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The association between first trimester placental biomarkers, third trimester placental MR T2*, and postnatal placental histological findings

Master's thesis

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Abstract

Introduction: Assessing placenta function during pregnancy is challenging. Currently, indirect measures including ultrasound fetal weight and Doppler blood flows are used. Direct measures using placental T2* MR-imaging correlates with placental pathology, however this method is time-consuming. Therefore, the aim of this study is to identify and investigate the association between first trimester biomarkers (Pregnancy-Associated-Plasma-Protein-A (PAPP-A), Human Choriogonadotropin (hCG), Soluble fms-like-tyrosine-kinase-1 (sFlt-1), Placental Growth Factor (PlGF), and ratio between sFlt-1 and PlGF (sFlt-1/PlGF ratio)), third trimester placental T2* and placental histological findings.

Methods: This retrospective cohort study included 184 singleton pregnancies from an existing database based on the presence of first trimester blood tests, third trimester placental T2* and placental histological samples. Z-scores for biomarkers and placental T2* were calculated based on a normal subset. Abnormal placenta histology examination (PHE) associated to placental dysfunction was defined according to the Amsterdam Placental Workshop Consensus Statement. Independent t-test/Mann-Whitney and Pearson's correlation were performed to compare means/medians of the Z-scores between groups, and Receiver Operating Characteristic (ROC) analysis, including corresponding Area Under the Curve (AUC) calculations was used to investigate the predictive performance of parameters and placental histology outcome, respectively.

Results: In abnormal PHE, the average PAPP-A-, sFlt-1- and placental T2* Z-scores were significantly lower compared to normal PHE ($p=0.017$, $p=0.024$, $p<0.001$ respectively). PAPP-A- and sFlt-1 Z-score were mutually associated with placental T2* Z-score ($r=0.14$ and $r=0.28$ respectively). In ROC analysis, the inclusion of PAPP-A- and sFlt-1 Z-scores did not improve the predictive performance of placental T2* Z-score with regard to abnormal placental histology outcome (AUC=0.78, 95% CI -1.13 – -0.56, $p<0.001$ versus combined AUC=0.78, 95% CI -1.15 – -0.51 $p<0.001$).

Discussion: Differences in first trimester placental biomarker concentrations were observed between the abnormal- and normal PHE groups. However, the biomarkers did not improve the performance of third trimester placental T2* Z-score in the prediction of abnormal placental histology outcome.

Keywords: PAPP-A, hCG, sFlt-1, PlGF, sFlt-1/PlGF-ratio, preeclampsia-ratio, birthweight, preeclampsia, placental T2*-weighted MRI, placental histology

1. Introduction

Placental dysfunction affects 10 to 15% [1] of pregnancies worldwide and is associated with several obstetric complications including preeclampsia (PE), low birthweight (BW) and preterm delivery [2]; thereby increasing the risk of perinatal morbidity and mortality [1]. In the first trimester, combining maternal characteristics, and first trimester biophysical- and biochemical markers can be used to estimate the risk of placental dysfunction in later pregnancy [3, 4]. In later pregnancy, indirect assessment of placental dysfunction currently utilizes a combination of ultrasound estimates of fetal weight and Doppler ultrasound of fetal and maternal vessels [1, 4]. Despite this, it has been demonstrated that normal outcomes associated with these assessments do not preclude the risk of placental dysfunction [2, 5]. It is therefore pertinent to obtain a more robust tool for placental assessment in order to enhance perinatal health outcomes.

Placental biomarkers may be used as a tool to assess placental dysfunction. Abnormal levels of first trimester placental biomarkers are associated with the development of placental dysfunction [6]. However, the predictive performance is moderate, possibly explained by the clinical heterogeneity of placental dysfunction. Well-described biomarkers include PAPP-A and hCG which are already utilised in the routine first trimester screening for aneuploidy [7]. sFlt-1 and PlGF are antiangiogenic and proangiogenic factors produced by the placenta, respectively [8, 9]. While sFlt-1 concentrations are increased in pregnancies complicated by preeclampsia, the opposite is observed for PlGF [10]. The ratio between sFlt-1 and PlGF can therefore be used to anticipate the likelihood of preeclampsia [10, 11].

Another tool available in the assessment of placental function, is placental T2*-weighted magnetic resonance imaging (MRI) [12]. Placental dysfunction is associated with placental hypoxia, which can be depicted by placental T2* weighted imaging as it is sensitive to the content of deoxyhemoglobin in tissue [13]. Several studies have demonstrated the feasibility of this method and the correlations to clinical signs of placental dysfunction including low birth weight, preeclampsia and abnormal placental histology [12, 14, 15], however, the cost and availability of MRI scanners may be a limiting factor in most centers. Moreover, MRI exposure in the first trimester of pregnancy also raises safety concerns for the fetus [16].

To the best of our knowledge, the association between first trimester placental biomarkers, placental T2* and abnormal placental histology remains to be described. Therefore, the aim of

this study was to identify and investigate the association between potential first trimester biomarkers namely Pregnancy-Associated-Plasma-Protein-A (PAPP-A), Human Choriogonadotropin (hCG), Soluble fms-like-tyrosine-kinase-1 (sFlt-1), Placental Growth Factor (PlGF), and sFlt-1/PlGF ratio, third trimester placental T2* and placental histological findings.

2. Materials and methods

2.1 Study population and design

A total of 184 singleton pregnancies who had undergone a combination of blood tests at 8-11 weeks' gestation, T2*-weighted placental MRI at 28-37 weeks' gestation, and postnatal placental histological examination (PHE) were retrieved from an existing Placental MRI research database. Blood tests, MRI scans and PHE were performed at Aalborg University Hospital between April 2012 and December 2019. PHE was considered abnormal in the presence of either maternal vascular malformation (MVM), high grade fetal vascular malformation (FVM) or villitis of unknown etiology (VUE), according to definitions stipulated by Khong et al. 2016 [17]. This study included 85 participants with abnormal-, and 99 participants with normal PHE. Group comparisons were performed based on 1) histological subtype groupings and 2) groups based on normal/abnormal PHE combined with/without clinical manifestations of placental dysfunction, including low BW (<22%) [18] and PE, defined as a systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg at least 4h apart in previously normotensive women after 20 weeks of gestation, and proteinuria of > 300 mg. A normal subset of pregnancies were identified in order to calculate Z-scores for the various parameters. This subset was defined as BW ≥ 15% for gestational age (GA), term deliveries (37-42 weeks' gestation) and normal PHE.

This study was approved by the Regional Committee on Biomedical Research Ethics (N20150015, N20090059, N20170052) and registered according to the General Data Protection Regulation in the North Denmark Region (2015-34). All participants provided oral and written informed consent.

2.2 First trimester blood tests

Serum samples were procured as part of the standard prenatal screening for aneuploidy from 8 to 11 weeks' gestation and subsequently stored at -80 degrees Celsius before undergoing biochemical analyses. The Department of Clinical Biochemistry at Aalborg University Hospital conducted the analysis for all samples using an automated immunoassay system (Kryptor Com-

pact, Thermo Fisher Diagnostics). An experienced technician (SLA), blinded to clinical outcomes, retrospectively determined the levels of PAPP-A, hCG, sFlt-1, PlGF, and sFlt/PlGF-ratio.

2.3 Placental histological examination

PHE was conducted by an experienced placental pathologist (ACP) utilizing nomenclature established in the Amsterdam Placental Workshop Consensus Statement [17]. ACP was single blinded for first trimester biomarker- and third trimester MRI results, but not clinical outcomes. Abnormal PHE reported in this study included MVM, high grade FVM, and VUE.

2.4 Placental MRI

MRI of the placenta was conducted in a GE Discovery MR450 1.5 T system (GE Healthcare, Milwaukee, USA). To avoid compression of the vena cava, participants were placed on their left in a supine position [12]. An established placental MRI protocol- not exceeding 30 minutes- was then performed. T2* weighted scans using a gradient recalled echo sequence (repetition time, 70.9 ms; 16 echoes ranging from 3.0 to 67.5 ms in steps of 4.3 ms; field of view, 350 ×350 mm and matrix 256x128) resulted in an in-plan resolution of 1.37 ×2.73 mm [12]. Three eight mm slices of the placenta distributed evenly in the transverse orientation were produced [12]. These three slices were acquired during individual breath-holds of 12s.

2.5 MRI analyses

As described in Sinding et al. 2021 [12], a custom-made program developed and written in MATLAB (The MathWorks Inc., Natick, MA, USA) was used to process MR images. Free-hand region of interests (ROIs) covering the entire placenta were drawn prospectively by a single observer (MS) in the transverse orientation [12]. Absolute T2* values were determined through the fitting of averaged signals within each ROI against echo time using a mono-exponential decay function, which employed M0 and T2* as free parameters, and a non-linear least-squares fitting algorithm [12]. The mean T2* value of each placenta was calculated by averaging fitted T2* measurements of the three transverse placenta slices.

2.6 Statistical analyses

All statistical analyses were performed using Stata version 17.0 (StataCorp LP, College station, TX). Standardized PAPP-A-, hCG-, PlGF-, sFlt-1, sFlt-1/PlGF-ratio and placental T2* values (Z-scores) were calculated using a linear regression model to adjust for GA based on the normal subset of pregnancies, as previously defined (n=62). Normally distributed continuous data was presented as mean (± SD), and groups were compared by independent t-test. Similarly, non-

normally distributed continuous data were presented as median (interquartile range), and groups were compared by Mann-Whitney U test. Categorical variables were represented as n (%) using Chi square test for group comparisons. Associations between selected variables were investigated using pearson's correlation at a significance level of 0.05. Receiver Operating Characteristic (ROC) curves based on logistic regressions to predict abnormal PHE were constructed to compare the performance of selected parameters, with corresponding Area Under the Curve (AUC) calculations. Additionally, one-way ANOVA was performed to compare mean Z-scores among 1) histology subtype groups: normal PHE, MVM, high grade FVM and VUE and 2) groups based on abnormal PHE and clinical manifestations of placental dysfunction: Group 1 (normal PHE without clinical manifestations), group 2 (normal PHE with clinical manifestations), group 3 (abnormal PHE without clinical manifestations), and group 4 (abnormal PHE with clinical manifestations). P-values <0.05 were considered statistically significant.

3. Results

3.1 Demography and clinical characteristics

Between the abnormal- and normal PHE-group, no significant differences in maternal age, maternal ethnicity, body mass index (BMI), parity, cigarette smoking, diabetes, gestational age at MRI and gestational age at first trimester screening were observed (Table 1).

3.2 Association between placental T2, biomarkers, and gestational age*

In the subset of normal pregnancies (n=62), a significant linear relationship was observed between GA (weeks), and the absolute values for the following parameters: PAPP-A (R^2= 0.57 and p<0.001), hCG (R^2=0.12 and p=0.005), PlGF (R^2=0.50 and p<0.001), sFlt-1/PlGF-ratio (R^2=0.16 and p=0.005) and placental T2* (R^2=0.844 and p<0.001). Although linear, the relationship between GA (weeks) and sFlt-1 was not significant (R^2=0.03, p=0.240) (Figure 1).

3.3 Abnormal PHE versus normal PHE

PAPP-A-, sFlt-1- and placental T2* Z-scores were significantly lower in the abnormal PHE group compared to the normal PHE group (median (IQR)); -0.43 (-0.87 – 0.14) versus -0.14 (-0.52 – 0.38), p=0.017 and -0.45 (-0.90 – 0.03) versus -0.21 (-0.58 – 0.16), p=0.024 and -1.65 (-2.76 – -0.79) versus -0.04 (-0.99 – 0.40), p<0.001, respectively. Although median sFlt-1/PlGF-ratio Z-score was also lower in the abnormal PHE group compared to the normal PHE group, this finding was only near-significant; -0.46 (-0.92 – -0.10) versus -0.27 (-0.73 – 0.10), p=0.070 (Table 2, Figure 2).

In the abnormal PHE group, percentage of reported preeclampsia was significantly higher (p=0.004), and median BW Z-score was significantly lower (p<0.001) compared to the normal PHE group (Table 2).

Positive correlations were identified between PAPP-A Z-score and placental T2* Z-score (r=0.14, p=0.048) and between sFlt-1 Z-score and placental T2* Z-score (r=0.28, p<0.001).

Two ROC models were constructed to predict abnormal PHE; the first model utilised only placental T2* whereas the second model utilized all parameters for which there was observed a significant difference in the median between abnormal- and normal PHE groups (PAPP-A-, sFlt-1-, and placental T2*- Z-scores). Upon comparing AUC for each model, it was found that the inclusion of PAPP-A- and sFlt-1- Z-scores did not improve the predictive performance of placental T2*-Z-score (AUC=0.78, 95% CI -1.13 – -0.56, p<0.001 versus combined AUC=0.78, 95% CI -1.15 – -0.51 p<0.001).

3.4 Placenta histological subtypes (normal PHE, MVM, FVM and VUE)

Placental T2* was the only parameter that showed a significant difference in mean Z-scores (MD) across placenta histological subtypes (Figure 3). This was seen between high grade FVM and normal PHE (MD=-1.50, p<0.001), and between MVM and normal PHE (MD=-1.65, p<0.001). No significant differences in mean Z-scores were observed between placenta histological subtypes with regards to first trimester biomarkers. Despite this, the following trends were observed; Mean Z-score for PAPP-A, hCG, sFlt-1 and sFlt-1/PlGF-ratio were consistently the lowest in the VUE group. Mean PlGF Z-score was lowest in the FVM (high grade) group while mean placental T2* Z-score was lowest in the MVM group. Conversely, mean Z-scores were observed to be highest in the normal PHE group, for all parameters notwithstanding hCG.

3.5 Outcomes of PHE and clinical manifestations of placental dysfunction

Placental T2* was the only parameter that showed a significant difference in mean Z-scores across groups (Figure 4). This was seen between the following groups: 4 versus 1 (MD=-2.53, p<0.001), 3 versus 1 (MD=-0.98, p=0.001), and 4 versus 3 (MD=-1.55, p<0.001). Additionally, near significant differences in mean placental T2*- and mean sFlt-1/PlGF-ratio Z-scores were observed between groups 2 and 1 (MD=-1.33, p=0.063), and groups 4 and 1 (MD=-0.62, p=0.089) respectively. Regarding first trimester biomarkers, no significant differences in mean Z-scores were observed between groups.

4. Discussion

This study demonstrated reduced first trimester placental biomarker concentrations (PAPP-A, sFlt-1) and third trimester placental T2* in pregnancies with abnormal PHE. However, the predictive performance of PAPP-A and sFlt-1 in regard to abnormal PHE did not improve the predictive performance of third trimester placental T2*.

4.1 Methodological considerations

Strengths of this study were the well-described study population selected from an existing Placental MRI research database. Moreover, the PHE examinations were performed by a single experienced pathologist (ACP) strictly adhering to the criteria put forth in the Amsterdam placental workshop group consensus statement [17], and blinded to serum biomarker- and placental T2* outcomes. However, the pathologist was not blinded for clinical outcomes such as BW and PE. Despite the introduction and implementation of the Amsterdam placental workshop group consensus statement by Khong et al. in 2016 [17], a study by N.J. Sebire in 2017 [19] has illustrated a three-fold over reporting of placental lesions in cases of preeclampsia (PE) where pathologists were not blinded to clinical information, compared to reporting blinded for outcome. Another limitation of this study is the potential source of bias in the form of motion-related artifacts (fetal movement and maternal respiration, and involuntary uterine contractions) relating to MR imaging [14]. Despite this, it has been repeatedly demonstrated that placental T2* has a strong reproducibility [15]. Finally, group comparisons pertaining to placental histological subtypes, and outcomes of PHE including clinical manifestations of placental dysfunction, involved cohorts with smaller sample sizes. This could potentially result in reduced statistical power and an increased risk of type I errors [20].

4.2 First trimester biomarkers and third trimester placental T2 in normal pregnancies*

This study demonstrated significant correlations between GA, first trimester biomarkers and third trimester placental T2* in a subset of normal pregnancies. PAPP-A, sFlt-1 and PlGF increased with GA, while hCG, sFlt-1/PlGF-ratio and placental T2* decreased with the progression of GA. These findings are mostly in line with previous studies conducted on normal pregnancies [8, 10, 21–24]. It should be noted that while we do not observe the initial exponential increase in hCG levels at the start of pregnancy (due to blood sample collection starting around GA 8), this study observed the subsequent fall in hCG levels that take place late in the first trimester [24].

Similarly, while it is documented that both sFlt-1 and PlGF increase with the progression of GA in the first trimester [25], the variation in sFlt-1/PlGF-ratio in the first trimester of normal pregnancies remains to be described, due to focus on its clinical application for diagnosing PE in later trimesters. Thus we are unable to verify the accuracy of our finding in regard to sFlt-1/PlGF-ratio. To compensate for the variation in GA at which serum biomarkers and placental T2* were obtained, absolute values were converted into Z-scores. Previous studies have frequently adjusted for maternal factors, encompassing, but not limited to: smoking status, BMI and parity [8, 26]. However, due to the small size of our normal subset group (n=62), this was deemed counter intuitive.

4.3 Abnormal PHE versus normal PHE

In this study, only significant differences between groups were observed for PAPP-A, sFlt-1 and placental T2*, whereas there was a near-significant reduction in sFlt/PlGF-ratio between groups.

Regarding PAPP-A, several studies have investigated the association between low first trimester PAPP-A and obstetric complications such as low BW and PE [27]. However, the association between first trimester PAPP-A and isolated abnormal PHE has only been sparsely described. In line with our results, Odibo A et al. [28] has demonstrated reduced surface area and volume of the placental terminal villi in pregnancies with reduced first trimester PAPP-A.

Regarding sFlt-1, our study demonstrated a significantly lower sFlt-1 Z-score in the abnormal PHE group. This finding was surprising as previous literature suggests that the concentration of sFlt-1 is increased in pregnancies complicated by placental dysfunction [29], as sFlt-1 is an anti-angiogenic factor [29]. In a study by Triunfo et al. [30], it was also demonstrated that first trimester sFlt-1 mom values were increased in placentas with signs of placental underperfusion. Likewise, a study by Ogge et al. [31], demonstrated higher third trimester sFlt-1 levels in pregnancies with abnormal placentas. However, in line with our study, Schiffer et al. [6], demonstrated reduced first trimester sFlt-1 levels in pregnancies complicated by MVM. A possible explanation for this discrepancy in sFlt-1 levels could be the different trimesters at which samples were collected. As highlighted by Stepan et al. [32], sFlt-1 concentration increases regularly during the third trimester but is prematurely elevated in pregnancies that later develop PE, typically 5 weeks before the onset of symptoms. It is thus not implausible that this study found reduced levels of sFlt-1 in the first trimester.

Regarding placental T2* values, the reduction observed in pregnancies with abnormal PHE is in line with a previous study by Sinding et al. [12], where pathological findings were observed to be closely related to placental T2* values [12]. The reduced placental T2* value may be explained by both placental hypoxia and abnormal tissue morphology associated to placental dysfunction including infarction and fibrosis, which may reduce the intrinsic T2 relaxation time, resulting in a lower placental T2* value [12, 33].

Triunfo et al. [34], reported an increased first trimester sFlt-1/PlGF-ratio in pregnancies with histological signs of placental underperfusion. However, in line with our study, Schiffer et al. [6], recently reported a trend towards reduced sFlt-1/PlGF-ratio in pregnancies complicated by MVM and clinical manifestation of placental dysfunction.

This study could not demonstrate any difference in first trimester hCG or PlGF between the abnormal- and normal PHE groups. This is in line with a study by De Moreulia et al. [35], who demonstrated no association between first trimester PlGF and placental lesions in preeclamptic pregnancies. Conversely, previous studies by Schiffer et al. [6] and Fillion et al. [36] demonstrated a trend of lower first trimester PlGF in pregnancies with MVM compared to their counterparts, and that third trimester PlGF is a strong predictor of MVM, respectively.

In the ROC analysis, the inclusion of PAPP-A and sFlt-1 did not result in an improvement of placental T2*'s AUC; in fact, it actually resulted in a wider confidence interval. From this we can infer that there was no significant improvement in the model's overall predictive performance for histological outcome following the additional inclusion of PAPP-A and sFlt-1. This implies that, despite a positive correlation between PAPP-A and placental T2*, PAPP-A cannot be mutually employed as a substitute for MRI screening, as initially suggested.

4.4. Placenta histological subtypes (normal PHE, MVM, FVM and VUE)

In the group comparison based on histological subtypes, there was a significant difference in mean placental T2* between the MVM group, and the FVM (high grade) group, compared to the normal PHE group respectively. This trend was not observed between the VUE group and the normal PHE group. To the best of our knowledge, no studies have investigated placental T2* and histological placental subtypes. As described above, reduced placental T2* values may be explained by both placental hypoxia and morphological changes [37], however the specific contribution of different placental morphological changes associated with MVM and FVM cannot be elucidated from this study.

No association between first trimester serum biomarkers and histological subtypes could be demonstrated. This finding is in line with a study by Moreuil C. et al. [35], where no apparent causation between serum biomarkers and placental histological subtypes were found.

4.5 Outcomes of PHE and clinical manifestations of placental dysfunction

In the group comparison based on PHE and clinical manifestations, only significant differences between groups were demonstrated regarding the placental T2* value. In pregnancies of abnormal PHE with clinical manifestations, the placental T2* value was significantly reduced compared to the other groups. This could be explained by the severity of these pregnancies, and is in line with previous studies demonstrating reduced placental T2* values in pregnancies complicated by low BW [14], preeclampsia [38], and abnormal placental histology [37]. Surprisingly, it was noted that pregnancies of normal PHE with clinical manifestations showed a trend towards a lower mean placental T2* value compared to pregnancies with abnormal PHE without clinical manifestations; implying that the development of clinical manifestations is not only determined by placental hypoxia and abnormal histology, but on factors that are as of yet, not illuminated.

Regarding sFlt-1, PlGF, and their ratio, the lack of difference between groups was surprising and in contrast to previously published studies [32]. In a study by Fillion et al. [36], it was demonstrated that third trimester PlGF is a better predictor of PE associated with MVM than a predictor of PE without MVM, hypothesizing that PlGF is a stronger marker of MVM than PE. This contrasts with the findings of this study, where no differences in PlGF across groups could be demonstrated. Unlike Fillion et al. [36], that explored third trimester PlGF concentrations, our study explored first trimester PlGF concentrations, which may explain the discrepancy in findings between studies. In a study utilizing first trimester serum biomarkers, Triunfo et al. [34] also demonstrated the association between low PlGF levels and obstetric complications later in pregnancy. Additionally, elevated sFlt-1 levels were observed in cases of late onset PE that exhibited histological findings compatible with placental malperfusion [34]. The aforementioned elevation in sFlt-1 levels seen in the previous study were already present at the time of sample collection (first trimester) [34]. Our study does not corroborate this finding.

4.6 Further investigation

As this study only focused on first trimester serum biomarkers, it would be intriguing in future studies to investigate the predictive performance of third trimester biomarkers regarding abnormal histological outcome; as this might mitigate the conflicting results observed across studies in this field. Exploring the integration of placental T2* imaging with concurrent serum biomarkers collected at the same GA may further elucidate correlations that could ultimately eliminate the need for MRI in certain scenarios. Further research in this direction is crucial for unlocking new insights that can enhance both scientific knowledge and clinical practices in obstetrics and maternal-fetal medicine.

5. Conclusion

In conclusion, this study demonstrated reduced first trimester placental biomarker concentrations (PAPP-A, sFlt-1) and third trimester placental T2* in pregnancies with abnormal PHE. However, the predictive performance of PAPP-A and sFlt-1 regarding abnormal placental histology outcome was poorer than that of placental T2*, and did not contribute to an improvement in placental T2*'s ROC model.

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Figure 1

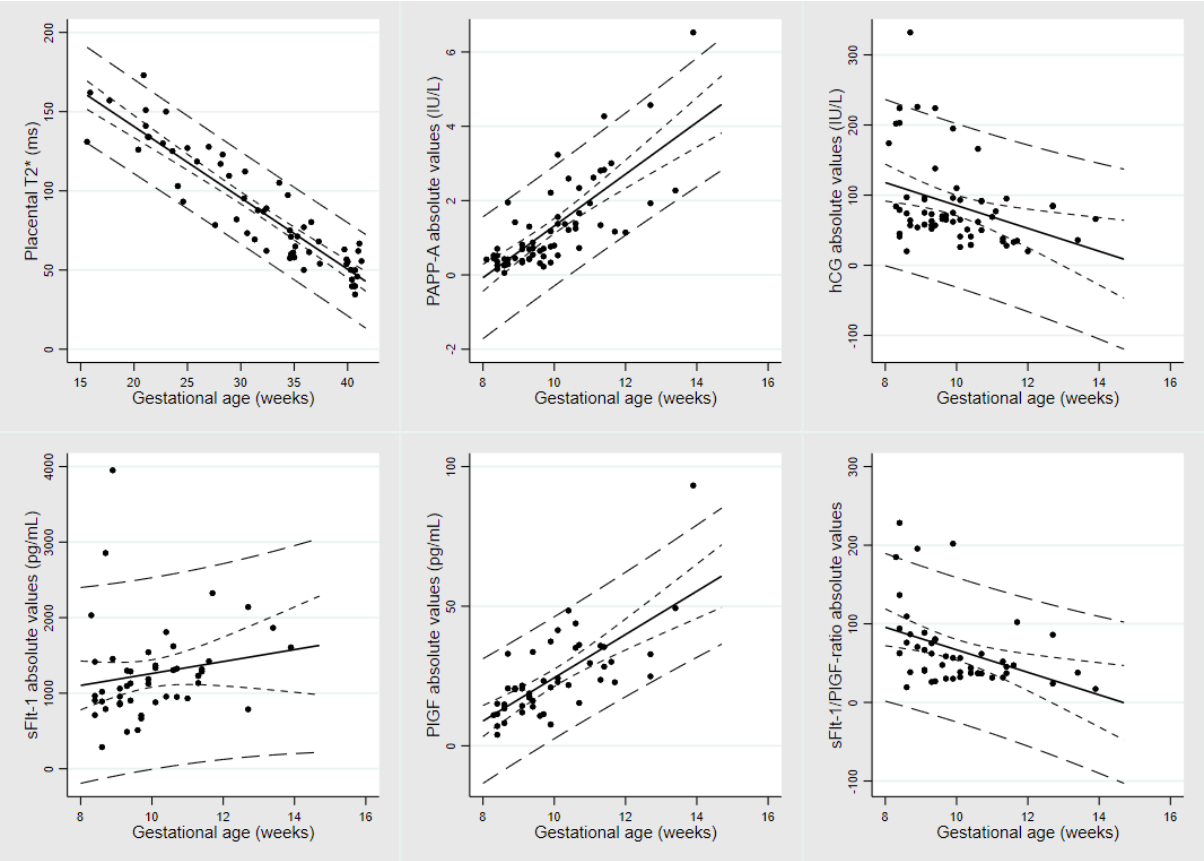

Figure 1. The linear relation between PAPP-A, hCG, sFlt-1, PlGF, sFlt-1/PlGF-ratio, placental T2*, and gestational age (weeks). The solid line represents ordinary least squares fit. Shorter dash lines indicate the 95% confidence interval while longer dash lines indicate the 95% prediction interval.

Table 1: Maternal and pregnancy characteristics

Characteristics	Normal PHE (n=99)	Abnormal PHE (n=85)	p-value
Maternal age (years)	29.727 (±4.447)	29.706 (±4.988)	0.976
Maternal ethnicity (%)			
Caucasian	93 (93.94)	80 (94.12)	
African-american			
African	1 (1.01)		
Asian	1 (1.01)	1 (1.18)	0.530
Greenlandic		2 (2.35)	
Oriental	3 (3.03)	2 (2.35)	
Mixed	1 (1.01)		
BMI (kg/m2)	26.029 (±4.452)	26.636 (±5.540)	0.411
Parity (%)			
Nulliparous	47 (47.47)	47 (55.29)	0.290
Cigarette smoker (%)			
Current smoker	12 (12.12)	15 (17.65)	0.291
Diabetes (%)			
GDM, non-insulin-demanding	5 (5.10)	6 (7.06)	0.579
Gestational age at MRI (weeks)	33.6 (28.3 – 36.9)	33 (28.7 – 36.3)	0.730
Gestational age at double test (weeks)	9.6 (8.9 – 10.6)	9.4 (8.9 – 10.1)	0.472

Table 1. Maternal and pregnancy characteristics for normal- and abnormal placenta histology examination (PHE) groups, with corresponding probability value (p-value) where n= number of observations, BMI refers to body mass index, and GDM refers to gestational diabetes mellitus. Normally distributed continuous data presented as mean (± SD), non-normally distributed continuous data presented as median (interquartile range) and categorical variables represented as n (%).

Table 2: Obstetrical outcomes

Characteristics	Normal PHE (n=99)	Abnormal PHE (n=85)	p-value
PAPP-A conc. (z-score)	-0.138 (-0.521 – 0.383)	-0.433 (-0.869 – 0.144)	0.017
hCG conc. (z-score)	-0.402 (-0.652 – 0.293)	-0.335 (-0.694 – 0.295)	0.722
PlGF conc. (z-score)	-0.018 (-0.526 – 0.585)	-0.039 (-0.408 – 0.632)	0.929
sFlt-1 conc. (z-score)	-0.206 (-0.579 – 0.156)	-0.450 (-0.901 – 0.031)	0.024
sFlt-1/PlGF (z-score)	-0.274 (-0.729 – 0.102)	-0.458 (-0.917 – -0.100)	0.070
Placental T2* (z-score)	-0.035 (-0.986 – 0.404)	-1.651 (-2.759 – -0.786)	0.000
Preeclampsia (%)	1 (1.01)	9 (10.59)	0.004
Gestational age at birth (weeks)	40.1 (38.6 – 41.1)	38.4 (35.6 – 40.1)	0.000
Birth weight (z-score)	-0.800 (-1.58 – -0.01)	-1.698 (-2.33 – -1.14)	0.000
Placenta histology (%) *MVM* *FVM (high grade)* *VUE*		 58 (68.24) 19 (22.35) 8 (9.41)	

Table 2. Obstetrical outcomes for normal- and abnormal placenta histology examination (PHE) with corresponding probability values (p-value) shown in red where statistically significant. n= number of observations, MVM refers to maternal vascular malformation, FVM (high grade) refers to fetal vascular malformation (high grade) and VUE refers to villitis of unknown etiology. Normally distributed continuous data presented as mean (± SD), non-normally distributed continuous data presented as median (interquartile range) and categorical variables represented as n (%).

Figure 2

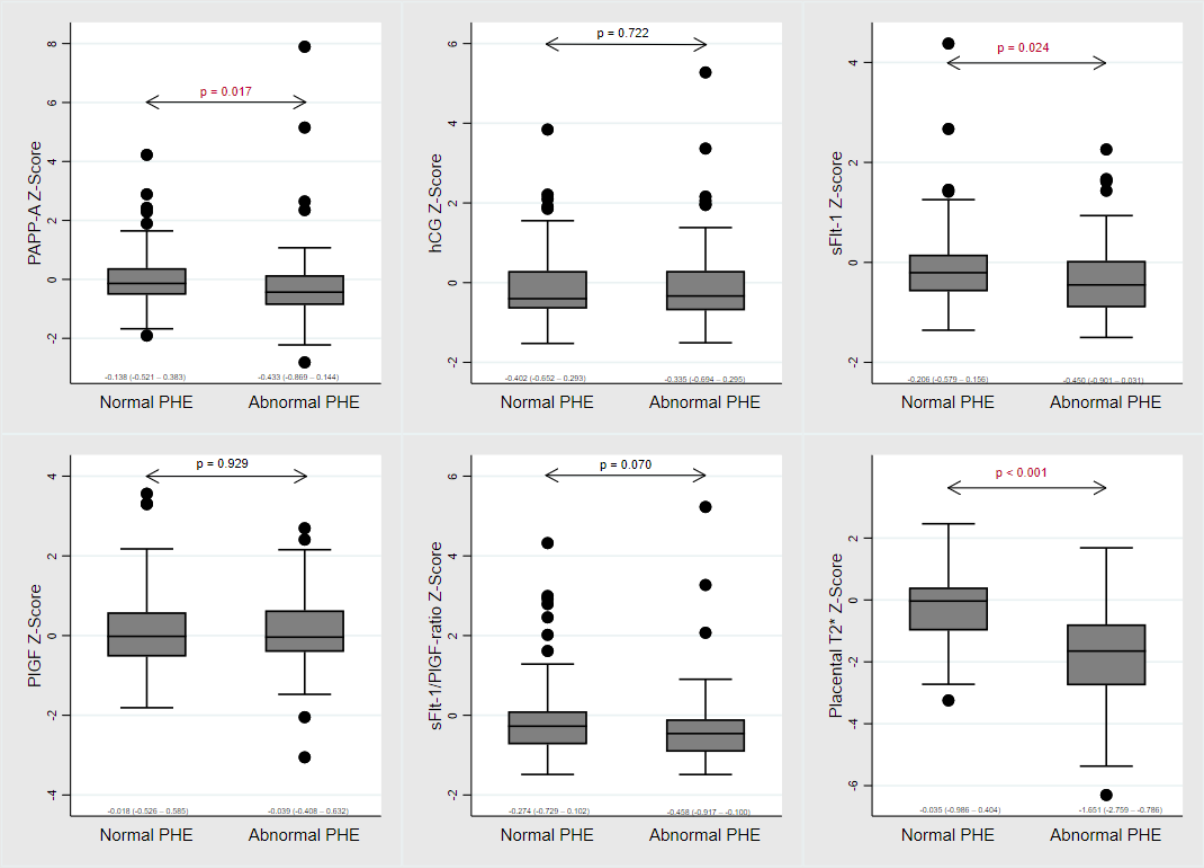

Figure 2. Box plots comparing medians in PAPP-A-, hCG-, sFlt-1-, PlGF-, sFlt-1/PlGF-ratio-, and placental T2* Z-scores between the normal PHE group and the abnormal PHE group. The respective median and interquartile range for each group is depicted below each box. Significant differences in medians between these groups for PAPP-A-, sFlt-1- and placental T2* Z-scores were observed and are shown in red. A corresponding near-significant difference in sFlt-1/PlGF-ratio Z-scores was also noted.

Figure 3

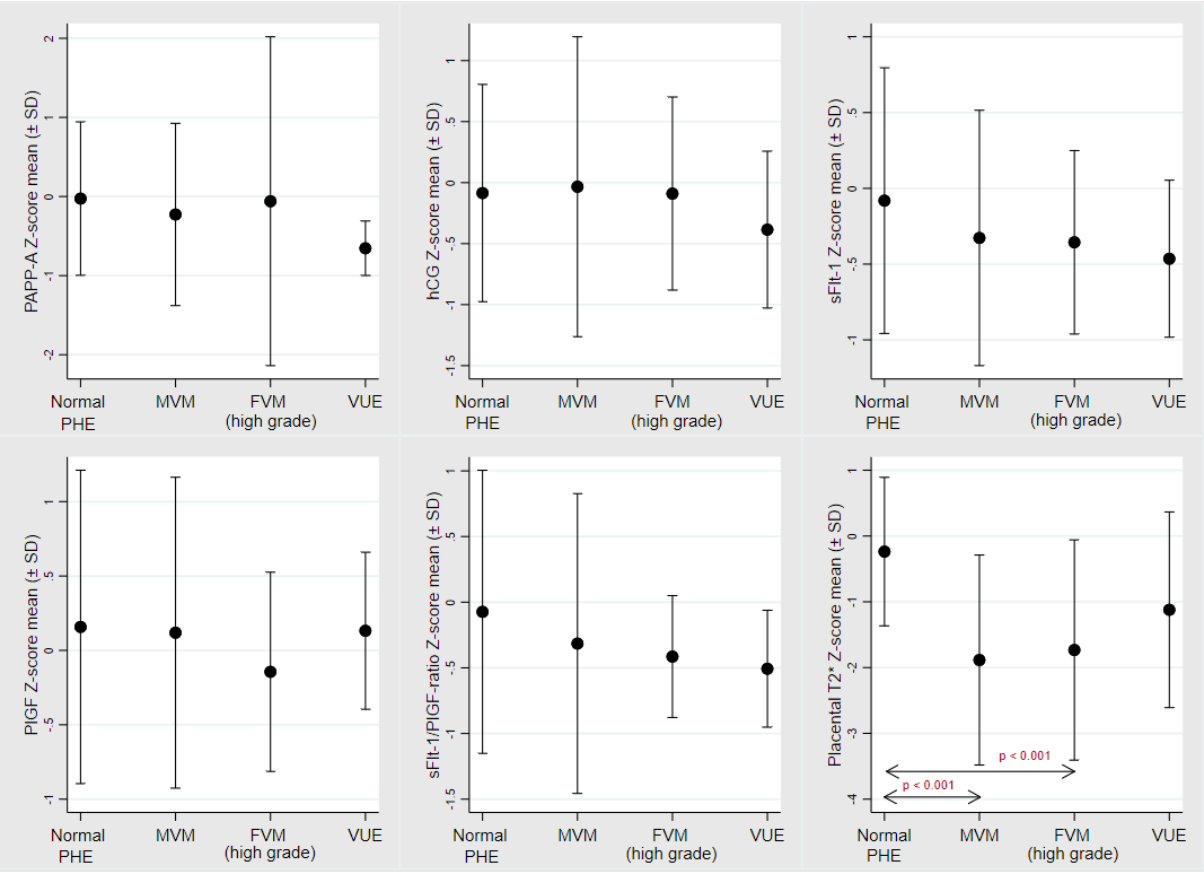

Figure 3. Error bars comparing mean and standard deviation of PAPP-A-, hCG-, sFlt-1-, PlGF-, sFlt-1/PlGF-ratio-, placental T2* Z-scores for each of the following placenta histology subtypes: normal PHE, maternal vascular malformation (MVM), high grade fetal vascular malformation (FVM) and villitis of unknown etiology (VUE). Differences in mean Z-scores were tested across the individual groups for each of the various parameters. Significant differences between groups marked by double-headed arrows.

Table 3: One way ANOVA results for PHE, MVM, FVM (high grade) and VUE

Parameters (z-scores)	Groups	Mean (± SD)	Variance between groups	Difference in means between groups, MD
*T2**	*Normal histology*	-0.236 (± 1.129)	F= 19.79 (0.00)	
	MVM	-1.885 (± 1.596)		2-1: -1.648 (0.000)
	FVM (high grade)	-1.733 (± 1.674)		3-1: -1.496 (0.000) 3-2: 0.152 (1.000)
	VUE	-1.121 (± 1.487)		4-1: -0.885 (0.486) 4-2: 0.764 (0.848) 4-3: 0.612 (1.000)
PAPP-A	*Normal histology*	-0.026 (± 0.970)	F= 0.94 (0.425)	
	MVM	-0.227 (± 1.152)		2-1: -0.201 (1.000)
	FVM (high grade)	-0.059 (± 2.078)		3-1: -0.034 (1.000) 3-2: 0.168 (1.000)
	VUE	-0.654 (± 0.344)		4-1: -0.628 (0.885) 4-2: -0.427 (1.000) 4-3: -0.595 (1.000)
hCG	*Normal histology*	-0.086 (± 0.890)	F= 0.29 (0.830)	
	MVM	-0.034 (± 1.229)		2-1: 0.518 (1.000)
	FVM (high grade)	-0.090 (± 0.791)		3-1: -0.004 (1.000) 3-2: -0.056 (1.000)
	VUE	-0.385 (± 0.643)		4-1: -0.300 (1.000) 4-2: -0.352 (1.000) 4-3: -0.296 (1.000)
sFlt-1	*Normal histology*	-0.081 (± 0.876)	F= 1.41 (0.243)	
	MVM	-0.327 (± 0.843)		2-1: -0.246 (0.558)
	FVM (high grade)	-0.356 (± 0.605)		3-1: -0.275 (1.000) 3-2: -0.029 (1.000)
	VUE	-0.464 (± 0.518)		4-1: -0.383 (1.000) 4-2: -0.137 (1.000) 4-3: -0.108 (1.000)
PlGF	*Normal histology*	0.158 (± 1.053)	F= 0.38 (0.765)	
	MVM	0.119 (± 1.045)		2-1: -0.039 (1.000)
	FVM (high grade)	-0.144 (± 0.670)		3-1: -0.302 (1.000) 3-2: -0.263 (1.000)
	VUE	0.132 (± 0.528)		4-1: -0.026 (1.000) 4-2: 0.013 (1.000) 4-3: 0.276 (1.000)
sFlt-1/ PlGF- ratio	*Normal histology*	-0.073 (± 1.078)	F= 1.05 (0.372)	
	MVM	-0.315 (± 1.142)		2-1: -0.252 (1.000)
	FVM (high grade)	-0.414 (± 0.464)		3-1: -0.341 (1.000) 3-2: -0.099 (1.000)
	VUE	-0.507 (± 0.445)		4-1: -0.433 (1.000) 4-2: -0.182 (1.000) 4-3: -0.093 (1.000)

Table 3. One way ANOVA results ((mean ± SD), variance between groups with corresponding probability value (p-value), and differences in means between groups, ‘’MD’’ with corresponding probability value (p-value) for normal placenta histology examination (PHE)-, maternal vascular malformation (MVM)-, fetal vascular malformation (FVM)-, and villitis of unknown etiology (VUE)- groups with regards to named parameters. Significant differences in means between groups shown in red.

Figure 4

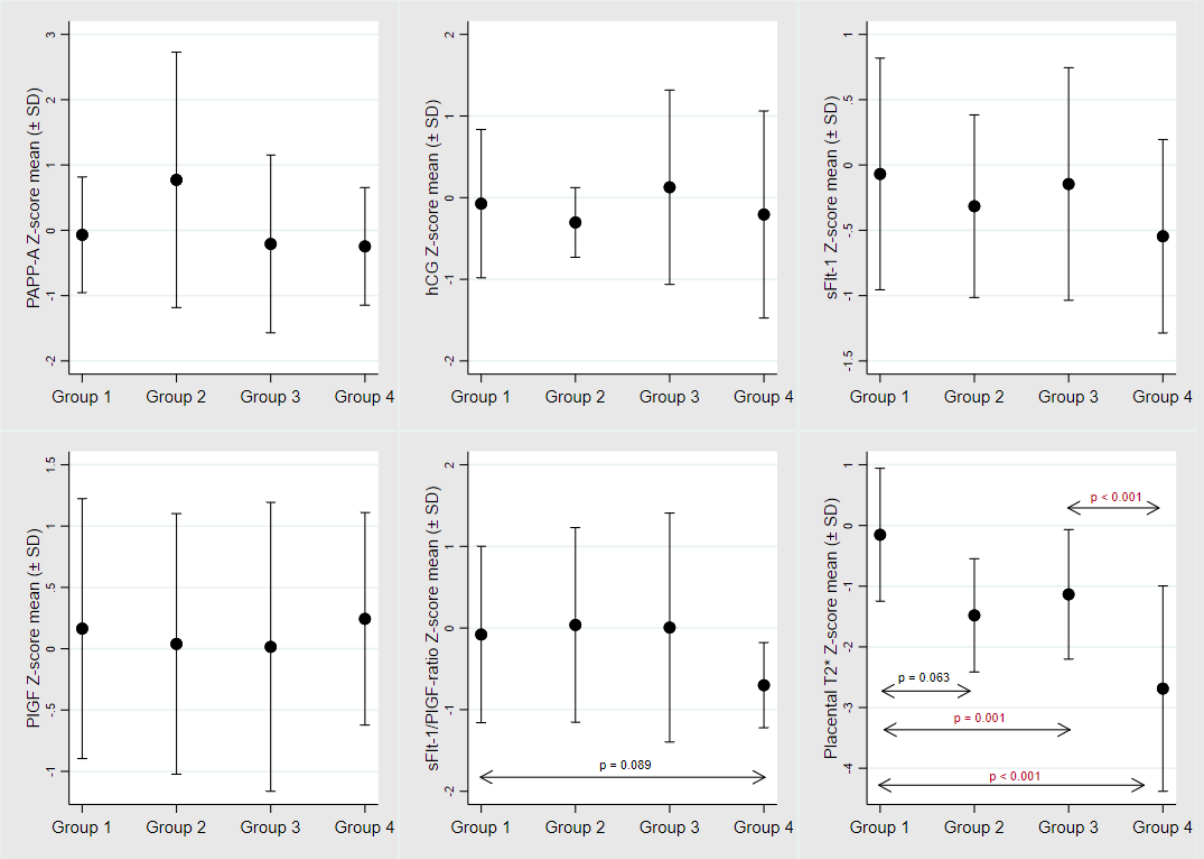

Figure 4. Error bars comparing mean and standard deviation of PAPP-A-, hCG-, sFlt-1-, PlGF-, sFlt-1/PlGF-ratio-, and placental T2* Z-scores between groups. Group 1: normal PHE without clinical manifestations, group 2: normal PHE with clinical manifestations, group 3: abnormal PHE without clinical manifestations, and group 4: abnormal PHE with clinical manifestations. Differences in mean Z-scores were tested across the individual groups for each of the various parameters. Significant differences between groups marked in red by double-headed arrows, trends marked similarly in black.

Table 4: One way ANOVA results for outcome of PHE and clinical manifestations of placental dysfunction

Parameters (z-scores)	Groups	Mean (± SD)	Variance between groups	Difference in means between groups
*T2**	*Normal histology % CS*	-0.153 (± 1.10)	F=32.30 (0.000)	
	Normal histology + CS	-1.481 (± 0.933)		2-1: -1.328 (0.063)
	Abnormal histology % CS	-1.135 (± 1.067)		3-1: -0.982 (0.001) 3-2: 0.346 (1.000)
	Abnormal histology + CS	-2.688 (± 1.692)		4-1: -2.534 (0.000) 4-2: -1.207 (0.173) 4-3: -1.553 (0.000)
PAPP-A	*Normal histology % CS*	-0.070 (± 0.886)	F= 1.50 (0.217)	
	Normal histology + CS	0.772 (± 1.956)		2-1: 0.842 (0.476)
	Abnormal histology % CS	-0.209 (± 1.361)		3-1: -0.139 (1.000) 3-2: -0.981 (0.313)
	Abnormal histology + CS	-0.246 (± 0.901)		4-1: -0.177 (1.000) 4-2: -1.019 (0.270) 4-3: -0.037 (1.000)
hCG	*Normal histology % CS*	-0.074 (± 0.909)	F= 0.61 (0.607)	
	Normal histology + CS	-0.304 (± 0.427)		2-1: -0.230 (1.000)
	Abnormal histology % CS	0.127 (± 1.190)		3-1: 0.201 (1.000) 3-2: 0.431 (1.000)
	Abnormal histology + CS	-0.206 (± 1.268)		4-1: -0.133 (1.000) 4-2: 0.097 (1.000) 4-3: -0.334 (1.000)
sFlt-1	*Normal histology % CS*	-0.069 (± 0.887)	F= 1.98 (0.120)	
	Normal histology + CS	-0.316 (± 0.699)		2-1: -0.247 (1.000)
	Abnormal histology % CS	-0.145 (± 0.890)		3-1: -0.077 (1.000) 3-2: 0.170 (1.000)
	Abnormal histology + CS	-0.546 (± 0.741)		4-1: -0.477 (0.105) 4-2: -0.230 (1.000) 4-3: -0.400 (0.525)
PlGF	*Normal histology % CS*	0.164 (± 1.060)	F= 0.25 (0.865)	
	Normal histology + CS	0.039 (± 1.062)		2-1: -0.125 (1.000)
	Abnormal histology % CS	0.015 (± 1.178)		3-1: -0.149 (1.000) 3-2: -0.024 (1.000)
	Abnormal histology + CS	0.244 (± 0.866)		4-1: 0.080 (1.000) 4-2: 0.204 (1.000) 4-3: 0.228 (1.000)
sFlt-1/ PlGF ratio	*Normal histology % CS*	-0.079 (± 1.080)	F= 2.46 (0.066)	
	Normal histology + CS	0.038 (± 1.192)		2-1: 0.117 (1.000)
	Abnormal histology % CS	0.006 (±1.403)		3-1: 0.085 (1.000) 3-2: -0.032 (1.000)
	Abnormal histology + CS	-0.700 (± 0.522)		4-1: -0.621 (0.089) 4-2: -0.738 (1.000) 4-3: -0.706 (0.108)

Table 4. One way ANOVA results ((mean ± SD), variance between groups with corresponding probability value (p-value), and differences in means between groups, ‘’MD’’ with corresponding probability value (p-value) for groups 1-4 with regards to named parameters. Group 1: normal PHE without clinical manifestations, group 2: normal PHE with clinical manifestations, group 3: abnormal PHE without clinical manifestations, and group 4: abnormal PHE with clinical manifestations. Significant differences in means between groups shown in red.