Placenta-associated biomarkers and pregnancy outcome in HPA-1a alloimmunization: A prospective cohort study

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Abstract

Introduction: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) results from parental incompatibility in human platelet antigens (HPA) and subsequent maternal sensitization. The HPA-1a epitope is also expressed on placental tissue. Chronic placental inflammation and lower birth weight is observed more often in HPA-1a alloimmunized pregnancies, suggesting a placental component in the pathophysiology of FNAIT. Today, prediction of FNAIT severity is limited. The aim of the study was to investigate whether dysregulated maternal angiogenic proteins are associated with neonatal outcome in HPA-1a alloimmunized pregnancies.

Material and Methods: Eighty-seven HPA-1a negative pregnant women were identified from a large prospective screening study in Poland (PREVFNAIT) including 43 HPA-1a immunized and 44 non-immunized controls. Placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) were measured in maternal plasma from 2nd and 3rd trimester by enzyme-linked immunosorbent assay and levels/ratios were compared between study groups, using uni- and multivariable analyses. Main outcome measures were either classic FNAIT-related (severe thrombocytopenia, petechia, intracranial hemorrhage), placenta-related (small for gestational age) or a composite variable combining them all.

Results: There were no significant differences in plasma concentrations of sFlt-1, PlGF, sEng nor sFlt-1/PlGF ratio when comparing immunized and non-immunized pregnancies. Among HPA-1a alloimmunized pregnancies, increasing levels of the sFlt-1 protein in 3rd trimester were significantly associated with lower neonatal platelet count (multivariable linear regression, $p=0.024$). Increased sFlt-1 and sFlt-1/PlGF ratio in 3rd trimester were significantly associated with higher odds of a composite adverse neonatal outcome in alloimmunized pregnancies (multivariable logistic regression, $p=0.029$ and $p=0.019$, respectively).

1   **Conclusion**: An anti-angiogenic profile in HPA-1a alloimmunized mothers is associated with

2   a composite adverse neonatal outcome. This suggests that sFlt-1 and the sFlt-1/PlGF ratio may

3   assist in predelivery risk stratification and clinical management decisions for FNAIT.

4   **Keywords**: sFlt-1, PlGF, placenta, small for gestational age, intracranial hemorrhage, fetal and

5   neonatal alloimmune thrombocytopenia

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1. INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is defined by thrombocytopenia in the fetus or newborn caused by maternal alloantibodies directed against human platelet antigens (HPA) on fetal platelets. Feto-maternal incompatibility of HPAs usually stems from paternal antigens, but can also be present from donor egg or sperm, during *in vitro* fertilization [1]. Incompatibility in the HPA-1 system is the most common cause of FNAIT in Whites [2, 3]. The alloantibodies cross the placenta and may target fetal platelets for destruction, rendering the fetus/neonate at risk of bleeding. Clinical signs range from petechia to intracranial hemorrhage (ICH), with the latter typically leading to death or lifelong neurological impairment [4-6]. Although these alloantibodies may inflict platelet destruction in the fetus, a wider range of clinical implications have emerged. Lower birthweights and an increased risk of small for gestational age (SGA) newborns from HPA-1a alloimmunized mothers have been observed in both retrospective and prospective studies, some reporting a global tendency whereas others find associations only in boys [7-11]. Together with findings of various chronic placental inflammations among HPA-alloimmunized pregnancies [12, 13], this indicates an important placental component in the pathophysiology of FNAIT. Indeed, the HPA-1a epitope is not only expressed on platelets, but also on placenta tissue [14-16]. An *in vitro* study showed that anti-HPA-1a antibodies partially inhibited adhesive and migratory capacity of a first trimester trophoblast cell line [17], supporting that anti-HPA-1 antibodies may influence placentation.

Although never tested in a placebo-controlled trial, weekly antenatal treatment with off-label intravenous immunoglobulins (IVIg) is widely used to treat HPA-1a alloimmunized women at risk of FNAIT [4, 5, 18]. Today, prediction of FNAIT severity is limited to prior obstetric history [19, 20] and maternal anti-HPA-1a antibody levels [21, 22], with only limited predictive strengths. Better predictors of clinical outcome to target ante- and perinatal management are warranted [18]. Placental dysfunction, in particular syncytiotrophoblast (STB) stress, is

believed to promote excessive release of proinflammatory factors, including anti-angiogenic proteins into the maternal blood circulation. This results in elevated maternal levels of circulating soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) [23, 24]. Excess sFlt-1 levels reduce the level of free circulating placental growth factor (PlGF) [23-25]. An elevated ratio of anti-angiogenic to angiogenic protein levels in maternal circulation is associated with development of pregnancy disorders linked to placental dysfunction, including preeclampsia [26, 27] and fetal growth restriction [28-30]. Whether increased STB stress and release of anti-angiogenic factors also play a part in the pathophysiology of HPA-1a alloimmunization has not been explored. A recent publication showed that the sFlt-1 level in cord blood correlated with neonatal platelet count in an FNAIT cohort, suggesting that placenta-related biomarkers may be relevant in predicting FNAIT severity [31].

The aim of the study was to investigate whether dysregulated circulating maternal angiogenic proteins during pregnancy associates with neonatal outcome in HPA-1a alloimmunized pregnancies.

2. METHODS

Study design

Pregnant women were recruited to the "*Prevention of fetal /neonatal alloimmune thrombocytopenia in Polish newborns*" (PREVFNAIT) study from September 2013 to March 2017. This involved HPA-1 typing of 24 259 pregnant women in Poland to identify HPA-1a negative women at risk of delivering a neonate with FNAIT. The PREVFNAIT study was a collaboration between The Institute of Hematology and Transfusion Medicine in Warsaw, Poland and the Immunology research group at UiT The Arctic University of Norway, and is detailed by Dębska *et al* [32]. The screening for HPA-1a, molecular diagnostics of mothers,

1 fathers and newborns, collection of clinical data and all samples for the biobank was performed

2 in Warsaw. Plasma from HPA-1a negative women were screened for anti-HPA-1a antibodies

3 at 2nd and 3rd trimester, as well as six weeks post-partum. HPA-1a alloimmunized women

4 received obstetrical follow-up in accordance with local hospital procedures and some HPA-1a

5 alloimmunized women were treated antenatally with intravenous immunoglobulins (IVIg).

6 The screening study was approved by The Bioethical Committee at the Institute of Hematology

7 and Transfusion Medicine, Warsaw, Poland (Approval no: 38/2013), and the PREVFNAIT

8 biobank established in Norway was approved by the Regional Committee for Medical Research

9 Ethics, North Norway (REK 2014/83). Informed written consent was obtained from all

10 participants.

11 For the present study, all HPA-1a negative women with data on HPA-1a alloimmunization and

12 available plasma samples from 2nd and/ or 3rd trimester, were considered for inclusion. A

13 woman was defined as immunized if anti-HPA-1a antibodies were detected at least once during

14 pregnancy or within the first six days after delivery. If no maternal anti-HPA-1a antibodies

15 were detected during pregnancy, or anti-HPA-1a antibodies were only detected > 6 days

16 postpartum these women were included as non-immunized controls. Twin pregnancies,

17 pregnancies with HPA-1 compatible neonates or unknown neonatal HPA-1 status, pregnancies

18 ending with miscarriage, lack of available plasma samples or if the woman wished to withdraw

19 from the study, were not included.

20 The study population is shown in **Figure 1**. Of the total 24 259 women HPA-1 typed, 24 236

21 women were screened during PREVFNAIT and 23 HPA-1a negative women were included due

22 to previous FNAIT history (n=16) or delivered a neonate with clinical symptoms of

23 thrombocytopenia (n=7). Of these, 606 HPA-1a negative women were identified. Following

24 the exclusion criteria, maternal anti-HPA-1a antibodies were detected by MAIPA or by PAKLx

assay in 53 of 441 included women. Plasma samples were available from 43 alloimmunized

women with singleton pregnancies. Among these, 15 women received antenatal IVIg treatment.

Samples from 44 non-immunized women were included as controls. Women with available

placenta samples (n=11) were prioritized when selecting controls and the remaining samples

were randomly chosen and matched by numbers. In total, 87 pregnancies were included.

Data collection

Clinical maternal and neonatal data were obtained from medical records. Data on previous

pregnancy history, general health, and lifestyle were provided through self-report.

Biochemical analysis

Laboratory assays performed at the Institute of Hematology and Transfusion Medicine (IHTM)

in Warsaw included HPA-1 phenotyping of mother, father, and neonate by flow cytometry [33]

and genotyping by real-time polymerase chain reaction [34], using DNA isolated from EDTA

blood. Detection of anti-HPA-1a antibodies in maternal plasma was performed using a

monoclonal antibody immobilization of platelet antigens (MAIPA) assay [35] and

quantification by quantitative MAIPA [36]. Plasma samples from mothers who delivered

newborns with severe thrombocytopenia, and who tested negative in MAIPA, underwent

retrospective testing using PAKLx assay (LIFECODES, Immucor). EDTA-plasma were stored

locally at -70°C after sampling, shipped in batches on dry ice to UiT The Arctic University of

Norway in Tromsø, and stored at -70°C until analysis.

PlGF, sFlt-1 and sEng were measured in maternal EDTA-plasma from 2nd and 3rd trimester by

commercially available enzyme-linked immunosorbent assay (ELISA). Bioactive (free) PlGF

was measured with the ELISA kit for human PlGF (DPG00; R&D systems, Minneapolis, USA),

sFlt-1 with the human VEGF R1/Flt-1 kit (DVR100C; R&D systems, Minneapolis, USA) and

sEng with the human Endoglin/CD105 ELISA kit (DNDG00; R&D systems, Minneapolis,

USA). If samples from the preferred time points (week 16-20 and week 32) were not available, samples taken at earlier or later time points were used (week 28, n= 5 pregnancies and close to delivery, n= 3 pregnancies). Most women (79/87, 90.8%) had plasma samples from both 2nd and 3rd trimester. The samples were run in duplicates. Three samples had duplicate OD-values in PlGF or sFLT-1 measurements with a coefficient of variation (CV) >30% and thus excluded, while eight had a CV between 20 and 30% (n=2 for sFlt-1 (2nd trimester), n=2 for PlGF (2nd trimester) and n=4 for PlGF (3rd trimester)).

The sFlt-1/PlGF ratio was used to assess the frequency of immunized pregnancies and non-immunized controls with a ratio above 85, a commonly used cut-off to predict risk of early-onset PE [37, 38] and adverse outcome of early-onset FGR [39].

Definitions and outcome measures

Small for gestational age (SGA) was defined as a birthweight less than the 10th percentile [40]. A composite FNAIT-associated adverse neonatal outcome (yes/no) was defined as having at least one of the following outcomes: severe thrombocytopenia (platelet count below 50 x 10^9/L), skin bleedings, fetal/neonatal ICH or being SGA.

The highest level of anti-HPA-1a antibody (IU/mL) measured during pregnancy or before antenatal IVIg treatment commenced was used. For three pregnancies, detectable, but non-quantifiable anti-HPA-1a antibodies were detected in MAIPA ("weak response") and given an arbitrary value 0.01 IU/mL. Pregnancies where anti-HPA-1a antibodies could only be detected by Pak Lx were excluded from antibody level analyses (n= 6). The natural logarithm (ln) of anti-HPA-1a antibody concentrations were used to improve the overall model of fit.

Statistical analysis

All statistical analyses were performed using SPSS (Version 29.0). Quantitative data were skewed and described by median and range. Qualitative data were described by frequency and

1 percentages. Pairwise comparisons were performed using nonparametric Mann-Whitney test.

2 Fisher's exact test was used to assess relationships between categorical variables. Comparisons

3 of protein concentrations with more than two groups were performed using Independent-

4 samples Kruskal-Wallis Test and subsequent pairwise comparisons between groups were

5 Bonferroni-corrected. P-values < 0.05 (two-tailed) were considered statistically significant.

6 A multivariable linear regression model was used to analyze the relationship between any of

7 the three biomarkers levels (independent variable) and platelet count at birth (dependent

8 variable) among immunized pregnancies. Covariates in the model included parity (nulli- or

9 multiparous), GA at time of sampling and anti-HPA-1a antibody level.

10 A logistic regression model was used to assess whether any of the biomarkers were associated

11 with SGA (dependent variable), with one of the three biomarkers as independent variable.

12 Covariates included parity, GA at sampling and immunization status (yes/no) when analyzing

13 the whole population, while parity, GA at sampling and anti-HPA-1a antibody level were

14 adjusted for in the model with immunized pregnancies only. To study the relation between

15 biomarkers and the overall severity of FNAIT, a logistic regression model was used, including

16 the composite adverse neonatal pregnancy outcome as dependent variable, and any of the

17 biomarkers as independent variable. Covariates in the model included parity (nulli- or

18 multiparous), GA at time of sampling and anti-HPA-1a antibody level.

19 None of the participants reported that they smoked during pregnancy, and this co-variate was

20 therefore not included.

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3. RESULTS

Clinical characteristics comparing immunized (treated and untreated) and non-immunized women are shown in **Table 1**. Adjusted median birthweight was significantly lower in neonates from immunized women, but the frequency of SGA did not differ between the groups. All SGA neonates (n=4) in the immunized group and most SGA neonates in the non-immunized group (3/4) were boys.

Clinical characteristics comparing IVIg treated and non-treated immunized women are shown in **Table 2**. Of the 15 IVIg treated pregnancies, 14 mothers had a prior history of FNAIT. Maternal anti-HPA-1a antibody levels were significantly higher in IVIg treated pregnancies compared to non-treated immunized pregnancies. The median adjusted birthweight was significantly lower in neonates from the IVIg treated group compared to immunized untreated pregnancies.

GA at time of sampling was similar between the two groups for 2nd or 3rd trimester samples (p=0.802 and 0.505, respectively). There were no significant differences in plasma concentrations of sFlt-1, PlGF, sEng nor sFlt-1/PlGF ratio when comparing immunized and non-immunized pregnancies (**Table S1**). To visualize the longitudinal changes in biomarker concentrations of sFlt-1, PlGF and sEng from 2nd to 3rd trimester, paired dot-plots are shown in **Figure S1.** The median change in sFlt-1 concentration was twice as high for the immunized group compared to controls (4036 vs 1983 pg/mL, p = 0.067). The three pregnancies with the highest sFlt-1 concentrations and sFlt-1/PlGF ratio in the 3rd trimester (**Figure S1A and S1D**), as well as the lowest PlGF levels in 2nd trimester (**Figure S1B**) among immunized pregnancies were all IVIg treated. There was a significant difference in PlGF concentrations in the 2nd trimester between the three groups (immunized treated, immunized non-treated and non-immunized, Kruskal-Wallis Test, p = 0.034), with IVIg treated women having significantly

lower PlGF levels in 2nd trimester compared to non-immunized controls (p=0.029, **Figure S2B**). All 2nd trimester samples except one were taken before IVIg treatment commenced.

Immunized women with SGA neonates (n=4) had three times as high sFlt-1/PlGF ratio in 3rd trimester compared to non-immunized controls with SGA neonates (n=4, median 76 vs 23, p = 0.3). Among immunized pregnancies, maternal 3rd trimester sFlt-1 was twice as high in pregnancies with SGA neonates (n=4, median 23 493 pg/mL) compared to pregnancies without SGA (n=38, median 11 266 pg/mL, p=0.5). Third trimester sFlt-1/PlGF ratio was also more than twofold in plasma from immunized mothers with SGA neonates compared to immunized non-SGA (median 76 vs 30, p=0.6). However, none of the biomarkers were significantly associated with the odds of having a SGA neonate among immunized pregnancies, nor the whole population (multivariable analysis, data not shown).

Within the immunized group, increasing levels of the sFlt-1 protein in 3rd trimester were significantly associated with lower platelet count at birth, after adjusting for parity, GA at time of sampling and antibody level (multivariable linear regression, p=0.024). Antibody level (p=0.001) and parity (p=0.048) were significant covariates.

FNAIT-associated adverse neonatal pregnancy outcome as a composite variable was identified in 11 (26%) of the HPA-1a immunized pregnancies (**Table S2**), compared to 4 (9%) among controls. All adverse outcomes among controls were SGA. Among immunized pregnancies in 3rd trimester, the sFlt-1 level was twice as high (median 21 427 pg/mL) in pregnancies with adverse outcome compared to immunized pregnancies with no adverse outcomes (median 10 495 pg/mL, p=0.008). Similarly, the median concentration of sEng was twice as high in the adverse outcome group compared to immunized unaffected pregnancies (median 14 ng/mL vs 7 ng/mL, p=0.022). PlGF levels were significantly lower in the adverse group (median 251) compared to unaffected pregnancies (median 506, p=0.039). The sFlt-1/PlGF ratio in 3rd

trimester was significantly higher among immunized pregnancies with adverse outcomes (median 129) compared to unaffected immunized pregnancies (median 24, p=0.005). There were no significant differences in biomarker concentrations in 2nd trimester between the immunized group with and without adverse outcomes. Results from binary logistic regression, assessing the odds of adverse outcome within HPA-1a alloimmunized pregnancies, n=43 are shown in **Table 3**.

Increased sFlt-1 and sFlt-1/PlGF ratio in 3rd trimester were significantly associated with higher odds of a composite adverse neonatal outcome in alloimmunized pregnancies (**Table 3**, multivariable logistic regression, p=0.029 and p=0.019). An sFlt-1/PlGF ratio > 85 at 3rd trimester was significantly associated with adverse composite neonatal outcome in HPA-1a alloimmunized pregnancies (**Table 3,** multivariable logistic regression, p = 0.021).

There were five neonates with ICH among immunized pregnancies (**Table S3**). In 2nd trimester there were no significant difference in median sFlt-1, PlGF, sEng or sFlt-1/PlGF ratio between immunized untreated pregnancies with (n=2) and without neonatal ICH (n=24). In the 3rd trimester, the median sFlt-1 level was 2.6 times higher and the median PlGF level significantly lower in pregnancies with neonatal ICH (n=4) compared to immunized untreated pregnancies without ICH (p = 0.042 and p = 0.019). Pregnancies with neonatal ICH had six times higher median sFlt-1/PlGF ratio compared to immunized untreated non-ICH pregnancies (p=0.007). Furthermore, an sFlt-1/PlGF ratio > 85 was significantly associated with neonatal ICH (p=0.022). The median sEng level was significantly higher in pregnancies with ICH compared to immunized untreated pregnancies with no ICH (p=0.003). The gestational age between immunized untreated pregnancies with and without ICH in the neonate did not differ significantly (2nd trimester, p=0.145 and 3rd trimester, p=0.110).

4. DISCUSSION

This is the first study exploring whether maternal placenta-associated biomarkers in HPA-1a alloimmunized pregnant women associates with neonatal outcome. There were no significant differences in crude plasma concentrations of sFlt-1, PlGF, sEng nor sFlt-1/PlGF ratio when comparing immunized and non-immunized pregnancies. However, we demonstrate that higher sFlt-1 level and sFlt-1/PlGF ratio in 3rd trimester is associated with higher odds of a composite FNAIT-associated adverse neonatal outcome. IVIg treated pregnancies had significantly lower PlGF levels in early 2nd trimester compared to non-immunized controls. Our results suggest that sFlt-1 levels and sFlt-1/PlGF ratio may improve clinical prediction of severe FNAIT outcome.

The major strength of our study is its prospective design with a large cohort of HPA-1a incompatible pregnancies, combined with an appropriate control group. Longitudinal measurements of biomarkers combined with extensive laboratory and clinical data add to the quality, also allowing for multivariable analyses.

The number of HPA-1a alloimmunized pregnancies were only 43, which limits the statistical power of subgroup analyses. For this reason, GA at time of delivery was not included as a covariate but is accounted for in SGA. We lacked data on several maternal risk factors, such as preeclampsia and body mass index which are known to influence placental biomarkers. There were significantly more nulliparous women among non-immunized controls compared to the immunized group. This is anticipated since the chance of a mother becoming alloimmunized increases with each pregnancy, and because some of the immunized women were included due to previous FNAIT pregnancy. Nulliparous women have higher risk of preeclampsia [41], which could be in line with the higher sFlt-1/PlGF values for some of the non-immunized women (**Figure S1**). Differences in biomarker levels and associations with neonatal outcomes

1 between immunized and non-immunized women may therefore have been even larger if parity

2 was more evenly distributed.

3 As expected, the rate of caesarean section was higher in the immunized group, because women

4 with anti-HPA-1a antibodies are often recommended delivery by caesarean section [2], also

5 explaining the lower GA at delivery among immunized pregnancies. Boys were

6 overrepresented in the immunized group compared to controls, a phenomenon which has been

7 observed also in other FNAIT cohorts [11]. The role of birthweight and male fetal sex in HPA-

8 1a alloimmunization was recently discussed in a separate paper from the PREVFNAIT cohort

9 [8]. We did not find indications that levels of placental biomarkers related to HPA-1a

10 alloimmunization was influenced by sex of the fetus, however acknowledging sample size

11 limitations.

12 Due to the discovery of placental involvement in FNAIT pathophysiology, the catchment area

13 of FNAIT is expanding beyond thrombocytopenia and ICH. A composite FNAIT-associated

14 adverse neonatal outcome for this study was therefore generated. This way of assessing FNAIT

15 outcome has not been done before. Although there are still knowledge gaps on how HPA-1a

16 alloimmunization and placenta function relates, the inclusion of a placenta-associated clinical

17 outcome, as represented by SGA, thus seems justifiable in the assessment of FNAIT severity.

18 However, even though each of these severe outcomes are associated with FNAIT, we currently

19 do not know whether they share the same biological pathway.

20 The process of ensuring uteroplacental blood supply to the fetus includes unblocking of spiral

21 artery plugs around week 9-11, which rapidly elevates oxygen tension to the villous tree [23].

22 Premature unplugging due to reduced or dysfunctional mass of extravillous cytotrophoblasts

23 may cause oxidative stress in turn leading to impaired placentation processes [23, 46]. We

24 hypothesize that interference of maternal anti-HPA-1a antibodies with EVCTs may reduce the

mass, causing premature unplugging of the spiral arteries, thus leading to STB stress and excessive release of sFlt-1 into the maternal circulation. A previous FNAIT study demonstrated that plasma from immune pregnant mice had significantly less PLGF and more soluble sFlt-1 compared to control mice [14]. In our study, sub-analysis revealed that the IVIg treated women had significantly lower PlGF levels in early 2nd trimester compared to non-immunized controls. Our finding aligns well with previous studies demonstrating that women with early-onset preeclampsia have lower PlGF levels already from mid first trimester to beginning of 2nd trimester [43, 44]. This decrease in circulating PlGF observed among IVIg treated pregnancies is most likely mediated by excess levels of sFlt-1 binding to PlGF, as pointed out in a recent study [45]. The sFlt-1 ELISA kit is designed to detect unbound sFlt-1 which thus can explain why the IVIg pregnancies had a slightly lower sFlt-1 level in 2nd trimester. The 15 IVIg treated pregnancies represent a sub-cohort of more severe HPA-1a alloimmunization, 14 of them with a known history of previous severe FNAIT. We suggest that the low PlGF level among IVIg treated pregnancies reflect this, in addition to indicating signs of placenta dysfunction, rather than being an effect of the treatment itself. All except one of the 2nd trimester samples were taken before the IVIg treatment commenced. Whether the steep increase in sFlt-1 and sFlt-1/PlGF ratio from 2nd to 3rd trimester among IVIg treated pregnancies (Figure S1A and S1D) could be related to the treatment itself is not known.

Today, prediction of FNAIT severity is limited to prior obstetric history regarding risk of ICH [42], and maternal anti-HPA-1a antibody levels regarding risk of severe neonatal thrombocytopenia [21, 22]. In our study we found 3rd trimester sFlt-1 to be the best maternal placental biomarker to predict composite adverse neonatal outcome. Furthermore, of the pregnancies with an ICH neonate, 3/4 had an sFlt-1/PlGF ratio > 85 in 3rd trimester. Whether 85 is a clinically useful cut-off value in the context of FNAIT prediction remains to be determined in a larger study population. Our results suggest that assessing maternal placental

biomarkers in HPA-1a alloimmunized pregnancies may contribute to identifying the pregnancies at higher risk of FNAIT-associated neonatal complications. In conclusion, we demonstrate a more anti-angiogenic maternal protein profile in HPA-1a alloimmunized mothers with FNAIT-associated adverse neonatal outcome. This novel finding supports the importance of placental dysfunction in HPA-1a alloimmunization pathophysiology, but also suggests that circulating maternal sFlt-1 concentration and/or sFlt-1/PlGF ratio may play a future role in predelivery risk stratification and clinical management decisions for FNAIT.

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AUTHOR CONTRIBUTIONS

EB and AH conceived and designed the PREVFNAIT project. EB, AH, MU, KG, MTA, GB, HT and AO were the members of the Steering Committee of the PREVNAIT project. For the current sub-study, HT, NHN, AH, MTA and MN conceived and designed the study. NHN designed and performed all the ELISA assays, analyzed, and interpreted results and drafted the manuscript. MN planned ELISA assays and revised the manuscript. MTA planned ELISA assays and revised the manuscript. KG, EB and MU recruited, diagnosed and followed all Polish PREVNAIT participants. ELB planned ELISA assays, analyzed results, and revised the manuscript. AH interpreted the results and revised the manuscript. ACS planned ELISA assays, interpreted results and revised the paper. HT analyzed and interpreted results and edited the paper. All authors approved the final version.

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