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Establishing a Differential Marker Profile for Pregnancy Complications Near Delivery

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Keywords
Preeclampsia · Intrauterine growth restriction · Tumor necrosis factor alpha · Preterm delivery · Blood pressure · Proteinuria

Abstract
Objective: The aim of this work was to define a differential marker profile for pregnancy complications near delivery. ***Methods:*** We enrolled pregnant women who were referred to the outpatient pregnancy clinic of the University Medical Center, Ljubljana, Slovenia, due to symptoms of pregnancy complications and women with a history of pregnancy complications attending the high-risk hospital clinic for close surveillance. They were evaluated for prior risk and were tested for biophysical and biochemical markers at the time of enrolment. Biochemical markers included the pro- and anti-angiogenic markers, along with additional previously reported markers of potential value, all tested by various formats of immuno-diagnostics. Biophysical markers included blood pressure, sonographic markers, and EndoPAT. Statistical differences were determined with Kruskal-Wallis and Mann-Whitney tests for continuous parameters, and Pearson χ^2 for categorical values. $p < 0.05$ was considered significant. ***Results:*** The cohort included 125 pregnant patients, 31 developed preeclampsia (PE) alone (13 were <34 weeks' gestation), 16 had intrauterine growth restriction (IUGR) alone (12 were <34 weeks), 42 had both IUGR and PE (22 were <34 weeks), and 15 had an iatrogenic preterm delivery (PTD; 6 were <34 weeks). Twenty-one were unaffected and delivered a healthy baby at term. Mean arterial blood pressure and proteinuria were significantly higher in PE and PE+IUGR but not in pure IUGR or PTD. In PE, IUGR, and PE+IUGR, the levels of soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) were significantly higher, while placental growth factor (PlGF) was very low compared to unaffected controls and PTD. PE, IUGR, and PE+IUGR also had a high anti-angiogenic ratio (sFlt-1/PlGF) and a low proangiogenic ratio of PlGF/(sFlt-1+Eng). Levels of inhibin A were significantly higher in pure PE across subgroups but had many extreme values, which made it a poor differentiator. Higher uterine artery Doppler pulsatility indexes were detected in

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PE, IUGR, and PE+IUGR, with similar resistance indexes and peaks of systolic velocity. A significantly different marker level between PE and IUGR was found using arterial stiffness that was 10 times higher in PE; concurrently with an increase of the reactive hyperemia index, both were accompanied by a slight increase in placental protein 13. Higher tumor necrosis factor alpha (TNFα) differentially identified iatrogenic very early PTD (<34 weeks). ***Conclusion:*** Arterial stiffness can serve as a major marker to differentiate PE (with/without IUGR) from pure IUGR near delivery. TNFα can differentiate iatrogenic early PTD from other complications of pregnancy and term IUGR.

Introduction

Preeclampsia (PE) [1], intrauterine growth restriction (IUGR) [2], and preterm delivery (PTD) [3] are major obstetrical complications, affecting approximately 20% of all pregnancies [4, 5]. The specific clinical symptoms of each complication manifest mostly a few weeks prior to the time of delivery, with certain overlap of symptoms, thus presenting a challenge for patient management when admitted to obstetrics and gynecology clinics at hospitals prior to delivery. Over the last 10 years new markers have been identified to improve the accuracy of the clinical management of these complications [5–8].

PE is a hypertensive disorder associated with proteinuria that emerges from mid gestation in previously normotensive women [9–11]. Differentiating PE from IUGR is a challenge [12, 13], especially in cases that develop early, since in both cases the fetuses are small, the blood flow from the uterine arteries to the placenta is altered due to increased uterine artery impedance, and both are associated with premature delivery [2, 7, 8]. Iatrogenic PTD, often defined by having a short cervix, is frequently accompanied by premature rupture of the membranes, while IUGR lacks these symptoms. These complications often result in small for gestational age babies born before term [3, 7, 8, 14].

Traditional methods to predict the above pregnancy complications include measuring body mass index (BMI) [15], blood pressure, urine protein, or blood level of lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT), in addition to secondary symptoms such as the reversible loss of vision, headaches, upper gastric pain, etc. [9–11]. New predictive methods offer the use of immuno-diagnostics to measure various blood biomarkers and use sonographic values, creating a promising avenue for the risk prediction of the specific complication. The challenge remains to identify the specific complication near delivery, when symptoms overlap, and the diagnosis could be confused.

Research has shown that near the time of delivery there is a rise in anti-angiogenic factors, including soluble fms-like tyrosine kinase 1 (sFlt-1) [13, 16] and soluble endoglin (sEng) [17], a drop in the proangiogenic placental growth factor (PlGF) [13, 18], and slight changes in the vascular endothelial growth factor (VEGF) [18]. These have been associated with an increase of the anti-angiogenic sFlt-1/PlGF ratio [15, 19], and a decrease in the proangiogenic PlGF/(sFlt-1+sEng) ratio [20, 21]. Increases of inhibin A [22] and placental protein 13 (PP13) [23] near delivery were also reported. Elevation of tumor necrosis factor alpha (TNFα) has been detected in PTD [24] as well as IUGR [25].

Biophysical markers have also been widely used. Parameters such as the resistance index (RI) to the flow in uterine arteries, the average uterine artery pulsatility index (PI), and the peak systolic velocity (PSV) have been identified [2, 7, 8, 12, 26, 27]. A more recent approach involves the assessment of endothelial dysfunction and arterial stiffness using EndoPAT [28]. Developed for risk assessment of cardiovascular diseases (CVDs), the EndoPAT peripheral arterial tone (PAT) [29–31] measures the arterial stiffness of peripheral vessels [31–33].

The purpose of this study was to evaluate known predictive biomarkers near delivery through immune-diagnostics and biophysical measurements to determine whether they can be successfully utilized to provide differential risk profiles of PE, IUGR, PE+IUGR, and iatrogenic PTD to support clinical management. The availability of such information could help clinical research leaders to identify in a clear way the different and overlapping groups of pathologies, and to set up better cohorts and groups to study the etiologies of these pathologies.

Methods and Sample

Sample

Patients were enrolled between 2012 and 2015. The cohort included pregnant patients, who were referred to the outpatient pregnancy clinic of the Department of Perinatology, University Medical Centre of Ljubljana, Slovenia, due to pregnancy complications. The women were recruited after providing their written informed consent. Unaffected controls were women who attended the clinic for close surveillance due to a history of pregnancy complications and agreed to sign the informed consent. All patients were at 24 weeks of gestation or more but not in labor when included in the study.

The inclusion criteria were viable singleton pregnancies without major fetal abnormalities and the agreement to undergo all test procedures and deliver at the medical center. The exclusion criteria were maternal age below 18 years, multiple pregnancies, fetal abnormalities, or preexisting renal, hematological, autoimmune, or severe CVD conditions.

GA was determined from the last menstrual period and verified by evaluating the records of the routine first-trimester ultrasound measure of the fetal crown-rump length [35]. Pregnancy complications were defined as outlined below.

Preeclampsia

PE was defined as a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg, or both, measured on two occasions at least 4 h apart in a previously normotensive woman [9–11, 36], and proteinuria of 1^+ (on the dipstick) in urine [9–11]. In the absence of proteinuria, any of the following signs were used: thrombocytopenia (platelets <50,000/μL) [37], hemolysis (LDH >1.31 IU/dL) [38], renal insufficiency, impaired liver function (AST >0.34 IU/dL), ALT (>0.24 IU/dL) [39], or pulmonary edema. In addition, we recorded complaints of cerebral or visual problems after 20 weeks of gestation [9–11] but these were not used for PE diagnosis.

Intrauterine Growth Restriction

IUGR was defined as the estimated fetal weight <5th percentile, or abdominal circumference <5th percentile combined with oligohydramnios (AFI <5 cm), and/or an umbilical artery PI >95th percentile [40, 41].

Preterm Delivery

PTD was defined as delivery before 37 weeks [3] that was not related to IUGR and PE. After assigning women to subgroups based on the nature of the complication, the groups were further subdivided into delivery before 34 weeks (early cases), delivery between 34 weeks and 0 days, to delivery at 36 weeks and 6 days (intermediate), and delivery at 37 weeks and later (term cases).

Serum Biomarkers

After signing the informed consent at the clinic appointment, maternal blood samples were collected into vacutainer tubes that were left to clot at room temperature and then centrifuged at room temperature for 10 min at 1,000 *g* and stored at −70 °C in sterile cryo-vials until analysis. No sample underwent more than one freeze-thaw cycle before analysis.

Elecsys System

The concentrations of PlGF and sFlt-1 were determined by the automated Elecsys system (Cobas e411 system, Roche Diagnostics, Germany), as previously described [20], based on electro-chemiluminescence technology according to the manufacturer's instructions. The sFlt-1 assay covers a range of 10–85,000 pg/mL and the PIGF assay's range was 3–10,000 pg/mL, with an assay coefficient of variation of <2% for both markers' assays, and an interassay coefficient of variation of 2.3–4.3% for the sFlt-1 and 3.6–4.1% for the PlGF assay. The ratio of sFlt-1/PlGF was used to obtain the anti-angiogenic state [13, 19] and the ratio of PlGF/(sFlt-1+sEng) [19, 20] reflected the proangiogenic versus the anti-angiogenic state.

A personal LAB Microplate Analyzer (Adaltis, Italy) was used to determine sEng (Abcam, Cambridge, UK), PP13 (Hy-Laboratories, Rehovot, Israel), TNFα (IBL International GMBH, Hamburg, Germany), and VEGF (Abcam) according to the manufacturer's instructions. The minimal detectable concentration of sEng was <10 pg/mL with inter- and intra-assay coefficients of variation of <10 and <12%. For TNFα, the minimal concentration was 5 pg/mL with inter- and intra-assay coefficients of variation of 8.1 and 7.7%. For VEGF, the lower detection level was <10 pg/mL with inter- and intra-assay coefficients of variation of <12 and <10%. For PP13 the minimal detection level was 10 pg/mL with an inter- and intra-assay variation of 7.5 and 11%.

The Access 2 immunoassay system (Beckman Coulter, Pasadena, CA, USA) was used to determine inhibin A using the Access Inhibin A kit according to the manufacturer's instructions. The minimal detectable concentration of inhibin A was <1 pg/mL with inter- and intra-assay coefficients of variation of 4%.

Biophysical Parameters

At the time of the clinical appointment, after the provision of informed consent, all biophysical markers were measured.

Blood Pressure

Blood pressure was measured with arm-adjusted cuffs, using an automated device. The diastolic and systolic values were recorded and were used to obtain the mean arterial blood pressure (MAP), which was calculated as: (systolic + diastolic × 2)/3 [36].

Aix % and the reactive hyperemia index (RHI) were measured with the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel) at the time of blood drawing according to the manufacturer's instructions. The test was administered in a supine position in a quiet, temperature-controlled room (21–24 °C) after a 15-min rest to ensure a relaxed cardiovascular steady state and to allow adjustment to the room temperature. Patients removed jewelry and were asked to remain still and silent during the measurement with uncrossed legs. Blood pressure was measured to determine the occlusion pressure for the RHI measurement.

Description of the Use of the EndoPAT

The system estimates the endothelial response to a reactive hyperemia elicited by 5-min occlusion of the brachial artery. The device detects plethysmography pressure changes in the fingertips caused by the arterial pulse and translates this into a PAT [29, 30]. The EndoPAT corrects for systemic changes in vascular tone during the test by simultaneous measurement from the unoccluded, contralateral arm. An index based on the two arms is calculated to adjust for changes [33]. In addition, the EndoPAT also calculates the augmentation index (Aix %), i.e., a measure of arterial stiffness indicative of peripheral vascular resistance. The test is easy to perform, not operator dependent, and the analysis is completely automatic [34].

Plethysmography probes were placed on the index finger of each hand. A blood pressure cuff was placed on the non-dominant upper arm (test arm), whereas the other arm served as a control. A baseline signal was established by 10-min measurements, after which the cuff was inflated 60 mm Hg above systolic blood pressure and no less than 200 mm Hg, and to a maximum of 300 mm Hg for exactly 5 min to provoke a transient ischemia. The release of the cuff led to increased blood flow that caused an endothelium-dependent dilation of the vascular bed, which was continuously recorded for a further 5–10 min.

All the data were automatically recorded and expressed as RHI and Aix % by the EndoPAT software. The Aix % is derived from pulse waveforms, which are calculated as the ratio of the difference between the early (P1) and the late systolic peaks of the waveform (P2); the relative early to late peaks ([P2 – P1]/P1) were expressed as a percentage. RHI was calculated as the ratio of the post- ($O2_1$) to pre-occlusion ($O1_1$) PAT amplitude of the tested arm divided by the post- ($O2_2$) to pre-occlusion ($O1_2$) ratio of the control arm. Official reference values for RHI and Aix are not available for pregnant women. Thus, we used values below 2, as determined in a population at risk of ischemic heart disease, which was defined as endothelial dysfunction and increased arterial stiffness between –10 and 10% [42].

Ultrasound

All patients underwent fetal biometry at admission, including measurements of the fetal head and abdominal circumference and femur length, measured according to the guidelines established by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) [43]. The estimated fetal weight was calculated according to Hadlock's formula based on four fetal measurements [44].

Maternal vessels were examined bilaterally, and the results were reported as the average of the right and left side measurements. The uterine artery PI was obtained by placing the Doppler transducer on the mother's abdomen, after a sagittal section of the cervix was obtained. The transducer tilted from side to side to identify the uterine arteries at the level of the internal os. A pulsed Doppler sampling gate of 2 mm was used to cover the entire vessel, and an angle insonation <30° with PSV of >60 cm/s was used to obtain the necessary waveforms before calculating the average of the PI in the left and right uterine arteries [45].

The uterine artery RI was measured with arteries visualized by power Doppler placed abdominally over the uterine artery to provide its RI. RI was calculated as: PSV – end diastolic velocity/PSV. It was performed at the point where this artery crosses the external iliac artery (beam/flow angles were kept at 30°) [46].

The PSV index of the uterine arterial blood flow was measured after the mothers had been resting for at least 20 min. Each uterine artery was visualized by power Doppler, using the minimum power setting needed to delineate the vessel walls. The vessel diameter was measured perpendicular to the vessel during systole, between the outer aspects of the lumen, and the waveform was recorded to obtain the time-averaged mean, PI, and to define the presence or absence of notching as described elsewhere [47]. This test was conducted at the end of the ultrasound examination.

Statistical Analyses

Descriptive Statistics

For categorical variables, summary tables are provided giving frequencies for categorical values and arithmetic means for continuous variables with 95% CI (Table 1a, b; online suppl. Table 1a, b; see www.karger.com/doi/10.1159/000502177 for all online suppl. material). Medians of markers (continuous values) were calculated with their 95% CI in the same way (Tables 2a, b; online suppl. Table 2a, b).

Inferential Statistics

Non-parametric tests for two or more independent samples were applied for evaluating the significant differences for a given parameter among the study groups for the continuous variables (participant characteristics, biomarkers, and vascular modulation). Statistical analysis was performed using the SPSS package version 24 (SPSS Inc., Chicago, IL, USA). The significance of the model was analyzed with the Kruskal-Wallis non-parametric test, and significant differences between the reference and the test groups were analyzed with the Mann-Whitney non-parametric test, * $p < 0.5$ and ** $p < 0.01$ (Table 1a, 2a, b; online suppl. Table 1a, b, 2a, b). Pearson's χ^2 tests were applied for correlations between the study groups for the categorical parameters (Table 1a, b; online suppl. Table 1a, b). Box plot graphs provided the graphic description of quartile distribution. Statistical significance was defined as $p \leq 0.05$.

Results

Characteristics

We enrolled 125 patients with a full dataset of whom 31 developed PE alone (13 were <34 weeks' gestation), 16 had IUGR alone (12 were <34 weeks), 42 had both IUGR combined with PE (22 were <34 weeks), and 15 had an iatrogenic PTD (6 were <34 weeks) not related to IUGR or PE. Twenty-one were unaffected and delivered a healthy baby at term. Furthermore, we divided patients into subgroups of early cases (delivery before 34 weeks), intermediate cases (delivering at gestational age between 34 weeks and 0 days and 36 weeks and 6 days), and term cases who were delivered at 37 weeks and later. The demographic and clinical characteristics of the entire cohort and the subgroups are depicted in Table 1a, b, and online suppl. Table 1a, b. All women were Caucasian, and there was no difference in maternal age, parity, or gravidity among the pathology groups as a whole or across pathology subgroups.

Body Mass Index

The BMI measured at enrolment was significantly higher in the PE and PE+IUGR groups compared to the unaffected control or PTD groups. The BMI of the IUGR group was in the mid-range (Table 1a). These differences were verified for all cases (Table 1a) as well as for all subgroups, including early cases (<34 weeks, Table 1b), term cases (online suppl. Table 1a), and intermediate cases (online suppl. Table 1b). Measurements at delivery showed low/no weight gain between enrolment and delivery (approx. 0.7 g/week or less).

Blood Pressure

Comparison of blood pressure values for the entire study cohort (Table 1a) indicated that the mean blood pressure of PE was 150/94 mm Hg and the blood pressure of PE+IUGR was 151/94 mm Hg (i.e., both comply with

Table 1. Descriptive statistics

a All participants

	Unaffected (n = 21)	PTD (<37 weeks) (n = 15)	PE (n = 31)	IUGR (n = 16)	IUGR+PE (n = 42)	p
Enrolment						
Gestational age at enrolment, weeks	34.0 [32.0–35.9]	31.2 [29.4–32.9]*	33.9 [32.3–35.6]	31.4 [29.1–33.6]*	31.8 [30.7–32.8]*	0.014
Maternal age, years	31.6 [29.5–33.8]	31.3 [29.7–32.9]	32.0 [29.9–34.1]	31.7 [29.7–33.7]	32.9 [31.1–34.7]	0.713
BMI	25.8 [23.7–27.9]	24.6 [22.9–26.4]	29.5 [26.5–32.6]	27.6 [24.2–31.0]	29.6 [26.9–32.4]	0.101
Gravidity	2.3 [1.8–2.9]	1.8 [1.4–2.2]	1.8 [1.2–2.4]	2.0 [1.4–2.6]	1.7 [1.4–2.0]	0.055
Parity	1.7 [1.3–2.0]	1.6 [1.2–2.1]	1.4 [1.0–1.8]	1.5 [1.1–1.9]	1.5 [1.2–1.8]	0.252
Systolic BP, mm Hg	112[107–118]	119 [109–128]	150 [145–155]**	131 [125–137]*	151 [146–156]**	<0.001
Diastolic BP, mm Hg	71 [66–76]	76 [68–84]	94 [91–97]*	80 [76–84]	94 [90–97]*	<0.001
MAP, mm Hg	85 [80–90]	90 [82–98]	113 [109–116]**	97 [93–101]*	113 [109–116]**	<0.001
Previous PE, %	4.8	6.7	6.5	6.3	9.5	0.965
IVF, %	4.8	0	6.5	0	11.9*	0.361
Chronic hypertension, %	0	0	19.4*	0	16.7*	0.032
Diabetes, %	0	0	3.2	0	4.8	0.787
Polycystic ovary, %	0	0	0	0	7.1*	0.204
Delivery						
Gestational age at delivery, weeks	39.1 [38.5–39.7]	33.8 [32.1–35.5]*	34.2 [32.6–35.9]*	31.7 [29.4–34.0]**	32.0 [31.0–33.1]**	<0.001
BMI	–	–	29.4 [27.7–31.1]	28.2 [25.8–30.7]	30.4 [28.9–31.9]	0.172
Systolic BP, mm Hg	112 [106–118]	119 [109–128]	143 [138–148]**	131 [123–140]*	140 [132–148]*	<0.001
Diastolic BP, mm Hg	71 [66–76]	76 [68–84]	87 [83–92]**	82 [74–90]*	83 [76–90]**	0.004
MAP, mm Hg	85 [80–90]*	90 [82–98]*	106 [102–110]**	98 [91–106]*	102 [95–108]**	<0.001
Vaginal delivery, %	76.2	69.2	45.2**	40.0*	14.6**	<0.001
Baby's birthweight, g	3,330 [3,133–3,528]	2,207 [1,872–2,542]*	2,306 [1,906–2,705]*	1,306 [834–1,778]**	1,449 [1,247–1,651]**	<0.001
Fetal death in utero, %	0	0	3.2	12.5	7.1	0.318
Delivery <34 weeks, %	0	40.0*	41.9*	75.0**	66.7*	<0.001
Delivery <37 weeks, %	0	100**	67.7*	75.0*	90.5**	<0.001
Modular						
U-creatinine, mmol/L	5.9 [4.0–7.9]	5.7 [3.7–7.7]	9.7 [7.2–12.3]*	6.1 [2.7–9.5]	11.8 [8.9–14.8]**	0.002
Proteinuria[1], 0–4	–	–	2.4 [1.8–2.9]	0.4 [0–0.9]*	2.7 [2.3–3.1]	<0.001
AST, IU/dL	–	–	0.90 [0.50–1.30]	0.37 [0.25–0.49]	0.64 [0.45–0.82]	0.110
ALT, IU/dL	–	–	1.06 [0.46–1.66]	0.48 [0.22–0.75]	0.64 [0.45–0.84]	0.294
LDH[1], IU/dL	–	–	4.1 [3.4–4.8]	3.0 [2.5–3.4]*	3.9 [3.4–4.4]	0.017

b All deliveries <34 weeks

	PTD (n = 6)	PE (n = 13)	IUGR (n = 12)	IUGR+PE (n= 22)	p
Enrolment					
Gestational age at enrolment, weeks	29.2 [26.8–31.6]	29.9 [27.5–32.3]	29.3 [27.7–30.8]	29.9 [28.9–30.9]	0.570
Maternal age, years	31.3 [27.8–34.8]	33.8 [33.0–37.7]	31.5 [29.2–33.8]	33.1 [30.7–35.5]	0.535
BMI	24.7 [21.0–28.4]	30.7 [26.2–35.2]	26.3 [23.9–28.8]	29.7 [26.1–33.4]	0.101
Gravidity	1.8 [1.0–2.6]	2.1 [0.6–3.5]	1.8 [1.1–2.4]	1.8 [1.3–2.2]	0.540
Parity	1.8 [1.0–2.6]	1.5 [0.7–2.4]	1.3 [0.9–1.7]	1.6 [1.2–2.0]	0.268
Systolic BP, mm Hg	112 [99–126]	152 [141–163]*	131 [124–138]	154 [147–160]*	<0.001
Diastolic BP, mm Hg	74 [54–93]	94 [89–100]*	78 [73–83]	95 [91–99]*	<0.001
MAP, mm Hg	87 [70-103]	114 [107–121]*	96 [91–101]	115 [110–119]*	<0.001
Previous PE, %	0	0	0	7.1	0.591
IVF, %	0	15.4*	0	10.7	0.498
Chronic hypertension, %	0	7.7	0	21.4*	0.146
Diabetes, %	0	0	0	3.6	0.771
Polycystic ovary, %	0	0	0	3.6	0.771
Delivery					
Gestational age at delivery, weeks	31.0 [28.0–34.0]	30.2 [27.8–32.6]	29.5 [28.0–31.1]	30.2 [29.2–31.2]	0.517
BMI[1]	–	27.3 [25.1–29.5]*	26.9 [24.9–29.0]*	30.4 [28.6–32.2]*	0.020
Systolic BP, mm Hg	112 [99–126]	148 [72–224]*	130 [123–136]*	139 [127–151]*	0.011
Diastolic BP, mm Hg	74 [54–93]	92 [85–98]	80 [71–90]	83 [75–91]	0.389
MAP, mm Hg	87 [70–103]	110 [81–140]*	97 [89–105]*	102 [94–110]*	0.041
Vaginal delivery, %	80.0	23.1*	27.3*	7.4**	0.003
Baby birthweight, g	1,669 [1,318–2,020]	1,276 [923–1,628]*	874 [627–1,121]**	1,171 [995–1,346]*	0.012
Fetal death in utero, %	0	7.7	8.3	3.6	0.825
Modular					
U-creatinine, mmol/L	6.3 [3.8–8.8]	10.5 [4.7–16.2]	6.4 [1.8–11.0]	10.7 [7.8–13.8]	0.102
Proteinuria[1], 0–4	–	2.8 [1.9–3.7]	0.5 [0.0–1.1]*	3.1 [2.7–3.5]	<0.001
AST, IU/dL	–	1.27 [0.43–2.11]	0.40 [0.24–0.56]	0.70 [0.44–0.96]	0.188
ALT, IU/dL	–	1.65 [0.31–2.99]	0.54 [0.17–0.91]	0.68 [0.45–0.92]	0.243
LDH, IU/dL	–	4.80 [3.27–6.34]	3.08 [2.49–3.67]	4.14 [3.51–4.77]	0.084

Data are presented as the mean [95% CI] or percentage. Non-parametric tests for independent samples were applied for evaluating the significant differences for a given parameter among the study groups for the continuous variables. p values were generated using the Kruskal-Wallis non-parametric test. Comparisons between the reference group (the unaffected group in **a**, or the PTD group in **b**) and the clinical complication test groups were performed with the Mann-Whitney non-parametric test. The Pearson χ^2 test was used to compare categorical values against the reference groups. * $p < 0.5$, ** $p < 0.01$. [1] Comparison between the tested groups.

Table 2. Median biomarkers and vascular modulation

a All participants

	Unaffected (n = 21)	PTD (<37 weeks) (n = 15)	PE (n = 30)	IUGR (n = 16)	IUGR+PE (n = 43)	p
Gestational age at testing, weeks	34.0 [32.0–35.9]	31.2 [29.4–32.9]*	33.9 [32.3–35.6]	31.4 [29.1–33.6]*	31.8 [30.7–32.8]*	0.014
sFlt-1, pg/mL	3,009 [1,897–4,090]	3,026 [1,753–4,173]	15,207 [9,602–18,015]**	9,430 [7,381–12,335]*	18,149 [13,134–21,811]**	<0.001
PlGF, pg/mL	524 [223–681]	693 [308–980]	101 [69–153]*	76 [43–117]*	62 [48–87]*	<0.001
sFlt-1/PlGF	5 [3–31]	6 [2–9]	177 [106–301]*	195 [55–310]*	265 [168–382]*	<0.001
sEng, pg/mL	10,218 [6,734–13,790]	8,468 [6,104–9,460]	23,397 [19,639–33,139]*	23,862 [18,343–31,734]*	33,070 [25,170–39,022]*	<0.001
PlGF/(sFlt-1 + sEng)	0.033 [0.011–0.077]	0.056 [0.029–0.111]	0.002 [0.001–0.005]*	0.002 [0.001–0.004]*	0.001 [0.001–0.002]*	<0.001
VEGF, pg/mL	7 [5–13]	10 [6–25]	7 [6–9]	11 [6–14]	7 [6–8]	0.158
Inhibin A, pg/mL)	724 [491–904]	330 [261–928]	2,097 [1,546–2,660]*	1,269 [760–2,348]*	1,876 [1,239–2,295]*	<0.001
PP13, pg/mL	363 [295–608]	341 [264–520]	479 [348–651]	344 [258–425]	371 [276–512]	0.415
TNFα, pg/mL	28 [14–156]	146 [53–410]	34 [19–104]	69 [18–333]	42 [22–103]	0.220
Uterine artery Doppler						
RI	0.56 [0.52–0.61]	0.57 [0.52–0.62]	0.64 [0.58–0.71]*	0.75 [0.66–0.78]**	0.73 [0.71–0.77]**	<0.001
PI	0.68 [0.66–0.70]	0.70 [0.64–0.61]	0.80 [0.60–1.17]*	1.35 [1.05–1.66]**	1.42 [1.25–1.56]**	<0.001
PSV	59.7 [58.3–60.8]	59.1 [58.3–62.0]	60.8 [57.7–63.9]	60.0 [56.9–62.1]	60.4 [59.0–62.6]	0.829
EndoPAT						
RHI	1.61 [1.59–1.65]	1.47 [1.44–1.62[	1.69 [1.67–1.97]	1.85 [1.81–2.07]*	1.82 [1.66–1.91]*	0.048
Aix, %	−4.0 [−8.0 to 5.0]	−4.5 [−12.0 to 2.0]	9.0 [8.9 to 12.0]**	−1.0 [−7.0 to 10.0]	8.0 [4.0 to 15.0]**	0.035

b All deliveries <34 weeks

	PTD (n = 6)	PE (n = 10)	IUGR (n = 12)	IUGR+PE (n = 28)	p
Gestational age at testing, weeks	29.2 [26.8–31.6]	29.9 [27.5–32.3]	29.3 [27.7–30.8]	29.9 [28.9–30.9]	0.570
sFlt-1, pg/mL	2,970 [1,180–4,761]	25,692 [8,976–42,408]**	11,880 [7,501–16,260]*	19,866 [15,128–24,604]**	0.001
PlGF, pg/mL	762 [182–1,343]	215 [0–479]*	70 [27–113]*	103 [39–167]*	0.009
sFlt-1/PlGF	6 [0–13]	521 [246–796]**	307 [174–439]*	460 [273–647]**	0.004
sEng, pg/mL	6,498 [4,792–8,204]	34,067 [26,536–41,598]**	23,550 [16,171–30,928]*	34,409 [27,640–41,178]**	<0.001
PlGF/(sFlt-1+sEng)	0.090 [0–0.182]	0.009 [0–0.025]**	0.002 [0–0.004]**	0.003 [0–0.005]**	0.004
VEGF, pg/mL	14 [0–29]	25 [0–59]	16 [3–29]	9 [5–12]	0.544
Inhibin A, pg/mL	457 [0–1,015]	3,216 [2,212–4,220]**	1,503 [1,019–1,987]*	2,384 [1,711–3,057]**	0.001
PP13, pg/mL	505 [231–780]	439 [279–598]	387 [223–552]	451 [311–591]	0.609
TNFα, pg/mL	770 [0–1,950]	185 [0–420]*	149 [34–264]*	161 [2–320]*	0.051
Uterine artery Doppler					
RI	0.57 [0.49–0.65]	0.65 [0.56–0.74]*	0.76 [0.72–0.80]**	0.73 [0.70–0.76]**	0.003
PI	0.69 [0.57–0.80]	1.20 [0.83–1.57]*	1.62 [1.35–1.90]**	1.43 [1.27–1.58]**	0.003
PSV	58.5 [57.9–59.2]	59.9 [52.6–67.2]	60.7 [58.7–62.8]	60.9 [58.5–63.3]	0.461
EndoPAT					
RHI	1.5 [1.4–1.6]	1.7 [1.6–1.7]	1.9 [1.8–2.1]*	1.7 [1.7–1.9]	0.047
Aix, %	−3.0 [−17.0 to 22.0]	9.0 [8.9 to 9.1]*	3.0 [−12.0 to 32.0]	6.0 [−15.0 to 25.0]	0.048

Data are presented as the mean [95% CI]. Non-parametric tests for independent samples were applied for evaluating the significant differences for a given parameter among the study groups for the continuous variables. p values were generated using the Kruskal-Wallis non-parametric test. Comparisons between the reference group (the unaffected group in **a**, or the PTD group in **b**) and the clinical complication test groups were performed with the Mann-Whitney non-parametric test. * $p < 0.5$, ** $p < 0.01$.

the definition of PE [11]). The blood pressure values of PE and PE+IUGR were both significantly higher for IUGR alone (131/80 mm Hg), which was significantly higher compared with PTD (119/76 mm Hg) and unaffected controls (112/71 mm Hg; Table 1a).

At delivery the mean blood pressure of PE was 143/87 mm Hg and the blood pressure of PE+IUGR was 140/83 mm Hg. The relatively lower blood pressure at delivery reflects the impact of anti-hypertensive drugs (mainly methyldopa), which, according to the guidelines of the medical center, should be given to all patients admitted with hypertensive disorders. Such changes were also detected for the subgroups, as detailed in Tables 1b and online suppl. Table 1a, b.

Proteinuria

Proteinuria values were significantly higher in the PE and PE+IUGR groups compared to the pure IUGR group (Tables 1a, b; online suppl. Table 1a, b). Protein in urine was not assessed in unaffected and PTD cases.

Chronic Hypertension

Chronic hypertension was much more prevalent in the PE and PE+IUGR groups (superimposed PE).

Other Risk Factors

Other risk factors, including conception by in vitro fertilization, previous history of PE, chronic diabetes mellitus, or polycystic ovary syndrome, were not significantly different between the groups (Table 1a, b; online suppl. Table 1a, b).

Birthweight

Baby birthweight of PE and PTD in the entire study cohort was reduced compared to unaffected controls (Table 1a). In the IUGR+PE and pure IUGR groups, baby birthweights were further reduced compared to pure PE and PTD (Table 1a). In the comparison of the subgroups (Table 1b; online suppl. Table 1a, b), baby birth weight was not significantly different between pure PE and PTD, while pure IUGR and PE+IUGR always showed a significantly reduced birthweight.

GA at Delivery

GA at delivery revealed that a large proportion of the cases delivered prematurely, before 34 weeks or between 34 and 37 weeks, indicating a link between complications of all kinds and early delivery.

Differences across Markers in Clinical Groups and Pathology Subgroups

Serum Biomarkers

Anti- and Proangiogenic Markers. For the entire cohort, the proangiogenic factor PlGF was significantly lower and the anti-angiogenic factors sFlt-1 and sEng were significantly higher in the pure PE, pure IUGR, and PE+IUGR groups compared to PTD and the unaffected controls, when examined for the group as a whole or for the subgroups. The level of sFlt-1 was always higher in pure PE compared to pure IUGR, although the differences were not always significant (Table 2a, b; online suppl. Table 2a, b). The ratio of pro-/anti-angiogenic markers (PlGF/[sFlt-1 ± sEng]) was an order of magnitude lower for pure PE, pure IUGR, and PE+IUGR compared to the unaffected controls (Table 2a, b; online suppl. Table 2a, b). The anti-angiogenic ratio of sFlt-1/PlGF was significantly higher in pure PE, pure IUGR, and PE+IUGR (Table 2a, b; online suppl. Table 2a, b). In the early delivery groups, it was interesting that the ratio was highest in pure PE than in pure IUGR (Table 2a, b; online suppl. Table 2a, b), and that in any category it was higher in the early compared to the term groups (Table 2a, b; online suppl. Fig. 3a, b).

VEGF. The level of VEGF was not significantly different between clinical groups in any of the early, intermediate, or term periods (Table 2a, b; online suppl. Table 2a, b).

Inhibin A. Inhibin A had a similar pattern of increased values for pure PE, pure IUGR, and PE+IUGR, similar to the profile of the anti-angiogenic factors. It was consistently higher for cases with PE in all subgroups, although the value spread made it hard to differentiate pure PE from IUGR with and without PE (Tables 2b; online suppl. Table 2a, b).

PP13. PP13 exhibited a trend towards elevation in the PE group (Table 1a, b, 2a, b; online suppl. Table 2a, b), but only reached significantly higher values in the intermediate group for pure PE and PE+IUGR (Table 1a, b; online suppl. Table 2a, b).

TNFα. TNFα was significantly lower in PE, IUGR, and PE+IUGR compared to PTD <34 weeks (Table 2b), and was significantly higher in the pure IUGR and PE+IUGR groups >37 weeks (Table 2a; online suppl. Table 2b).

Biophysical Markers

MAP. MAP was significantly higher in the pure PE and PE+IUGR groups across any of the term, early, and intermediate subgroups (Table 1a, b; online suppl. Table 1a, b).

Doppler RI. Doppler RI was higher in pure PE and much higher in pure IUGR and in PE+IUGR compared to the unaffected controls and the PTD group for the entire cohort (Table 2a), and for the early subgroup (Table 2b), but not for the term or intermediate subgroups (online suppl. Table 2a, b).

Uterine Artery Doppler PI. Uterine artery Doppler PI was highest in both pure IUGR and IUGR+PE, and then in pure PE for the entire cohort and for the early subgroups (Tables 2a, b). Values were not significantly different for the other term and intermediate subgroups (online suppl. Table 2a, b).

Doppler PSV. Doppler PSV was not different between the study groups (Tables 1a, b, 2a, b; online suppl. Table 2a, b).

RHI. For the entire cohort, RHI of the EndoPAT measures was significantly higher for pure PE and for PE+IUGR (Table 2a), but such differences were not maintained for the analysis of early, intermediate, or term subgroups (Tables 2b; online suppl. Table 2a, b).

Aix. The Aix % measure of EndoPAT was significantly higher for pure PE in the early subgroup (Table 2b), the intermediate subgroup (online suppl. Table 2a, b), and the entire cohort (Table 2a). In the latter and at term Aix % was also significantly higher for PE+IUGR (Tables 2a; online suppl. Table 2a).

Box Plot Analysis

Serum Biomarkers

In addition to the differences in the medians already shown, the box plots also depict the interquartile differences (Fig. 1, 2; online suppl. Fig. 3a, 4a). For the proangiogenic factors, the median was lower for pure PE, pure IUGR, and PE+IUGR when the entire cohort was examined or across subgroups. Values were distributed compactly around the medians (Fig. 1b, 2b; online suppl. Fig. 3b, 4b).

For the anti-angiogenic factors sFlt-1 and sEng, the picture was the opposite, with higher values for the pure PE, pure IUGR, and PE+IUGR cases in the entire cohort and across subgroups. There was a very wide spread of values between the medians to the upper and lower quartiles (Fig. 1a, 2a, for sFlt-1, Fig. 1d, 2d for sEng; online suppl. Fig. 3a, d, 4a, d).

The anti-angiogenic ratios (sFflt-1/PlGF) were higher for pure PE, pure IUGR, and PE+IUGR, and had a wide, even spread above and below the medians. Interquartile ranges were far from the medians and the spread was similar above and below the medians (Fig. 1c, 2c; online suppl. Fig. 3c, 4c).

The proangiogenic ratio of PlGF/(sFlt-1 + sEng) indicated a significant reduction for pure PE, pure IUGR, and PE+IUGR. This was manifested for the entire cohort and also for the subgroups. The interquartile values were very near the medians on both sides (Fig. 1e, 2e; online suppl. Fig. 3e, 4e). Medians for VEGF were similar among PE, IUGR, and PE+IUGR, and the distributions around the medians were not significant except for pure IUGR (Fig. 1f, 2f; online suppl. Fig. 3f, 4f).

While inhibin A appears to be higher for pure PE across all subgroups, the interquartile distribution was not even for the lower and higher quartiles, and with many extreme values on either side. It made inhibin A a poor differentiator between pure PE, pure IUGR, and PE+IUGR (Fig. 1g, 2g; online suppl. Fig. 3g, 4g).

The PP13 profile had a large uneven distribution around the medians, but the differences between medians in the intermediate group were significant, with many extremely high outliers for pure PE at 34–37 weeks (online suppl. Fig. 4h). The large value spread made it difficult to use it for pathology differentiation (Fig. 1h, 2h; online suppl. Fig. 3h, 4h).

For TNFα, many cases of PTD had higher values. This was most apparent for the early cases <34 weeks (Fig. 2i). The value also tended to be high in term pure IUGR cases (online suppl. Fig. 3i). In early (<34 weeks) PTD (Fig. 2i) and in term (>37 weeks) pure IUGR cases (online suppl. Fig. 4i), the upper quartiles of TNFα were higher than the lower quartiles, probably reflecting diversity in the origin of the disorder.

For both RHI and Aix %, all values for pure PE were not only 10 times higher compared to unaffected controls or PTD, but they were all packed and unified around the medians. This "packing" and low diversity were extremely unified for the early cases (Fig. 2j, k), but much less so for the term cases or the entire group (Fig. 1j, k; online suppl. Fig. 3j, k).

Discussion

The aim of this study was to evaluate whether a large marker profile that is generated at the time of complications could be developed to improve the detection of major pregnancy complications near the time of delivery. This could potentially assist in directing clinical management. The clinical triage takes into consideration the health of the fetus (iatrogenic PTD, pure IUGR), the mother (pure PE), and both (PE combined with IUGR). Accordingly, we have tried to evaluate whether key biophysical and biochemical markers could help in identifying the severity of the complications in the context of gestational age, which is crucial for fetal development [12–14, 19].

Our results have indicated that for iatrogenic PTD, unassociated with PE or IUGR, and especially in cases presenting before 34 weeks' gestation, an extremely elevated TNFα level is a differential predictive marker to optimize the time for delivery. The diversity in the upper quartile may reflect a polymorphism of TNFα [48]. For PTD cases, this marker returned to normal in subgroups of delivery >34 weeks. Given the limited size of our cohort, we are not saying that TNFα is a "game changer" for early PTD, but it is worth testing in additional studies.

On the other end, in the case of IUGR, our results indicated that at term (gestational week ≥37) there might be a benefit of combining TNFα with sonographic assessment (mainly uterine artery PI). This might add value to the currently used sonographic assessment of fetal blood flow to the essential organs (heart, brain, liver, etc.). Such an additional combination of TNFα and uterine arteries PI should thus be considered when determining the best time for delivery in cases suspected of having term IUGR [3, 12, 47, 49]. This observation may also warrant testing in larger cohorts.

For pro- and-anti-angiogenic factors, our findings are consistent with large-scale studies showing that high levels of anti-angiogenic factors and low levels of the proangiogenic factors become evident from 5 weeks before delivery [13, 15–19]. Both ratios are larger in cases of pure

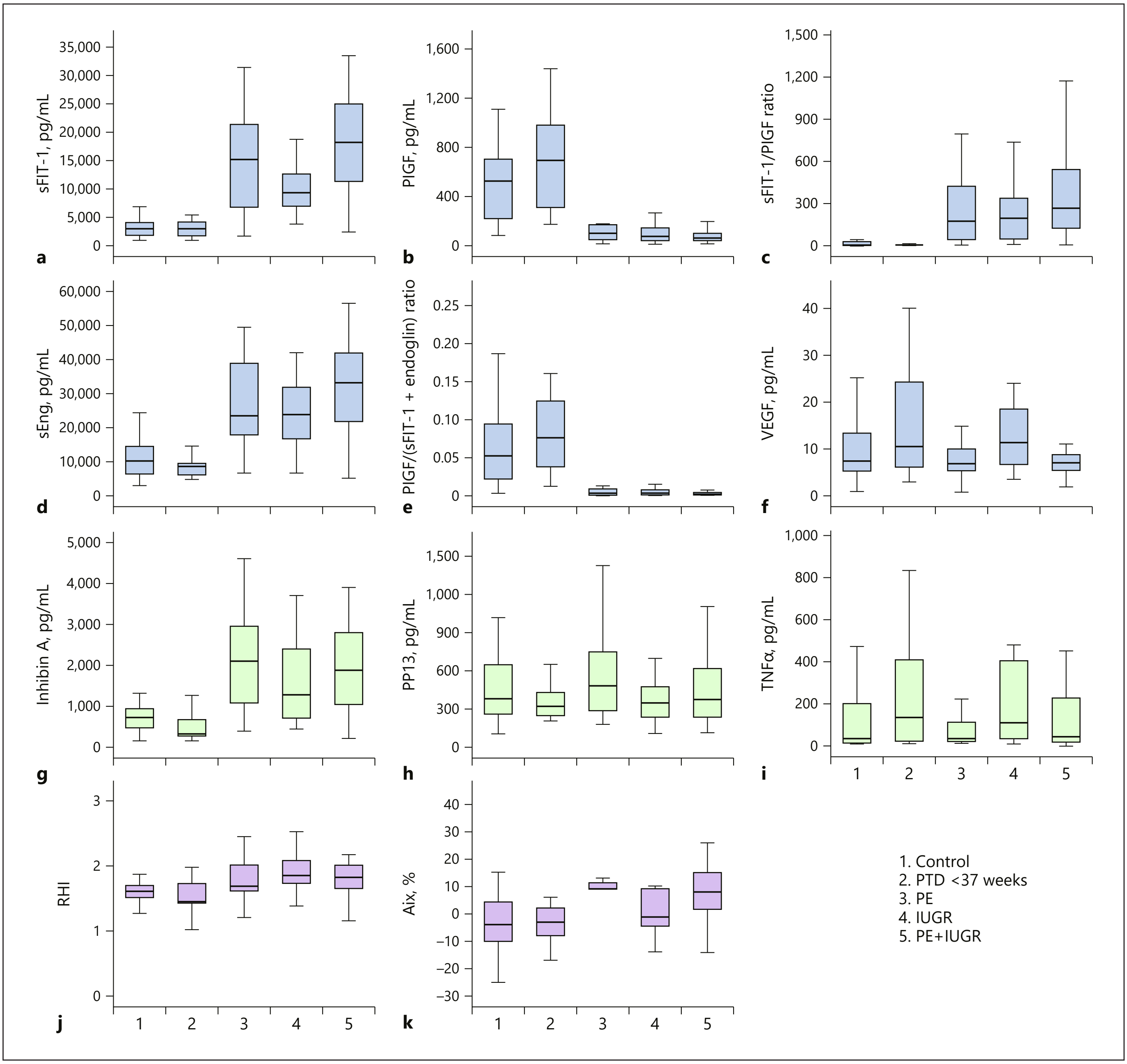

Fig. 1. Box plot analysis of marker levels of all patients. The results for the entire cohort compared to unaffected controls: sFlt-1 (**a**), PlGF (**b**), sFlt-1/PlGF ratio (**c**), sEng (**d**), PlGF/(sFlt-1 + endoglin) ratio (**e**), VEGF (**f**), inhibin A (**g**), PP13 (**h**), TNFα (**i**), RHI (**j**), Aix (**k**). The controls were women who delivered a healthy baby without complications at term. PE, pure preeclampsia not accompanied by fetal growth restriction; IUGR, pure intrauterine growth restriction not accompanied by preeclampsia; PE+IUGR, women who had the combined pathologies; PTD, preterm delivery (<37 weeks) unrelated to PE or IUGR. The values in each group include all patients with these pathologies.

PE compared to pure IUGR. There are multiple high-quality studies that have demonstrated high ratios of anti-/proangiogenic factors or low ratios of pro-/anti-angiogenic factors in pure PE, pure IUGR, and PE combined with IUGR [50, 51]. Such very clear and significant differences of these values were unequivocally shown here as well as in many other studies compared to the unaffected controls. Recent evidence has indicated that in

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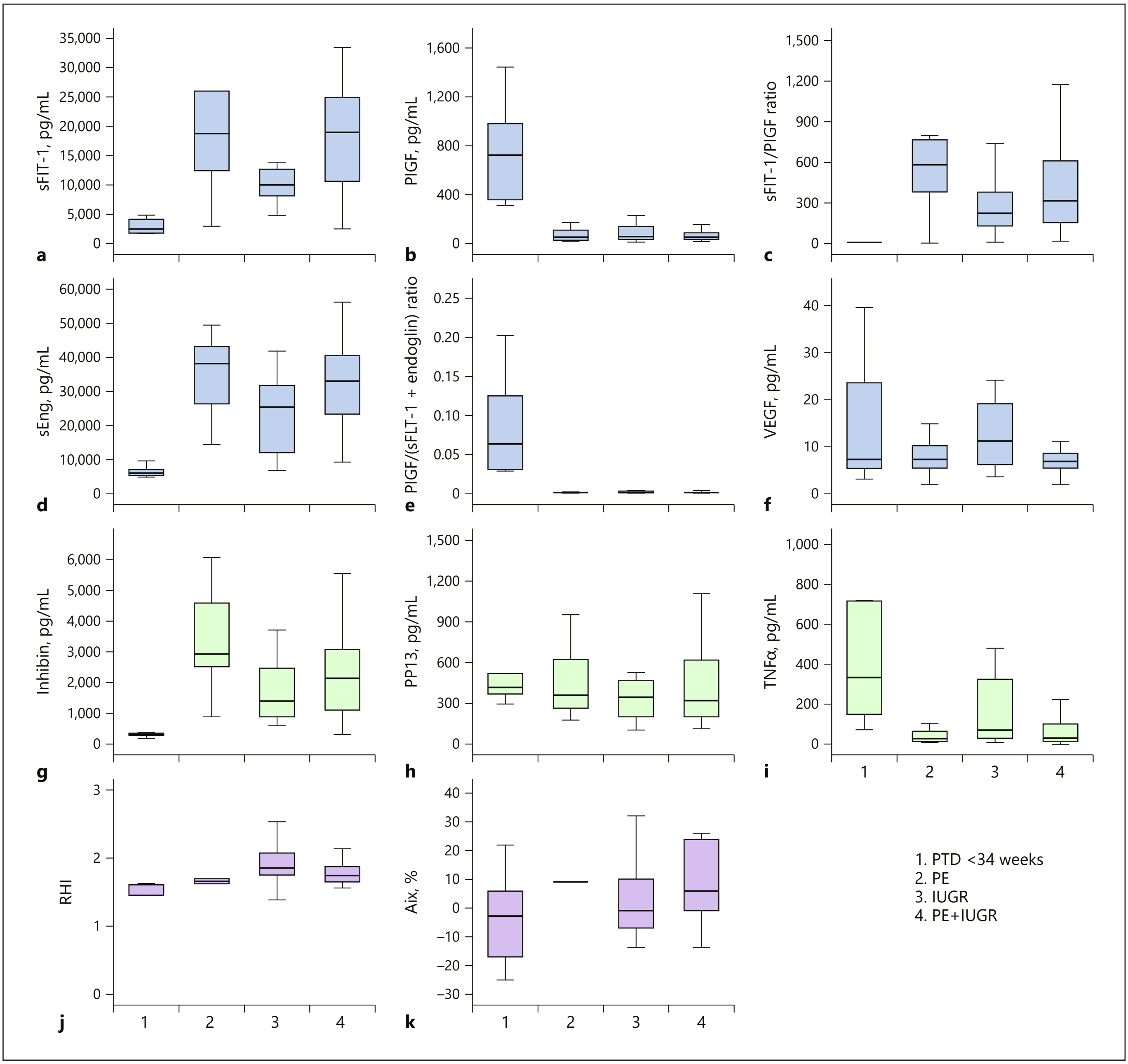

Fig. 2. Box plot analysis of marker levels for patients who delivered <34 weeks. The results for patients who delivered before 34 weeks compared to the PTD group that delivered before 34 weeks: sFlt-1 (**a**), PlGF (**b**), sFlt-1/PlGF ratio (**c**), sEng (**d**), PlGF/(sFlt-1 + endoglin) ratio (**e**), VEGF (**f**), inhibin A (**g**), PP13 (**h**), TNFα (**i**), RHI (**j**), Aix (**k**). PE, pure preeclampsia not accompanied by fetal growth restriction; IUGR, pure intra uterine growth restriction not accompanied by preeclampsia; PE+IUGR, women who had the combined pathologies; PTD, preterm delivery (<34 weeks) unrelated to PE or IUGR.

cases of pure PE the ratio is likely to be surmounted and that a sudden rapid increase can assist in selecting the time of delivery, whereas in IUGR the increase is more moderate. In this regard, our study echoed these findings for comparing cases and unaffected controls. Our study indicated that there may be additional markers that could aid differentiating between pure IUGR, pure PE, and PE+IUGR.

Of the Doppler markers [46, 47, 49] assessed here, PSV did not discriminate among any of the pathologies. Uterine artery resistance (RI) was also non-informative. Thus, for these markers, we have found, like several former studies, that both are not very useful tools for discriminating between the pathologies. This is said with caution, as in the case of early pure IUGR, RI might be useful. The PI was significantly higher in IUGR. In our study there was a considerable overlap between pure IUGR (fetal) and IUGR+PE (maternal and fetal pathologies combined). Accordingly, it appears that PI on its own may not be a good choice for a differential estimate of the pathologies. At the same time, it is likely to be a useful parameter to help show adverse effects on the fetus.

For EndoPAT, we found that the Aix % was an order of magnitude higher for pure PE, and was a strong differentiator of this complication for early pure PE (<34 weeks). Aix % is a parameter used to evaluate vasoconstriction and arterial stiffness, both known features of the early pure PE (a maternal disease). Between 34 weeks to term there was a slow growth of the EndoPAT parameters. For the pure PE group, RHI increased from 1.85 to 2.05 (11% growth), and Aix % increased from 9.0 to 11.5 (28% growth). While not longitudinally measured in the same patient, this cross-section analysis of values may tell us that there was an increase over time in both parameters. If so, it implies that the level of arterial stiffness is elevated. The measurements with the EndoPath sheds lights on the potential evolvement of a maternal defective cardiovascular system in pure PE and in the combined maternal and fetal pathology of PE+IUGR at this stage [2, 5].

A higher Aix % is associated with a faster pulse wave that moves away from the heart and then returns earlier, thus further augmenting Aix [52]. This effect is further increased with higher MAP, advanced maternal age, and faster heart rate, all of which are symptoms of PE. Since Aix % is also an independent risk factor for CVDs, its higher value in early PE may account for an increased risk for developing CVDs later in life after PE, mainly after early PE [53].

Although found to be higher, the Aix % value may be underestimated. The medical center guideline to treat hypertensive patients attending the clinic with an anti-hypertensive drug involves the frequent use of methyldopa. This is an anti-alpha-2-adrenergic agonist that blocks sympathetic activity, and decreases cardiac output, heart rate, and contractility. Accordingly, the drug reduces sympathetic output to the vascular system, which is followed by a decreased vascular tone, increased vasodilation, reduced vascular resistance, and decreased arterial pressure. All these effects may cause a reduction in the measurements of Aix %. Thus, it is possible that the actual Aix % value may be even higher than measured [56–58]. However, since the Aix % measured here was in any event an order of magnitude higher in the PE cases compared to unaffected and PTD cases, it implies that the effect of the anti-hypertensive drug was marginal [59]. Further investigations are needed to verify the true Aix % values without the methyldopa treatment.

Numerous studies have challenged the usefulness of blood pressure (systolic, diastolic, or MAP) measurements for the diagnosis of PE [15–19, 54]. Here, we have found that hypertension is a very strong marker of pure PE and of PE combined with IUGR. The values measured here appear to be higher in PE and PE+IUGR compared to pure IUGR. Therefore, measuring MAP appears to remain a tool with which to differentiate both from pure IUGR. Difficulties in obtaining correct blood pressure values may be driven by the use of devices that were not properly calibrated, the lack of cuff size adjustment to arm size, or other low-precision handling of such measurements [36, 54].

In this study, proteinuria determined by dipstick measurements successfully differentiated pure PE and PE with IUGR from pure IUGR, especially in the early cases (<34 weeks). Nevertheless, this parameter was difficult to interpret around term, as has been discussed elsewhere [55].

Strengths and Weaknesses

The study has developed some promising tools that might be useful as an approach for generating differentiating marker profiles to assist in increasing the accuracy of prediction of pregnancy complications near delivery. The prime study weakness was the small cohort size. Our study did not intend to introduce new methods of routine screening. Rather, it aimed to identify pointers to be verified in larger series of pregnant women, and to offer better tools for differential assessment of the complications of fetal or maternal etiologies, or the two combined. In these regards, the study, if repeated in larger cohorts, might help to develop future clinical value for mapping marker profiles to assist in clinical management. The cohort was small and warrants testing in larger studies. It may yet serve to set up better cohorts and groups to study the etiologies of these pathologies. Today, not all major pregnancy complications can be identified in screening methods in the 1st and 2nd trimesters. Therefore, markers near the time of delivery are required. There is a clear need for developing marker toolboxes to improve the differential profile of markers in different pregnancy complications near delivery, combining classical tests of pregnancy complications (blood pressure and urine protein), with state-of-the-art immune-diagnostic methods. Biochemical, imaging, and biophysical measures appear to be promising. In this context, the use of the EndoPAT provided an additional measure to evaluate arterial stiffness and endothelial dysfunction. Future studies will show its potential use for a proper clinical approach to manage pregnancy complications near delivery. Preferentially, the measurement in such studies should be made prior to any use of anti-hypertensive drugs.

One may ask why we use predictive markers as a potential method for developing future tools to direct clinical decision. Today a "gray zone" has been formed where predictive markers are often used in clinical management. For example, the ASPRE set of biophysical and biochemical markers and the respective algorithm are predictive tools, but they are currently used to direct the clinical decision of treating with aspirin [35]. In Germany, the PlGF/sFlt-1 ratio is used to identify which women can go home and return in a few days and which should be admitted to the hospital because severe PE/PE+IUGR is anticipated to outbreak shortly [45]. Accordingly, it is estimated that the development of a battery of tools that may differentiate between the pathologies by using predictive markers may turn into a useful toolbox to direct clinical management.

Conclusion

Elevated TNFα emerged as a promising marker for identifying iatrogenic early PTD (<34 weeks). When combined with sonographic measures of blood flow to fetal essential organs [25, 27], TNFα appears to be able to assist in orienting clinical decisions in cases of IUGR, especially around term.

Our findings also showed that anti-angiogenic and angiogenic factors, their ratios, or inhibin A are useful biomarkers to identify PE, IUGR, or the presence of both combined. Nevertheless, according to our findings they were not sufficiently powerful to differentiate these pathologies from one another. It remains to be seen how they can help in directing clinicians to select a treatment according the benefit of the fetus, the mother, or both.

The high values of Aix % measured by EndoPAT appears useful for pointing out the accurate prediction of pure PE, especially in its early form. It also assists in identifying PE combined with IUGR at term. In this context, additional studies are required to develop clinical implications for the benefits of this tool in terms of prenatal management near delivery to further disentangle fetal, maternal, or combined complications. Further studies are warranted with larger cohorts.

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Statement of Ethics

The National Medical Ethics Committee of the Republic of Slovenia approved the study (approval No. 104/04/12).

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Author Contributions

All authors contributed to the preparation of the manuscript. J.O., K.K., T.P.S., and N.T. prepared the clinical study protocol and obtained the ethics approval. T.P.S., V.F.V., and N.T. enrolled the patients to the ObGyn clinics, obtained the signatures for informed consent, and conducted all clinical evaluation and management. K.K. and V.F.V. performed the immunodiagnostic tests. T.P.S. directed all biochemical testing and blood pressure measurements. N.T. performed all ultrasound testing. K.K. and T.F. performed the measurements of EndoPAT. A.S.-N. and H.M. built the database and conducted the mathematical and statistical analysis and modeling together with K.H.N.

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