Multiplex analysis of circulating maternal cardiovascular biomarkers comparing preeclampsia subtypes

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Short title

CVD risk biomarkers phenotyping in preeclampsia

Table 1, Figures 5, Supplemental file 1

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Abstract

Preeclampsia, a hypertensive pregnancy disorder, links to increased long-term maternal cardiovascular disease (CVD). The risk is further increased with early-onset preeclampsia (EPE) and delivery of a growth restricted child. We hypothesized that circulating biomarkers associated with CVD risk differed between preeclampsia subtypes and controls. We compared EPE; n=37, delivery <week 34, late-onset preeclampsia (LPE); n=29, delivery ≥week 34, and normotensive controls (NC, n=49) using Olink Proseek multiplex CVD I assay (targeting 92 biomarkers). We stratified analysis to uteroplacental spiral artery acute atherosis presence in preeclampsia patients, sharing morphological similarities with atherosclerosis. We found 47 CVD-related biomarkers differing between the groups; 42 markers between NC and EPE, 28 markers between NC and LPE, and 9 markers between EPE and LPE. Among these 9 markers, ST2 protein, matrix metalloproteinase (MMP) 1, MMP3 and fractalkine (CX3CL1), were uniquely dysregulated in EPE. Principal component (PC) analysis of the differing markers identified 4 clusters (named PC1-PC4), that largely separated the preeclampsia and control groups as well as pregnancies with low and high circulating placental growth factor (PlGF). The combination of the single markers PlGF, ST2, MMP1, MMP3 and CX3CL1 had a high discriminatory property to differentiate between EPE and LPE. Preeclampsia with acute atherosis or with fetal growth restriction could be differentiated by Olink biomarkers as compared to preeclampsia without these features. We identified specific CVD-related biomarkers in pregnancy depending on preeclampsia subtypes and uteroplacental acute atherosis. Assessment of these pregnancy measured biomarkers' relation to long-term cardiovascular dysfunction and hard endpoints is warranted.

Keywords

Cardiovascular disease, preeclampsia, circulating biomarkers, multiplex

Introduction

Preeclampsia (PE) is a severe pregnancy complication, defined by new-onset hypertension and another sign of organ dysfunction after gestational week 20 (e.g. proteinuria or elevated liver enzymes), affecting 3-5% of all pregnancies and representing a major cause of maternal and neonatal morbidity and mortality.[1] Women with previous PE have a two-fold increased risk for future major maternal cardiovascular disease (CVD; e.g. stroke, myocardial infarction), and a four-fold risk of hypertension and heart failure with preserved ejection fraction. [2-4] The CVD risk is increased 7-8 fold when PE is complicated by premature delivery before week 34, early-onset PE (EPE), and fetal growth restriction (FGR). [4-6] The mechanisms for these associations are not fully understood. Presence of pre-pregnancy CVD risk factors in women later developing PE does only partly explain the association between PE and long-term CVD risk, [7, 8] indicating that pregnancy, and especially PE, may promote or induce additional permanent maternal cardiovascular changes. [2] Women with PE, and especially EPE, present with signs of endothelial dysfunction, hypertension and subclinical cardiac changes, both during pregnancy and postpartum. [4, 9, 10] Studies have measured levels of a single or a few CVD-related risk markers in PE during pregnancy, and some biomarkers have been stratified relative to PE subtype (e.g. EPE vs late-onset PE (LPE)). [11, 12] Differences in circulating cardiovascular biomarkers between PE subtypes and normotensive controls in pregnancy could reflect subtype-specific cardiovascular changes, reflecting different CVD risks. [13, 14] This biomarker phenotyping could be utilized to target women at highest risk of CVD, to tailor cardiovascular follow-up and preventive measures, [15] as suggested by us. [2]

Increased maternal CVD risk is seen after PE; but also after other subtypes of placental dysfunction, such as in FGR without preeclampsia. [2] Uteroplacental spiral artery acute atherosis is a pregnancy-specific lesion, resulting in downstream placental dysfunction, and sharing morphological features of early atherosclerosis. [16] We have proposed that acute atherosis represents a marker of increased

CVD risk, [17] and have identified an association to classical CVD risk markers in older pregnant women. [18]

In this study we assessed whether a multiplex panel of 92 circulating CVD-risk biomarkers differed between women with EPE, LPE, and normotensive pregnancies (NC: normal controls). Furthermore, we stratified this biomarker analysis to the presence or absence of acute atherosis, FGR, and the lowest quartile of circulating placental growth factor (PlGF), the latter suggested by us to represent a marker of placental dysfunction and placental cellular (syncytiotrophoblast) stress. [19]

Methods

Reasonable requests to access part of the anonymous data set can be sent to corresponding author, but access is restricted because of ongoing clinical follow-up, the sensitive nature of the data collected for this study, and the patient informed consents (and thereby ethical body approval). All procedures were in accordance with institutional guidelines.

Patient recruitment and blood sampling

Pregnant women scheduled for elective caesarean section were recruited from 2001 to 2014 to the Oslo Pregnancy Biobank. The Regional Committee for Medical and Health Research Ethics in South-Eastern Norway approved the study. All patients signed informed consent. Preeclampsia was defined as new-onset hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥1+ on dipstick or ≥30 total protein/creatinine ratio) at ≥20 weeks gestation. [20] EPE was defined as delivery prior to gestational week 34, LPE as delivery ≥ 34 weeks. Clinical characteristics of the patient groups are presented in Table 1. None of the patients had pregestational or gestational

diabetes. Further clinical details, [21, 22] including blood sampling [23] and blood pressure measurement devices, are presented in the Supplemental File.

Decidua basalis tissue evaluation for acute atherosis

In a subset of the cohort (n=79) the immunohistological diagnosis of decidua basalis (endometrium of pregnancy) acute atherosis was available. [24] Sampling of decidua basalis tissue and immunohistochemical acute atherosis identification was described previously. [24]

Multiplex biomarker analysis

EDTA plasma samples from NC (n=49), EPE (n=37), and LPE (n=29) were analyzed using the Proseek multiplex CVD I assay at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden, that had developed the proximity assay technology, targeting 92 CVD linked biomarkers. [25] Analytical details and acronyms/full names of markers are presented in the Supplemental Table S1. Plasma from 108 of the 115 pregnancies analyzed with Olink CVD biomarkers were also analyzed for serum protein PlGF concentration with immunochemiluminescence assay (Roche, elecsys 2010 Modular) and showed excellent correlation, as shown in Supplemental Figure S1; r=0.97 (95% CI 0.91-1.01).

Statistics and multivariate data analysis

Statistical analyses were performed using SPSS Statistics 25.0 (IBM). For continuous variables a non-parametric Kruskal-Wallis test adjusting for multiple testing (false discovery rate, FDR) was used to identify markers that were significantly different between the three study groups. Dunn's-Bonferroni post hoc test was applied to the significant different markers to determine significant

differences between groups. Unadjusted and adjusted logistic regression was performed on standardized variables using forced entry, adjusting for BMI, age, systolic (SBP) and diastolic blood pressure (DBP), all measured before 20 weeks gestation. Principal component (PC) analysis was performed on the markers significantly different between study groups in the initial Kruskal-Wallis test (diagnostic groups) or Mann-Whitney test (low vs. high PlGF). The PC was thereafter compared and associated to diagnostic groups or low versus high PlGF. A p-value <0.05 was considered significant.

Results

Single Olink biomarker analysis

Among the 92 Olink biomarkers, 89 were satisfactorily identified in the pregnant women. IL4, ITGB1BP2, and BNP were excluded from further analysis as the two former were not detected in measurable amounts. BNP was only measurable in one NC and half of the PE group samples, the former in line with previous Olink reports of non-pregnant populations. [26] NTproBNP was however measurable in all samples, in line with its higher concentration than BNP in plasma. We identified 47 of the 89 detected biomarkers as significantly different between the 3 pregnancy outcome groups (Supplemental Table S1).

Following the post hoc test, 42 biomarkers differed significantly between EPE and NC and 28 different between LPE and NC, Supplemental Figure S2. Three biomarkers were lower both in EPE and LPE groups (PlGF, TIE2, and FS) and 22 biomarkers were significantly higher for the EPE and LPE groups relative to NC (Supplemental Table S2 (summarize previous findings [27-70]) and Supplemental Figure S2): VEGFA, IL6, TNFR1, TRAILR2, GH, PTX3, CHI3L1, TIM, IL16, UPAR, RAGE, MMP7, CD40, tPA, HBEGF, ESM1, SPON1, CASP8, FABP4, LEP, CCL20, and

NTproBNP). The EPE and the LPE group differed for 9 biomarkers; 5 biomarkers were lower in the EPE group (PlGF, hK11, MMP1, CTSD and FS) and 4 were higher (MMP3, MPO, ST2, CX3CL1) (Figure 1). Among these, 5 markers were altered in the EPE group relative to both the NC and LPE groups (MMP3, MPO, ST2, CX3CL1 and MMP1). Two of the 9 markers were not different compared to NC (hK11 and CTSD), and the last two markers were different between all the three groups (PlGF and FS); lowest for the EPE group, intermediate value for the LPE group and the highest values in the control group.

To correct for potential confounding factors between the 9 markers different between EPE and LPE such as age (OR=0.64, p=0.087), BMI (OR=1.06, p=0.087), and blood pressure (SBP OR=1.43, p=0.286, DBP OR=0.89, p=0.720), we performed a logistic regression analysis correcting for these factors when comparing the study groups. After adjusting, we found that CX3CL1, ST2, MMP3, FS, CTSD, MMP1, hK11, and PlGF remained significantly different between the EPE and LPE group (Figure 2). When adjusting for smoking, or newborn sex, our conclusions remained the same (data not shown).

We also investigated whether the 9 markers dysregulated only in EPE were different in EPE with FGR (n=27) versus without FGR (n=9) using logistic regression adjusting for age, BMI, SBP and DBP, and found MMP3 higher (OR=3.29, 95% CI [1.02-10.6], p=0.046) and CTSD lower (OR=0.08, 95% CI [0.01-0.54], p=0.009) in the EPE FGR group.

Multivariate data analysis

We performed multivariate principal component analysis of the 47 markers differing between the diagnosis groups (NC, EPE, and LPE) to identify combinations of biomarkers contributing to best grouping of the dataset. We found four main principal components (PC1-PC4), explaining 46.8% of the variance in the Olink biomarker set. The relative contribution of the different markers to each PC is presented in Figure 3A. These PCs also identified clusters of the diagnosis groups (NC, EPE, and LPE: Figure 3B). In order to compare the PC between the diagnostic groups, we performed a logistic regression adjusting for the confounding factors maternal BMI, age, SBP and DBP (Figure 3C). Comparing EPE vs. LPE group, we identified PC2, PC3, and PC4 as most discriminatory of EPE. For the LPE group, PC1, PC2 and PC3 were the best biomarker discriminators, while all PC groups (PC1-4) were significantly associated with EPE compared to NC (Figure 3C). In order to investigate if some of the PCs predicted EPE compared to LPE better than any single markers, we performed a receiver operating characteristic analysis, indicating that PlGF displayed the best accuracy for identifying EPE, followed by PC3 and MMP3 (Figure 4). When testing individual markers (without PC groups), we found the best discrimination between EPE and LPE with the combination of the individual markers PlGF*ST2*MMP1*MMP1*CX3CL1, Area under the curve [95% CI] 0.98 [0.95-1.00], $p<0.001$ (Figure 4).

Olink biomarker analysis vs placenta function biomarkers

We wanted to investigate if circulating PlGF also subclasssified the Olink cardiovascular biomarkers, as low PlGF represents a marker of placenta dysfunction, [19] which can result in many clinical syndromes that individually associate with premature maternal CVD [2]. We therefore removed the Olink PlGF data from the initial PCA analysis, and performed a new PC analysis on the 37 markers differently regulated between women with the lowest quartile of circulating PlGF protein compared to the remaining higher PlGF protein quartiles. As hypothesized, PCs also

differed between the pregnant women with the lowest quartile of PlGF (quartile 1) and the women with the higher PlGF quartiles (quartile 2-4) (Supplemental Figure S3A-C).

We also subclassified the total Olink dataset to uteroplacental presence of acute atherosis, another biomarker of placental function. Acute atherosis was as previously reported [24] significantly most prevalent in the EPE group (Table 1), and controls positive for acute atherosis were too few for sensible analysis of an association to Olink biomarker patterns. We found 3 markers significantly (unadjusted p-values) different between PE with acute atherosis compared to without (FAS, TM and MMP10; Figure 5). After adjusting for confounders in logistic regression (BMI, age, gestational age at delivery, SBP and DBP), only FAS (p=0.017) and TM (p=0.019) remained significantly associated to acute atherosis presence.

Discussion

Our findings suggest a dysregulation of both single and clusters of CVD-related circulating biomarkers in pregnancy according to pregnancy phenotype. A CVD-related biomarker dysregulation was found both comparing early- and late-onset PE and normotensive pregnancies, as well as when phenotyping on circulating biomarker of placental dysfunction (low PlGF) [19] or presence of uteroplacental acute atherosis. The CVD biomarker dysregulation was as hypothesized most prominent in the EPE group, which epidemiologically has the highest increased risk of future CVD. Among 92 CVD-associated markers we identified i) 47 markers different between the NC, EPE and LPE groups, ii) 9 markers specifically different between the EPE and LPE group, iii) 4 PCs providing segregation of the three clinical outcome groups, iv) PlGF, followed by PC3 and MMP3 to have the best diagnostic performance for EPE vs. LPE comparing single or multivariate

analysis, v) 2 markers different for PE pregnancies with acute atherosis compared to PE without this maternal uteroplacental artery lesion.

We are unaware of previous analysis of pregnancy cohorts using this Olink CVD-linked multiplex biomarker analysis. Previous studies of non-pregnant cohorts have found Olink biomarker association with atherosclerosis in 70 year olds, [71] with dyslipidemia in the general population, [26] and in relation to prediction of cardiovascular mortality in end-stage renal disease. [72] Our results support our previous findings of a preeclampsia cardiovascular-related biomarker phenotype differing from NC (e.g. MR-proANP [73] and MR-proADM [22]). Other studies have identified that particularly women with EPE have altered cardiac structure and function, along with increased circulating levels of the well-established cardiovascular biomarker NTproBNP. [56, 74, 75]

The dysregulated CVD biomarkers identified in the preeclamptic pregnancies likely reflect heterogeneous physiological and pathological processes linked to the pregnancy stress of PE, and particularly to EPE, which is also linked to the highest degree of placental cellular stress (e.g endoplasmic reticulum stress). [76] Whether these biomarkers also can be used for targeting women at highest risk for long-term CVD, and therefore for intervention trials and personalized medicine, is an exciting possibility to explore in longitudinal studies. During pregnancy, the circulating biomarkers are likely stemming from cardiovascular (myocardial and vascular) and other (e.g. placenta) sources. Eight markers were uniquely altered in EPE compared to LPE, pointing towards specific processes relevant for this PE subtype possible connected to the highest future maternal CVD risk, which has been shown both for CVD-related deaths as well as for major cardiovascular events in population based studies from Norway, [6, 77] as well as in other countries. [2] Among the dysregulated single markers, (low) PlGF followed by (high) MMP3 were most strongly associated

with EPE compared to LPE. The PlGF finding is in line with previous angiogenic biomarker findings over the last 15 years, where low PlGF is shown to more precisely diagnose and predict EPE as compared to LPE. [29, 78] As we have argued previously, low PlGF in pregnancy is not a marker of PE per se, but represents a marker of syncytiotrophoblast dysfunction and placental cellular stress. [19] During pregnancy, placenta is the main source for circulating PlGF. The Olink biomarker panel was designed to identify high PlGF outside pregnancy, identifying persons with increased vascular inflammation, as vascular endothelium is the main source for PlGF outside pregnancy. [79] In patients with non ST-segment elevation acute coronary syndrome, elevated circulating PlGF predicts an independent increased risk for all-cause mortality or myocardial infarction. [31] We have suggested [2] that a (low) PlGF level in pregnancy is a very interesting biomarker to test for prediction of CVD endpoints, representing a link between placental dysfunctions and high risk of maternal CVD. This concept was recently confirmed by Benschop et al, demonstrating that low midpregnancy circulating PlGF is an independent risk marker of future cardiovascular structure and function 6-9 years after pregnancy, independent of PE or hypertension, [80] confirming the potential for low PlGF for individualized follow-up and CVD prevention strategies. The other marker especially associated with EPE in our study was MMP3, which is a risk factor of vascular disorder and coronary heart disease, [81] and that has recently been reported as elevated in EPE. [82]

Another interesting finding was the low levels of FS in EPE, in line with previous reports of lower levels in PE, and an increase with gestational age in uneventful pregnancies. [28, 83] Through neutralization of the TGF-β superfamily members, FS participates in various processes like cell growth, development, and differentiation, [84] and members of the FS family are suggested involved in CVD pathophysiology outside pregnancy. [30]

Biomarkers specifically dysregulated for EPE were ST2, CX3CL1, and MMP3 (all elevated) as well as MMP1 (lower). All these markers are previously described as dysregulated in PE, and specific for the maternal-fetal interface and trophoblast dysfunction. Combining these markers and PlGF displayed the highest discriminatory properties of EPE vs LPE (Figure 4). Supplemental Table S3 summarizes findings in PE and CVD pathophysiology. [81, 82, 85-98] The Supplemental discussion text summarizes these biomarkers' potential role in the PE and CVD pathophysiology and their expression levels in different tissues summarized in Supplemental Figure S4.

In line with the epidemiological evidence of further increase in maternal cardiovascular risk in EPE complicated with FGR, we identified higher MMP3 and lower CTSD in the EPE group with FGR as compared to without FGR. MMP3 and CTSD play important roles in physiological remodeling processes and trophoblast invasion. [85] Reduced MMP3 expression in the extravillous trophoblast at the fetal-maternal interface and higher CTSD expression in the placenta has been reported in PE. [59, 99] These opposite EPE findings in the circulating versus placental tissue markers may reflect some of the pathological processes of abnormal placentation and compensatory mechanism in early PE. [82]

In the multimarker approach we identified four PC clusters. PC3 was the cluster most discriminating EPE from LPE, consisting of biomarkers important for growth (CTSD, PlGF, FS), angiogenesis (TIE2, MMP3), cholesterol metabolism (LOX1, LEP, FABP4, GAL), and inflammation (IL18). PC1 did not distinguish between EPE and LPE, and these markers were mostly elevated in PE regardless of the onset-type, and consisted of markers involved in TNF signaling (e.g TRAILR2, TNFR1, CD40, FAS). The markers that predicted EPE best compared to

LPE, comparing both single and multivariate analysis, was as expected (low) PlGF, followed by PC3 and MMP3. Reflecting the individual biomarker findings, PlGF and MMP3 were both part of PC3.

Markers of inflammation, endothelial activation and lipids have been associated with the promotion of atherosclerosis lesion formation. We and others have shown higher presence of acute atherosis in the uteroplacental arteries from PE women, [24, 100] and here we identified higher levels of circulating FAS and TM in the presence of acute atherosis in PE; however these biomarkers did not differ in EPE compared to LPE. Interestingly, acute atherosis predisposes to spiral artery thrombosis which may lead to placental infarcts. [17] These results are in line with our previous results showing few associations between classical circulating CVD risk factors (e.g. dyslipidemia) and presence of acute atherosis. [18]

The major strength of this study is the clinically well characterized cohort with extensive pregnancy data and the wide range of markers included in the analysis enabling a multivariate approach. The numbers of pregnancies are however low relative to the number of markers. Further, the Olink biomarker levels are relative and not absolute values, but the excellent correlation identified between Olink and immunochemiluminescence assay for PlGF protein levels and concentrations (Supplemental Fig. S1), across clinical diagnosis groups, are reassuring for the interpretation of the remaining Olink biomarker findings. Gestational age differed between the three study groups, and might possibly have affected the levels of circulating markers. However, ST2, CX3CL1, MMP1 and MMP3 levels did not associate significantly with gestational age in the study groups. PlGF levels decrease significantly in the last trimester for all pregnancies, but earlier in the early- than late-onset preeclampsia and normal controls. Gestational-age matched blood samples would have

identified a greater difference in maternal PlGF concentrations between the groups than in the current study. Storage time differs in our samples before analysis, but importantly, no difference between the study groups. However, different proteins may have different stability over time, which may have impacted our results.

To conclude, we found circulating ST2, CX3CL1, MMP1 and MMP3 levels significantly different between EPE and LPE. Few markers were uniquely changed in LPE that were not also changed in EPE, supporting that LPE does not represent biomarkerwise a specific type of preeclampsia, but likely a less extreme variant of this heterogeneous syndrome. The uniquely EPE dysregulated markers may reflect differences in early placentation pathophysiology as well as possibly associate with differences in long-term CVD risk. Further work will benefit from exploring timing of the biomarker alterations in pregnancy as well as identifying their relation to surrogate and hard cardiovascular endpoints.

Perspectives

The findings of altered circulating levels of CVD risk associated markers in PE at term are in line with epidemiological findings of the increased CVD risk after PE. Particularly, ST2, CX3CL1, MMP1 and MMP3 are interesting to pursue in longitudinal studies, since these were altered in the EPE group only, which has the highest future CVD risk. Another longitudinal biomarker option to assess is NTproBNP, which is readily available in the clinical routine today as a biomarker of CV disease, and that was found elevated across both early and late-onset PE groups, as well as in the group with the lowest PlGF. The identified biomarkers may alone or in clusters provide useful information for targeting women for intensified prevention of CVD, as well as provide ideas for novel intervention strategies.

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Disclosure of interests

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Novelty and Significance

What Is New?

- Multiplex plasma analysis of CVD risk associated markers phenotyping of preeclampsia subtypes has not been published before

What Is Relevant?

- Several CVD risk associated markers differed in pregnancy plasma between preeclampsia subtypes and normotensive controls, and between groups defined by levels of placenta dysfunction markers
- Four individual CVD risk associated markers ST2, CX3CL1, MMP1 and MMP3 were altered specifically for the EPE subtype, potentially linked to the high risk of future CVD for this group

Summary

- Our data are consistent with a placental-cardiovascular axis in pregnancy, as tissue-based and circulating placental dysfunction markers associate with circulating CVD biomarker patterns.
- The dysregulated CV biomarkers identified at delivery may contribute to improved targeting of women for intensified prevention of CVD, as well as provide ideas for novel intervention strategies

Figure Legends

Figure 1. Individual levels of the 9 circulating Olink biomarkers differing significantly between early-onset preeclampsia (EPE) and late-onset preeclampsia (LPE) groups (delivery prior to or from gestational week 34, respectively). Data are given as NPX log 2. P value represents the overall group effect (FDR adjusted Kruskal-Wallis). Dunn's- Bonferroni post hoc test are used to compare groups *$p<0.05$, **$p<0.01$ ***$p<0.001$. Horizontal bars represent mean ± SD. Biomarker acronyms are explained in Supplemental Table S1.

Figure 2. Logistic regression analysis showing adjusted associations between the Olink-identified biomarkers different between study groups. Biomarker acronyms are explained in Supplemental Table S1. The figure is showing the odds-ratio (OR) (and 95% confidential intervals) of the biomarkers associated with early-onset preeclampsia (EPE; delivery prior to gestational week 34) compared to controls (NC), late-onset preeclampsia (LPE; delivery from gestational week 34) compared to NC and EPE compared to LPE, using standardized values.

Figure 3. Principal component (PC) analysis. A) Markers contributing to each of the four principal components (PC1-PC4) from the initial 47 markers differently regulated between diagnostic groups. B) Correlation plots between the different PC showing diagnostic groups in different colors. NC (blue); normotensive controls, LPE (green); late-onset preeclampsia (delivery from gestational week 34), EPE (red); early-onset preeclampsia (delivery prior to gestational week 34). C) Logistic regression analysis showing adjusted associations between the different PCs and diagnostic groups (acronyms as in Figure 3B). Biomarker acronyms are explained in Supplemental Table S1.

Figure 4. Receiver operating characteristic curves for predicting early-onset preeclampsia (EPE: delivery prior to gestational week 34) compared to late-onset preeclampsia (LPE: delivery from gestational week 34) by Olink multimarker (PC1-PC4) and single markers (FS, MMP1, hK11, PlGF, CTSD, MMP3, ST2, CX3CL1, MPO), and combination (PlGF, ST2, MMP1, MMP3 and CX3CL1) approach. Biomarker acronyms are explained in Supplemental Table S1.

Figure 5. Levels of the circulating markers FAS (tumor necrosis factor receptor superfamily member 6), TM (thrombomodulin), and MMP10 (matrix metalloproteinase-10) that were different between women with the presence of decidua basalis acute atherosis (with AA; n=28) and those without acute atherosis (no AA; n=15) in the preeclampsia patients using logistic regression. Data are given as NPX log 2. P values represent unadjusted models. Horizontal bars represent mean ± SD.

Table 1. Clinical characteristics of the patient groups during pregnancy

Clinical variables	NC (n=49)	LPE (n=29)	EPE (n=37)	Between groups (p=)
Maternal age at delivery (years)	33 (4)	34 (5)	32 (5)	0.147
Body mass index early pregnancy (kg/m^2)	22.3 (20.6, 23.7)	24.1 (21.0, 30.3)*	24.1 (22.0, 29.8)**	0.002
Systolic blood pressure <20 weeks (mmHg)	110 (103, 117)	115 (110, 123)	117 (108, 126)**	0.008
Diastolic blood pressure <20 weeks (mmHg)	67 (60, 71)	75 (67, 80)*	72 (65, 78)*	0.003
Systolic blood pressure at delivery (mmHg)	121 (115, 132)	160 (158, 173)***	162 (155, 180)***	<0.001
Diastolic blood pressure at delivery (mmHg)	71 (65, 80)	100 (96, 108)***	100 (95, 108)***	<0.001
Gestational age at delivery and sampling (weeks)	39.1 (38.9, 39.3)	35.9 (34.4, 38.1)*** †††	30.3 (27.9, 32.9)***	<0.001
Ever smoked during pregnancy n (%)	3 (6.1)	4 (13.8)	3 (8.3)	0.509
Primipara n (%)	25 (51.0)	18 (62.1)	22 (59.5)	0.917
Small for gestational age n (%)	1 (2.0)	13 (44.8)*** †††	34 (91.9)***	<0.001
Fetal growth restriction n (%)	0 (0)	9 (31.0)*** †††	27 (75.0)***	<0.001
Newborn weight (g)	3399 (299)	2603 (819)*** ††	1212 (439)***	<0.001
Newborn weight percentile	52.1 (28.3, 74.5)	32.5 (1.78, 50.5)*** ††	0.10 (0.01, 3.54)***	<0.001
Newborn sex (girl/boy)	21/28 (42.9/57.1)	13/16 (44.8/55.2) †	26/11 (70.3/29.7)*	0.027
Acute atherosis (All/Yes) n (%)	36/3 (8.3)	19/5 (26.3)	24/10 (41.7)**	0.010

Data given as mean (SD) when normal distributed and median (25^{th}, 75^{th}) when skewed distributed. *p<0.05, **p<0.01, ***p<0.001 compared to controls, †p<0.05, ††p<0.01, †††p<0.001 between LPE and EPE. NC: Normotensive controls; LPE: late-onset preeclampsia (delivery from gestational week 34); EPE: early-onset preeclampsia (delivery prior to gestational week 34).