 12 %]) some other type of haemorrhage, while 9 (23 %) (95 % CI [10 %; 36 %]) children were without clinical signs.

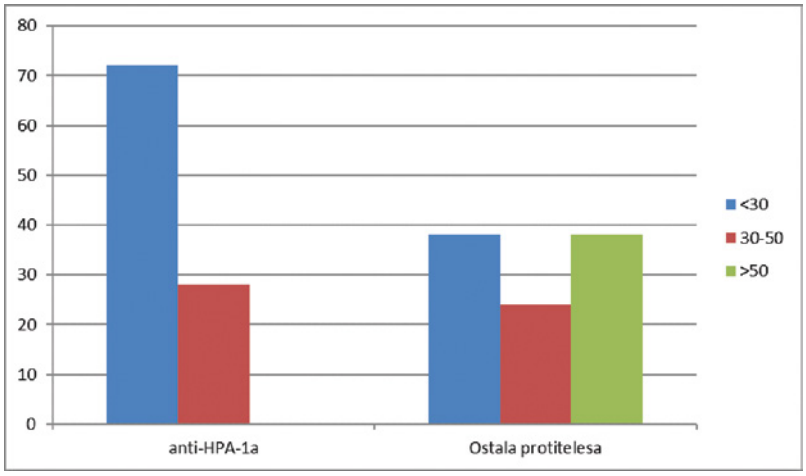

Figure 3: Proportion of children with respect to the level of thrombocytopenia, separately for FNAIT induced by anti-HPA–1a antibodies, and for FNAIT induced by other platelet antibodies.

Legend: Št.tr. – platelet count × 10^9/L; HPA – human platelet antigens.

Two children without clinical signs had evidence of anti-HPA–1a antibodies, 1 had anti-HPA–3a, 2 had anti-HLA, and 1 child had anti-gpIIb/IIIa antibodies. Three children without clinical signs had the evidence of platelet antibodies proven, however their specificity was not defined. Only 2 children with anti-HLA antibodies were free of clinical signs while 7 had petechiae.

Children with anti-HPA–1a antibodies had lower platelet counts compared to children with FNAIT caused by other platelet antibodies (the difference was statistically significant ($p = 0.015$)); the median of the lowest recorded platelet count was 16×10^9/L (IQR 19×10^9/L) vs. 36×10^9/(IQR 39×10^9/L). Figure 3 shows the proportion of children with varying levels of thrombocytopenia (platelet count $< 30 \times 10^9$/L, range $30–50 \times 10^9$/L and $> 50 \times 10^9$/L). In half of the children (median) with anti-HPA–1a FNAIT, the lowest platelet count was observed in less than 1 day (IQR 3) after birth, while for other platelet antibodies in half of the children (median) the lowest value was recorded less 2 days (IQR 6) after birth, but the difference was not statistically significant. The platelet count in children with FNAIT induced by anti-HPA–1a antibodies normalised more rapidly than in children with FNAIT caused by other platelet antibodies, however, the difference in distribution between the groups was not statistically significant (Table 3).

Twenty-five (64.5 %) (95 % CI [49 %; 80 %]) children did not require any therapy, 8 (20.5 %) (95 % CI [8 %; 33 %]) received a platelet transfusion, 4 (10 %) (95 % CI [1 %; 19 %]) a platelet transfusion and IVIG, 1 (2.5 %) (95 % CI [0 %; 7 %]) IVIG only, and 1 child (2.5 %) (95 % CI [0 %; 7 %]) received a platelet transfusion, IVIG and corticosteroids. Five women received IVIG therapy during pregnancy; two of them had anti-gpIIb/IIIa antibodies and the other three had anti-HPA–1a, anti-HPA–3a and anti-HLA, respectively. All five pregnant women had previously given birth to a child with thrombocytopenia; in her previous pregnancy, the one with anti-HLA antibodies gave birth to a newborn with thrombocytopenia, petechiae and subependymal haemorrhage.

The number of previous pregnancies and childbirths, the mode of labour, clinical picture, the lowest platelet count, the age of the child at the lowest platelet count, time to normalisation of platelet count, and thrombocytopenia therapy are presented in Table 2 and Table 3, separately for children who had FNAIT due to the presence of anti-HPA–1a antibodies or other platelet antibodies.

7.4 Discussion

Based on the results of studies monitoring HPA 1a negative pregnancies, the incidence of FNAIT is estimated at 1/1,000 to 2,000 live births (2,3,4). Given the fertility rate in Slovenia, 10 to 20

Table 2: Basic demographic and perinatal data of the included newborns.

	anti-HPA-1a and anti-HPA-1a + anti-HLA n = 15		Other platelet antibodies n = 24		Total n = 39	
Gender	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Male	12	(80)	15	(62)	27	(69.5)
Female	3	(20)	9	(38)	12	(30.5)
Birth weight in g						
Mean (SD)		3128 (410)		2960 (700)		3024 (604)
Median (IQR)		3250 (690)		3040 (1390)		3180 (760)
Gestation age in weeks						
Mean (SD)		38.4 (2)		38 (2.5)		38.1 (2.3)
Median (IQR)		39 (3)		39 (4)		39 (3)
Zaporedna nosečnost	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
1	6	(40)	9	(38)	15	(39)
2	6	(40)	7	(29)	13	(33)
3	2	(13)	6	(25)	8	(20.5)
≤ 4	1	(7)	2	(8)	3	(7.5)
Number of siblings in the family	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
1	8	(53)	12	(50)	20	(51)
2	6	(40)	7	(29)	13	(33.5)
3	1	(7)	4	(17)	5	(13)
≤ 4	0	(0)	1	(4)	1	(2.5)
Mode of delivery	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Vaginal	13	(87)	18	(75)	31	(79.5)
Caesarean section	2	(13)	6	(25)	8	(20.5)
Thrombocytopenia in previous pregnancy	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
No	13	(87)	19	(82)	32	(84)
Yes	2	(13)	4	(18)	6	(16)

Legend: SD – standard deviation; IQR – interquartile range; HPA – human platelet antigens; HLA – human leukocyte antigens.

cases of FNAIT would be expected annually. The results of the performed retrospective study have shown that that in Slovenia, FNAIT is proven on average in 2 infants yearly and that the incidence of FNAIT in Slovenia is 1/10,000 (95 % CI [0.6/10,000; 1.3/10,000]) live births, i.e. lower than the estimated incidence in prospective studies (2,3,4) and comparable with the FNAIT incidence in the studies of Norwegian and British researchers, which were designed in a similar way as our studies (1 per 7,700 to 8,000 live births) (5,6).

Although the incidence is calculated from the data obtained through a retrospective review and is therefore most likely underestimated, we believe that the set of patients under the given conditions was optimal and, therefore, the number of proven cases of FNAIT was real. Namely, the ZTM is the only institution in Slovenia where tests for the evidencing of platelet antibodies are carried out; considering the division of work in neonatology and the organization of obstetrics in Slovenia, it is very likely that children with FNAIT are treated in the three departments included in the survey

Although the calculated low incidence may be attributable to the shortcomings of retrospective analysis, the fact that in the 21-year period there were 229 requests for antibody identification to prove FNAIT, i.e. 10.9 yearly, cannot be ignored. Given the expected 10–20 FNAIT cases annually, identification requests should be at least as many or twice that number (12). Therefore, low incidence could also be a consequence of suboptimal active search of the disease.

Anti-HPA–1a were the most frequently proven antibodies, these being found in 15 children or in 68 % (95 % CI [49 %; 87 %]) of FNAIT due to HPA antibodies. The proportion of anti-HPA–1a induced FNAIT in our sample, which otherwise covers the majority of the population in the analysed period, is therefore lower than reported in the literature (75–90 %) (5,15,36,37,38). A lower proportion of FNAIT caused by anti-HPA–1a is also reported by Croatia, namely 50 % (12). The lower proportion of FNAIT caused by anti-HPA–1a antibodies could mean that the share of sensitised HPA–1a negative pregnant women in our wider environment is lower. Namely, the share of HPA–1a negative persons in our environment is similar as in populations where 75–90 % anti-HPA–1a–induced FNAIT are reported (39). A lower anti-HPA–1a-induced FNAIT could be a consequence of laboratory failure to detect low-avidity antibodies. The results of studies show that low-avidity anti-HPA–1a antibodies that fail to be detected by standard laboratory test can indeed cause FNAIT (40,41).

Anti-HLA antibodies exclusively were demonstrated in 9 (23 %) (95 % CI [10 %; 36 %]) cases. The aetiology of anti-HLA antibodies in the development of FNAIT has not been fully clarified, however is deemed not to be a cause for the occurrence of severe thrombocytopenia in the newborn, since the presence of anti-HLA antibodies in healthy pregnant women is quite common (up to 40 %) (24,36). Out of these nine pregnant women, 4 were primiparae. In the first pregnancy, when sensitisation occurs, the antibody HPA titre may be low and thus undetectable by laboratory tests. In the case of suspected FNAIT and the absence of anti-HPA antibodies, it is appropriate to determine the HPA antigens of the mother and the child or at least of the father by genotyping. If the mother and the newborn are incompatible antigen wise within the HPA system, FNAIT is possible as there may be low-avidity or scarce antibodies present, which failed to

Tabela 3: Comparison of clinical features, platelet count and treatment of newborns with FNAIT, separately for groups with anti-HPA-1 and other antibodies.

	anti-HPA-1a and anti-HPA-1a + anti-HLA n = 15		Other platelet antibodies n = 24		Total n = 39		Statistical significance test P value
Clinical picture	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	
Without symtpoms	2	(13)	7	(29)	9	(23)	Fisher's test p = 0.437
With symptoms	13	(87)	17	(71)	30	(77)	
The lowest platelet count (× 10^9/L)							
Mean (SD)	20 (13)		44 (36)		35 (32)		Mann-Whitney's test p = 0.015
Median (IQR)	16 (19)		36 (39)		29 (32)		
	2.2 (2)		3.8 (6.3)		3.2 (5.2)		Mann-Whitney's test p = 0.415
	1 (3)		2 (6)		1 (3)		
Time to normalisation of platelet count, Mean (SD), Median (IQR)							
Mean (SD)	11.5 (12.6)		25.7 (37)		19.1 (28.9)		Mann-Whitney's test p = 0.185
Median (IQR)	6 (4)		18 (20)		7.5 (21)		
Newborns' treatment[a]							
It was not necessary	8	(53)	17	(72)	25	(64.5)	Fisher's test p = 0.318
Platelet transfusion	5	(13)	3	(12)	8	(20.5)	
IVIG	1	(7)	0	(0)	1	(2.5)	
Platelet transfusion + IVIG	1	(7)	3	(12)	4	(10)	
Platelet transfusion + IVIG + KS	0	(0)	1	(4)	1	(2.5)	
Prenatal mother's treatment with IVIG[b]	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	
No	13	(93)	18	(82)	31	(86)	Fisher's test p = 0.628
Yes	1	(7)	4	(18)	5	(14)	

Legend: SD – standard deviation; IQR – interquartile range; HPA – human platelet antigens; HLA – human leukocyte antigens; Tr – platelets; IVK – intraventricular haemorrhage; IVIG – intravenous immunoglobulins; KS – corticosteroids

a – Fisher's exact test was used to compare the proportions of treated (those who received platelet transfusion, IVIG, KS or a combination of these therapies) and untreated children.

b – The data on the mother's prenatal treatment was obtained for 36 children.

be proven by standard laboratory tests. In our study, genotyping was performed in three out of 9 HLA-positive cases.

Positive history of thrombocytopenia in a sibling was established in 15 % (95 % CI [4 %; 26 %]) of children, similar to the study by Bussel et al. (43), where thrombocytopenia in 18 % of siblings was reported. Although a caesarean section is indicated in a pregnant woman with proven platelet antibodies, especially if she has already given birth to a child with thrombocytopenia previously, in our study, only 2 of 6 newborns with positive familial history were delivered with caesarean section.

The newborns with anti-HPA–1a antibodies had a lower platelet count than the newborns with FNAIT induced by other platelet antibodies. The rate of children with severe thrombocytopenia in the group with anti-HPA–1a-induced FNAIT was 100 % vs. 63 % of children with FNAIT induced by other platelet antibodies. Almost 70 % of children with FNAIT and proven anti-HPA–1a antibodies had a platelet count under 30. Similar rates are reported by other investigators (13,15,44). Ten children of mothers with proven platelet antibodies did not develop thrombocytopenia (10 of 54 mother samples, 18 % (95 % CI [8 %; 28 %]). Kjeldsen_Kragh et al. report even a higher proportion of children born to mothers with anti-HPA–1a antibodies, who had a normal platelet count, i.e. 50 % (4).

Given that anti-HPA–1a cause the most prominent thrombocytopenia, it would be expected that the time to normalisation of platelet count in children with anti-HPA–1a antibodies would be longer than in children with FNAIT induced by other platelet antibodies. In our study, the time to normalisation of platelet count in children with anti-HPA–1a antibodies was shorter, however, the difference was not statistically significant. The shorter time to normalisation of platelet count could be the result of treatment, as the share of children treated with platelet transfusion in the group of children with anti-HPA–1a antibodies was greater than in the group of children with other platelet antibodies, precisely 47 % (95 % CI [25 %; 70 %]) vs. 29 % (95 % CI [15 %; 49 %]) respectively. Most authors are in favour of treating newborns with FNAIT by platelet transfusions insofar as their platelet count is < 30 or they show signs of haemorrhage (8,28,29). In our study, platelet transfusion was administered in 13 of 20 children with a platelet count < 30.

Five women received IVIG during their pregnancy. Their children had various degrees of thrombocytopenia: 2 had mild, 1 moderate and 2 had severe thrombocytopenia, but none of them had an intracranial haemorrhage. Thus, despite the mother's treatment with IVIG, 40 % (95 % IC [12 %; 77 %]) of newborns had severe thrombocytopenia. Van der Lugt et al. have followed up the treatment outcome of women with IVIG; among a total of 22 newborns, 12 (55 %) developed severe thrombocytopenia and only one (4 %) had intracranial haemorrhage (44). Thus, IVIG treatment decreases the risk of intracranial haemorrhage although it does not normalise the platelet count, which has also been reported by other researchers (45).

The conducted research has some shortcomings. The retrospective collection of data is difficult and incomplete owing to the lack of documentation, non-uniform approach to recording findings in the documentation and various therapeutic approaches. Despite the fact that for 16 children documentation could not be obtained, we are satisfied with the collected clinical data for the remaining children. A 21-year period

is long enough for a retrospective data collection, and besides that, the available documentation provided answers to almost all the questions related to each child. Therefore we believe that the demographic data as well as the data on clinical picture and treatment of children with FNAIT in Slovenia are satisfactory. Due to the retrospective study design, we also could not manage to explain the aetiology of FNAIT in cases where platelet antibodies have been proven, but their specification has not been performed.

8 Conclusion

The article is the first review article on FNAIT, which presents the results of a retrospective review of aetiology, clinical picture and treatment of patients with FNAIT in Slovenia. Given the established incidence, which is lower than expected and most likely due to the lacking active search for FNAIT in newborns, the authors hope that their article will contribute to raising awareness of gynaecologists and neonatologists of this albeit rare but nevertheless potentially very serious disease.

In Slovenia, likewise elsewhere in the world, we do not have a national preventive programme for identification of HPA–1a negative pregnant women who are at risk of allosensitisation. Therefore, the current prenatal diagnostics and pregnancy management are based on anamnestic data on thrombocytopenia and intracranial haemorrhage in an older sibling. The present article shows that the management of pregnant women at risk and newborns with FNAIT in Slovenia is not uniform. Therefore, clinicians' knowledge of the disease and good cooperation with the laboratory are of key importance for the management of pregnant women at risk and the affected newborns. A register of HPA–1a negative platelet donors would contribute significantly to improving the treatment of newborns when they need platelet transfusion.

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