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Predicting Early Preterm Delivery and Late Fetal Growth Restriction by TNFα

Adi Sharabi-Nov[1,2], Vesna Fabjan Vodušek[3], Tanja Premru Sršen[3,4], Kristina Kumer[5,6], Teja Fabjan[5], Nataša Tul[4,7], Josko Osredkar[4,5,6], Kypros H Nicolaides8, Berthold Huppertz[9] and Hamutal Meiri[10]*

[1]*Department of Statistics, Tel Hai Academic College, Tel Hai 122103, Israel*

[2]*Department of Statistics, ZivMedical Center, Safed 131000, Israel*

[3]*Department of Perinatology, Division of Obstetrics and Gynecology, University Medical Center, Zaloška Cesta 2, 1000 Ljubljana, Slovenia*

[4]*Faculty of Medicine, University of Ljubljana, Vrazov Trg 2, 1000 Ljubljana, Slovenia*

[5]*Institute of Clinical Chemistry and Biochemistry, University Medical Center, Njegoševa 4, 1000 Ljubljana, Slovenia*

[6]*Faculty of Pharmacy, University of Ljublijana, Cesta 7, 1000, Ljubljana, Slovenia*

[7]*Women's Hospital, Pre˘cna Ulica 4, 6230 Postojna, Slovenia*

[8]*The Fetal Medicine Research Institute, King's College Hospital, 16-20 Windsor Walk, London SE5 8BB, UK*

[9]*Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz; Graz 8010, Austria*

[10]*TeleMarpe Ltd., 41 Beit El St., Tel Aviv 6908742, Israel*

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***Corresponding author:** Hamutal Meiri, TeleMarpe Ltd, 41 Beit El St, Tel Aviv 6908742, Israel

Abstract

We evaluated tumor necrosis factor alpha (TNFα) and uterine artery pulsatility index (UtA-PI) in the triage of patients with suspected preterm delivery (PTD), preeclampsia (PE), fetal growth restriction (FGR), and PE+FGR. The study included 125 pregnant women attending high-risk pregnancy clinics for triage of pregnancy complications. There were 31 pure PE cases, 42 cases of PE combined with FGR, 16 pure FGR cases, 15 PTD cases, and 21 term normal delivery controls. Maternal serum TNFα was determined by immune-diagnostic testing. UtA-PI was measured by Doppler sonography. Demographic, medical and pregnancy history, and mean arterial blood pressure (MAP) were extracted from the hospital medical records. Linear regression coefficients, and Box and Whisker plots were calculated and depicted using non-parametric statistics (Kruskal Wallis and Mann–Whitney). Spearman's regression coefficient assessed marker accuracy; $p<0.05$ was considered significant. It was found that high TNFα in cases <34 weeks gestation, when coupled to low UtA-PI and normal blood pressure are found in early PTD most likely linked to maternal inflammation. At term, high TNFα combined with high UtA-PI is associated with any FGR (with/without PE), possibly reflecting inflammation and maternal and fetal hypoxia due to the very long period of altered placental perfusion. Accordingly, TNFα, and Doppler UtA-PI could be used for the differential diagnosis of early PTD, and FGR (with/without PE) near delivery.

Keywords: Fetal growth restriction; Gestational week; Inflammation; Mean arterial blood pressure; Placental hypoxia; Placental perfusion; Preeclampsia; Pregnancy

Abbreviations: FGR: fetal growth restriction; GA: gestational week; MAP: mean arterial blood pressure; PE: preeclampsia; TNFα: Tumor necrosis factor alpha; PTD: preterm delivery; PPROM: premature rupture of the membranes; Endo-PAT: endothelial peripheral arterial tonometry; ISSHP: the International Society for the Study of Hypertension in Pregnancy

Introduction

Tumor necrosis factor alpha (TNFα) is as a major regulator of inflammatory responses in various inflammatory and autoimmune diseases, and is mainly generated by activated macrophages, T-lymphocytes, and natural killer cells Bradley [1], Horiuchi et al. [2]. TNFα binds to two different receptors, triggering signaling pathways involving other cytokines and chemokines, underlying inflammation and cell death Idriss & Naismith [3]. Inappropriate or excessive activation of TNFα signaling is associated with chronic inflammation and can eventually lead to

the development of complications such as autoimmune diseases. In pregnancy, TNFα influences hormone synthesis, placental architecture, and embryonic development, while increased TNFα levels in complicated pregnancies draw attention to trophoblast biology Romanowska-Próchnicka et al. [4]. Elevated TNFα may affect maternal-fetal interactions by altering the secretory profile of placental immunomodulatory factors, which in turn affect maternal immune cells Romanowska-Próchnicka et al. [5], Azizieh & Raghupathy [6]. While evidence linked TNFα to early placentation and growth, further evidence has shown increased levels near delivery Romanowska-Próchnicka et al. [5]. Overexpression of TNFα is linked to pro-inflammatory cytokines and the development of fetal growth restriction (FGR) in response to fetal hypoxia, possibly by decreasing the uptake of amino acids by the fetus Bartha et al. [7]. Increased TNFα was reported in preterm delivery (PTD) associated with premature rupture of the membranes (PPROM) and preeclampsia (PE) Gücer et al. [8], Bartha et al. [7]. In first trimester pregnancies, TNFα levels are twice higher in women at high risk of a subsequent development of PE, and at term it could reach five times higher levels in PE cases versus normal term delivery Trisnawati et al. [9].

In previous studies of this Slovenian cohort, we evaluated the uterine artery pulsatility index (UtA-PI) in differentiating PTD, PE and FGR. Using a UtA PI cutoff = 0.85, all cases of term controls and PTD <37 weeks gestation were significantly lower from all cases of FGR and PE+FGR Sharabi-Nov et al. [10]. Previous studies indicated that differential diagnosis of PE and PE+FGR are assisted by pro- and-anti-angiogenesis markers, inhibin A and the use of Endo-PAT (endothelial peripheral arterial tonometry) Sharabi-Nov et al. [11], Kumer et al. [12]. Here, we conducted a secondary analysis to explore whether the maternal serum level of TNFα, an inflammation markers could serve for the differential diagnosis of the complications alone, and whether vascular physiology that can be assessed by the blood flow through the uterine arteries measured by the Doppler pulsatility index (UtA-PI) and blood pressure measured by mean arterial pressure (MAP) could assist in the differential diagnosis of these complications from mid-gestation till term delivery.

Materials and methods

Samples and patients

Our dataset for this secondary analysis is based on patient records collected between 2012 and 2015 Sharabi-Nov et al. [11], Kumer et al. [12]. The National Medical Ethics Committee of the Republic of Slovenia approved the study (No. 104/04/12). The cohort database included women who signed their informed consent. Medical and delivery records were extracted from the outpatient clinics of the Department of Obstetrics and Gynecology of the University Medical Center of Ljubljana, Slovenia, attended for suspected PE, FGR, PE+FGR and PTD. Control term delivery (TD) cases were admitted due to a history of these complications in previous pregnancies. Excluded were women at gestational week <24, those in labor at enrolment, younger than 18 years, those with multifetal pregnancies, and major fetal malformation or chromosomal/genetic anomalies. We also excluded patients with pre-existing renal, hematological, autoimmune conditions, or chorioamnionitis along with mental disorders jeopardizing informed consent reliabillity.

TNFα measurements

TNFα was measured by immune-diagnostics. Ten ml blood were drawn into a vacutainer at enrolment, left for 1.5 h to clot at room temperature, then centrifuged at 1,500 x g for 15 min. Serum was collected and aliquots were kept at -70°C, and thawed once for testing in a LAB microplate analyzer (Adaltis, Italy). TNFα kits (International GMBH, Hamburg, Germany) were used according to the manufacturer's instructions. The minimal detectable concentration was 5 pg/ml with inter- and intra-assay coefficients of variation of 8.1% and 7.7%.

Blood pressure measurements

Data of the blood pressure were extracted from the medical records from measurements performed according to the FMF guidelines using arm-adjusted cuffs, and a pre-calibrated automated device (OMRON M6 Comfort, Omron Healthcare Co., Ltd., Kyoto, Japan) Poon et al. [13]. Mean arterial blood pressure (MAP) was calculated according to (systolic+diastolic*2)/3 Poon et al. [13].

Uterine artery pulsatility index

Data were extracted from the medical records from records made with trans-abdominal sonography with a GE Voluson U6 and GE Voluson 8Expert and a 2-7 MHz GE RAB6-D probe (GE Healthcare GmbH, Solingen, Germany). The A pulsed Doppler sampling gate of 2 mm was used to cover each vessel, and an angle of insonation <30° with peak systolic velocity of >60 cm/sec was used to obtain the necessary waveforms before calculating the average of the pulsatility index in the left and right uterine arteries Oros et al. [14], Sharabi-Nov et al. [10].

Definitions of the clinical complications

Preeclampsia (PE) was diagnosed according to the American College of Obstetricians and Gynecologists, and the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines at the time of admission (American College of Obstetricians and Gynecologists 2020, Magee et al. [15]). Diagnosis of PE required the presence of new-onset hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) at ≥ 20 weeks' gestation or chronic hypertension and either proteinuria (≥ 300 mg/24 h or protein-to-creatinine ratio ≥ 30 mg/mmol or ≥ 2+ on dipstick testing) or evidence of renal dysfunction (serum creatinine > 97 μmol/L), hepatic dysfunction (transaminases ≥ 65 IU/L) or hematological dysfunction (platelet

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count <100x103/mL). Fetal growth restriction (FGR) was defined according to ISUOG criteria Salomon et al. [16]. Preterm delivery (PTD) was defined as delivery <37 weeks' gestation Goldenberg et al. [17].

Results

Cohort Flow Chart and Characterization: This is a secondary analysis included 125 pregnant women who had blood samples drawn at the time of enrolment. There were 21 cases of TD, 15 PTD cases (6 delivered <34 weeks gestation, and 9 between 34 to<37 weeks). There were 31 PE cases (3 delivered <34 weeks, 8 between 34 to <37 weeks, and 10 at term). There were 16 FGR cases (12 early who delivered <34 weeks, and 4 at term). The group of FGR+PE (not counted in the former groups) included 42 cases (28 delivered <34 weeks, 10 between 34 to <37 weeks, and 4 at term) (Table 1). Among the groups who delivered <34 weeks, most parameters were similar. MAP was significantly higher at enrolment and at delivery in the PE and FGR+PE groups (Tables 1 & 2, A). Delivery by Caesarean section had a higher incidence in FGR+PE, while any of the FGR cases delivered smaller (lower birth weight) newborns (Table 1). Similar differences were recorded among women who delivered between 34 to <37 weeks (Tables 1&2, B), although patients with a higher BMI were found in the FGR+PE group. MAP was higher in any PE patient at enrolment and at delivery. At term delivery, FGR cases had lower birthweight. MAP was higher in any case of PE and FGR+PE at enrolment and at delivery (Tables 1&2, C).

Table 1: Basic Characteristic of pregnancies by complication and gestational age at delivery.

A. Delivery at <34 weeks gestation	**PTD n=6**	**PE n=13**	**FGR n=12**	**FGR+PE n=28**	**P**
Maternal age (y, Median [IQR])	31.5 [30.0-33.0]	35.0 [29.0-38.0]	30.5 [29.0-34.0]	33.0 [29.0-36.5]	0.535
BMI (kg/m^2, Median [IQR])	24.3 [24.0-27.2]	30.1 [26.2-35.2]	25.3 [24.1-26.7]	28.5 [25.5-29.8]	0.101
IVF (%)	0	15.4	0	10.7	0.498
GA at delivery (weeks, Median [IQR])	31.9 [30.4-31.2]	31.3 [30.3-32.4]	29.3 [27.9-31.4]	30.2 [28.6-32.4]	0.517
MAP at delivery (Median [IQR])	88 [87-93]	110* [108-113]	96 [94-104]	104* [98-109]	0.041
Vaginal delivery (%)	80	23.1*	27.3*	7.4**	0.003
Birthweight (g, Median [IQR])	1,790 [1,510-1,890]	1,400 [1,020-1,690]	900** [650-990]	1,100* [830-1,545]	0.012
B. Delivery at 34-36^{+6} weeks gestation	**PTD n=9**	**PE n=8**	**FGR n=0**	**FGR+PE n=10**	**P**
Maternal age (y, Median [IQR])	32.0 [29.0-33.0]	32.0 [29.5-35.5]		33.0 [28.0-36.0]	0.806
BMI (kg/m^2, Median [IQR])	23.8 [22.5-27.1]	26.4 [25.6-29.4]		30.1** [29.5-33.3]	0.032
IVF (%)	0	0		10	0.414
GA at delivery (weeks, Median [IQR])	35.7 [35.1-36.7]	35.4 [34.6-36.1]		34.8 [34.1-35.4]	0.227
MAP at delivery (Median [IQR])	98 [85-103]	106 [99-106]		102 [96-114]	0.242
Vaginal delivery (%)	62.5	62.5		10.0*	0.027
Birthweight (g, Median [IQR])	2,520 [2,315-2,835]	2,655 [2,503-2,985]		1,845** [1,680-2,120]	0.004
C. Delivery at ≥37 weeks gestation	**TD n=21**	**PE n=10**	**FGR n=4**	**FGR+PE n=4**	**P**
Maternal age (y, Median [IQR])	33.0 [29.0-35.0]	29.5 [25.0-30.1]	32.0 [28.5-36.0]	33.5 [28.5-36.5]	0.509
BMI (kg/m^2, Median [IQR])	25.8 [22.6-28.10]	29.9 [28.0-33.5]	33.4 [26.3-40.4]	21.7 [-]	0.088
IVF (%)	4.8	0	0	25	0.433
GA at delivery (weeks, Median [IQR])	39.3 [38.4-40.1]	38.8 [37.3-39.0]	38.0 [37.7-38.6]	37.9 [37.4-38.1]	0.129
MAP at delivery (Median [IQR])	87 [76-91]	109* [106-112]	106* [98-113]	90 [-]	0.006
Vaginal delivery (%)	76.2	60	75	75	0.888
Birthweight (g, Median [IQR])	3,500 [3,070-3,610]	3,250 [2,950-4,000]	2,550* [1,980-3,005]	2,710* [1,710-2993]	0.023

Descriptive Statistics of the cohort features: Median, interquartile range [IQR] and frequency (%) of deliveries <34 wks' gestation (A), deliveries at 34 to 36+6 wks' gestation (B), and deliveries ≥37 wks' gestation (C). Categorical values were compared with the Pearson Chi-Square test. Continuous values were compared with non-parametric tests for two or more independent samples. All groups were compared to the PTD group (A, B) or to the term delivery group (C). We used the Kruskal Wallis a-parametric test to compare the groups. *p<0.05, **p<0.01, ***p<0.001
TD - term delivery, PTD - preterm delivery, PE - preeclampsia, FGR - fetal growth restriction, IQR - interquartile range, MAP – mean arterial blood pressure, IVF – in-vitro fertilization, UtA PI – uterine artery pulsatility index, GA - gestational age, BMI - body mass index

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Table 2: Blood measurement values by complication and gestational age at enrolment.

A. Delivery at <34 weeks gestation	PTD n=6	PE n=13	FGR n=12	FGR+PE n=28	P
GA (wks, Median [IQR])	29.6 [28.9-31.0]	31.0 [30.0-32.1]	29.0 [27.6-31.1]	29.9 [28.3-32.1]	0.570
Maternal age (y, Median [IQR])	31.5 [30.0-33.0]	35.0 [29.0-38.0]	30.5 [29.0-34.0]	33.0 [29.0-36.5]	0.535
MAP (Median [IQR])	88 [87-93]	114*** [109-119]	97 [91-99]	113*** [106-123]	<0.001
UtA PI (Median [IQR])	0.65 [0.60-0.81]	1.16* [0.62-1.60]	1.59*** [1.44-1.66]	1.48** [1.25-1.62]	0.003
TNFα (pg/ml, Median [IQR])	335 [146-715]	31* [17-93]	51* [12-332]	39* [18-103]	0.050
B. Delivery at 34-36^{+6} weeks gestation	**PTD n=9**	**PE n=8**	**FGR n=0**	**FGR+PE n=10**	**P**
GA (wks, Median [IQR])	33.9 [31.6-34.7]	35.1 [34.3-35.9]		34.5 [33.9-35.1]	0.060
Maternal age (y, Median [IQR])	32.0 [29.0-33.0]	32.0 [29.5-35.5]		33.0 [28.0-36.0]	0.806
MAP (Median [IQR])	98 [85-103]	114** [106-118]		112** [110-114]	0.004
UtA PI (Median [IQR])	0.64 [0.63-0.70]	0.85 [0.56-0.98]		1.29* [0.89-1.50]	0.050
TNFα (pg/ml, Median [IQR])	53 [20-267]	122 [42-314]		59 [17-366]	0.853
C. Delivery at ≥37 weeks gestation	**TD n=21**	**PE n=10**	**FGR n=4**	**FGR+PE n=4**	**P**
GA (wks, Median [IQR])	33.6 [32.1-37.7]	38.5** [37.0-38.7]	37.7 [37.4-37.9]	37.6 [37.1-37.9]	0.018
Maternal age (y, Median [IQR])	33.0 [29.0-35.0]	29.5 [25.0-30.1]	32.0 [28.5-36.0]	33.5 [28.5-36.5]	0.509
MAP (Median [IQR])	87 [76-91]	111*** [108-117]	100* [94-106]	107* [88-114]	<0.001
UtA PI (Median [IQR])	0.68 [0.63-0.71]	0.77 [0.59-1.20]	0.80 [0.69-1.05]	1.11** [0.89-1.59]	0.040
TNFα (pg/ml, Median [IQR])	28 [13-178]	19 [16-114]	572* [63-1192]	485* [168-773]	0.060

Blood measurement values of the cohort participants at enrolment: Median and interquartile range [IQR] of deliveries <34 weeks gestation (A), deliveries at 34 to 36+6 weeks gestation (B), and deliveries ≥37 weeks gestation (C). Categorical values were compared with the Pearson Chi-Square test. Continuous values were compared with non-parametric tests for two or more independent samples. All groups were compared to the PTD group (A, B) or to the term delivery group (C). We used the Kruskal Wallis a-parametric test to compare the groups. *p<0.05, **p<0.01, ***p<0.001 TD - term delivery, PTD - preterm delivery, PE - preeclampsia, FGR - fetal growth restriction, IQR - interquartile range, MAP – mean arterial blood pressure, UtA PI – uterine artery pulsatility index, GA - gestational age

Uterine artery pulsatility index (UtA-PI): Among the groups who delivered <34 weeks (Table 2, A), UtA-PI at enrolment was higher in any of the PE, FGR and PE+FGR groups but no difference was found between TD and PTD, who were both significantly lower from any of the above. Among women who delivered between 34 to <37 weeks (Table 2, B), UtA-PI at enrolment was only higher in FGR+PE (no pure FGR cases in this time frame). Lower UtA-PI was measured in the TD and PTD cases who were indistinguishable. At term (Table 2, C), UtA-PI was higher in FGR+PE cases compared to TD.

Maternal serum TNFα: Figure 1 depicts the Box and Wiskers Plot of maternal blood levels of TNFα in the different groups divided according to the time of delivery. In the PTD group delivered <34 weeks, the group median was 321 pg/ml (95% Confidence Interval (95% CI): 145-715). This value was significantly higher compared to the level at GA between 34 to <37 weeks, which was 53 pg/ml (95 CI: 20-269), and also higher compared to TD cases, which was 22 pg/ml (95% CI: 14-145) (Figure 1A). Among the PE cases, the values of TNFα between 34 to <37 weeks were the highest for the group, 122 pg/ml (95% CI: 35-405), whereas TNFα values in cases <34 weeks and at term were lower, corresponding to 29 and 19 pg/ml, respectively (Figure 1B). However, the differences did not reach significance. FGR cases <34 weeks showed TNFα values of 50 pg/ml (95% CI: 12-312), which were ten times lower from 572 pg/ml (95% CI: 18-1349) in term FGR cases (Figure 1C). The group of combined FGR+PE also showed a reversed trend to the PTD group with 36 pg/ml (95% CI: 21-74) in cases delivered <34 weeks, reaching 42 pg/ml (95% CI: 17-366) at 34 to <37 weeks and ten times higher at term, 485 pg/ml (95% CI: 64-848) (Figure 1D).

Figure 2 shows the individual values of TNFα at the time of enrolment versus the outcome groups. It shows that for any of the FGR groups (pure FGR and FGR+PE), the regression line has a coefficient "r" with a clearly positive slope increasing with GA, with the respective values of r = 0.53 for pure FGR, and r = 0.37 for FGR+PE. Any of these is significantly different compared to the flat regression curves of the pure PE cases (r = 0.06) and the TD control cases (r = 0.04) (Fig. 2). By contrast, the regression line of the PTD cases shows a negative r-coefficient (r = - 0.19), reflecting that in these cases, TNFα values are decreasing over the second half of gestation. This regression coefficient of PTD is significantly different from the regression coefficients of the FGR and the FGR+PE groups.

TNFα and uterine artery pulsatility index in the second half of pregnancy: Figure 2 shows how different the two markers, TNFα (A) and UtA-PI (B), change with gestational age in the different groups. For the term delivery group, the values do not change during the evaluated period, with a slope of r = 0.04 for

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TNFαand a small increase (r=0.13) for UtA-PI. For the PTD group, TNFαdecreases from a higher value <34 weeks to a lower value at <37 weeks, with a moderate negative slope (r = -19). UtA-PI for this group is moderately increasing (r=0.23). For the PE group, TNFa has almost a horizontal curve over GA, whereas the slope for UtA-PI sharply decreases with r = -067. For the FGR+PE group, TNFα significantly increases between GA <34 to GA >37 weeks with an r = 0.37, whereas UtA-PI decreases with an r = -0.44. Pure FGR shows the clearest changes with TNFα increasing with an r = 0.59 and UtA- PI decreasing with an r = -0.81.

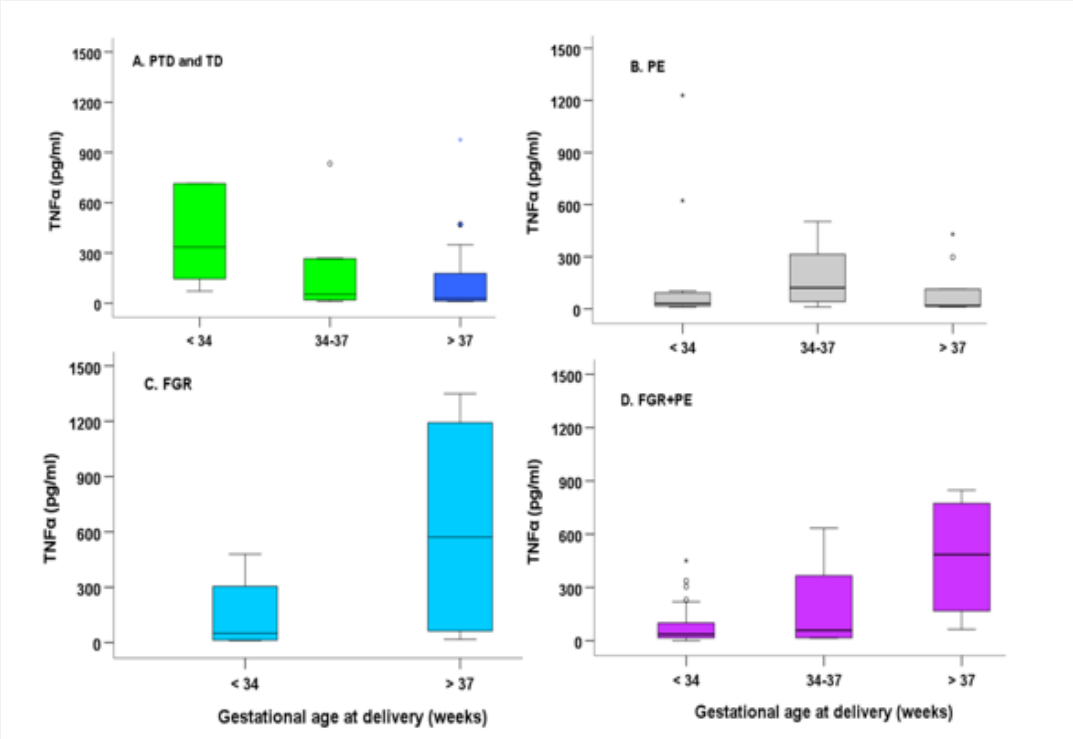

Figure 1: Box Plot of TNFα in the different groups according to gestational age at delivery.

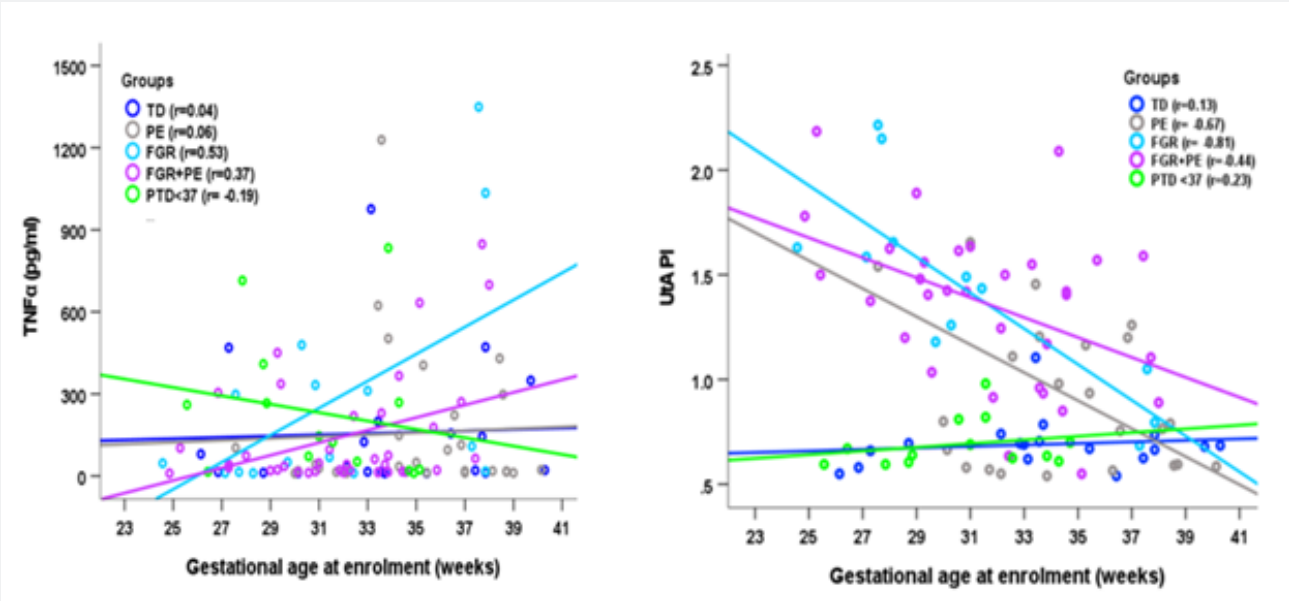

Figure 2: TNFα and UtA PI according to gestational age at enrolment.

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Slope of UtA-PI / TNFα by gestational age: We plotted the ratio of UtA-PI (x 100)/TNFα) against gestational age at enrolment (Fig. 3). For the PTD and TD groups the ratio remains flat at the evaluated gestational ages (r = 0). For the pure PE group, the slope slightly decreased (r = -0.20). By contrast, the slope for FGR and FGR+PE groups showed a steep decrease with increasing gestational age. Accordingly, a differential diagnosis can be offered, indicating high TNFa and low UtA-PI for early PTD cases (<34 weeks gestation) could mark these cases as unique groups compared to the patients with high UtA-PI who have low TNFα who subsequently developed pure FGR and FGR+PE. At term, when the PTD group already delivered, term FGR and to a lower extent term FGR+PE demonstrates higher TNFα compared to UtA-PI due to a continuous increase of TNFα in any FGR from the start of the third trimester to delivery at term. This was different from the level of TNFαin TD cases which was flat all through

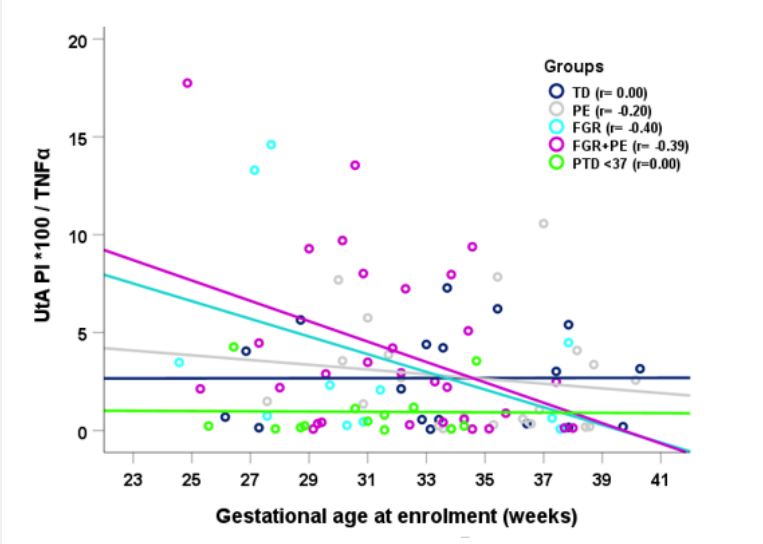

Figure 3: Ratio of UtA PI x 100 / TNFα according to gestational age at enrolment.

Discussion

Principal findings

The main finding is the presence of high TNFα in PTD cases <34 weeks gestation with low UtA-PI and without hypertension. This creates an avenue for differential diagnosis of early PTD versus pure FGR, pure PE or PE+FGR. At GA>37 (term delivery), compared with PE group, the pure FGR group presents significantly higher TNFα values combined with high UtA-PI. There were no significant trends in values of TNFα in PE cases.

Results in the context of what is already known in the literature

TNFα is an inflammatory marker Idriss & Naismith [3], Bradley [1], Horiuchi et al. [2], and it is known that PE is associated with significant inflammation Guan et al. [18]. In fact, pregnant women with documented inflammation due to the COVID-19 pandemic developed symptoms similar to PE Lai et al. [19]. Impaired TNFα in PE cases were previously found to have gene polymorphisms at position −308 G/A of the TNFα promotor associated with the mother's reduced tolerance to the growing fetus Chen et al. [20], Lin et al. [21]. Such TNFα polymorphism was so far identified in patients of Chinese or African ethnic origin Chen et al. [20], Lin et al. [21], Raguema et al. [22], while our cohort was of a pure Caucasian ethnicity. This may explain why we did not see any significant differences in TNFα levels in pure PE cases. Molvarec et al. [23] have found that the SNP – 308 G/A of TNFα has a role in the risk of developing severe FGR complicated by PE. Hence, there is a need to explore this polymorphism in our population.

Clinical and research implications

The bimodal changes in TNFα between PTD (high <34 weeks, low >34 weeks) and FGR (low <34 weeks, high >37 weeks) with high UtA-PI <34 weeks in early FGR but not in early PTD without hypertension, may well be used to identify and differentiate between early FGR and PTD.

The graphical representation of our findings (Figure 4) indicates that in PTD cases, early alterations of the tissues in the reproductive organs (such as microfractures of the fetal membranes) Mikkelsen et al. [24] may result in an increased maternal inflammatory response, and thus lead to an early increase of TNFα levels (red arrow in Fig. 4).

The high level of TNFα decreases during the third trimester of pregnancy and thus allows the segregation between the very early PTD cases <34 weeks gestation to later PTD cases. This may reflect unaltered placental perfusion with maternal blood, and a placental unstressed status Huppertz [25], Huppertz et al. [26].

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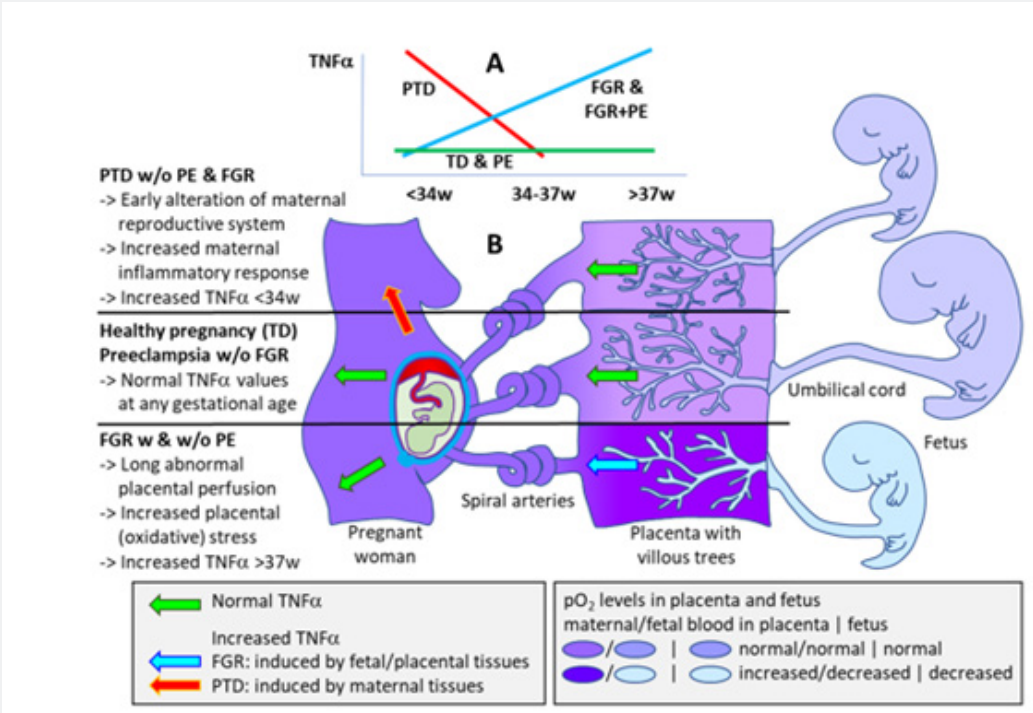

Figure 4: Graphical model of TNFα in pregnancy complications.

By contrast, in FGR cases (with and without PE), the long-lasting alterations of placental perfusion with maternal blood, due to an impaired transformation of uterine spiral arteries, may result in an increasing placental stress Bradley [1], Huppertz [25], Huppertz et al. [26]. Hence, a late increase of TNFα levels in such cases appears to be initiated by the placenta (blue arrow, Fig. 4). In FGR cases, high velocities of maternal blood flowing into the placenta induce damage to villous tissues and increased peripheral resistance in the placental vasculature Huppertz [25], Huppertz et al. [26]. This leads to a reduced transfer of oxygen and nutrients to the fetus, which in turn results in increased oxygen levels of the maternal blood in the intervillous space (hyperoxia) and at the same time hypoxia of fetal blood and the fetus (Figure 4) Huppertz [25], Huppertz et al. [26].

Strengths and limitations:

A strength of the study is coverage of the pregnancy epidemiology of Slovenia. The prevalence of PE in Slovenia (pure PE and FGR+PE combined) accounts for 2-3% of all deliveries. Having a cohort of 73 cases of PE (combining 31 cases of pure PE and 42 cases of PE+FGR) accounts for an approximate sample size of 2,433-3,650 pregnant women, corresponding to 15-20% of all deliveries in Slovenia, and around 40% of the deliveries in the medical center of Ljubljana. In this respect, our study has a power of 0.85-0.90. This appears reasonable, given we also included a group of preterm deliveries that were unrelated to PE and FGR (mainly spontaneous PTD cases, and had also a control group of unaffected cases at term. Of course, larger studies are needed.

A limitation of this study is the small cohort size. Although powered for the analysis, TNFα value standardization and their conversion to medians of the mean was not possible. Hence, our model was based on regression analysis and used the trend of changes for each group against gestational age and the ratio of the two markers (each of them behaved very differently) as a way to extract the values for the differential diagnosis of the use of TNFαand UtA-PI as aiding tools for differential diagnosis.

We measured the marker value at admission with a window between 24-41 weeks, and not in fixed gestational age. However, this mimics the real-life situation. Another limitation was the lack of repeated testing, which in principle could have helped to increase marker accuracy [27,28], (supplementary table),(Figure3,5).

Conclusions

This study showed how combining TNFα with UtA-PI could add to the differential clinical diagnosis of pregnancy complications from the 24th week of gestation towards delivery. High TNFα in cases <34 weeks gestation, when coupled to low UtA-PI and normal blood pressure reflects early PTD linked to maternal inflammation. At term, high TNFα combined with high UtA-PI is associated with any FGR (with/without PE), possibly reflecting inflammation and maternal and fetal hypoxia due to the very long period of altered placental perfusion. More and larger studies are warranted to verify the findings.

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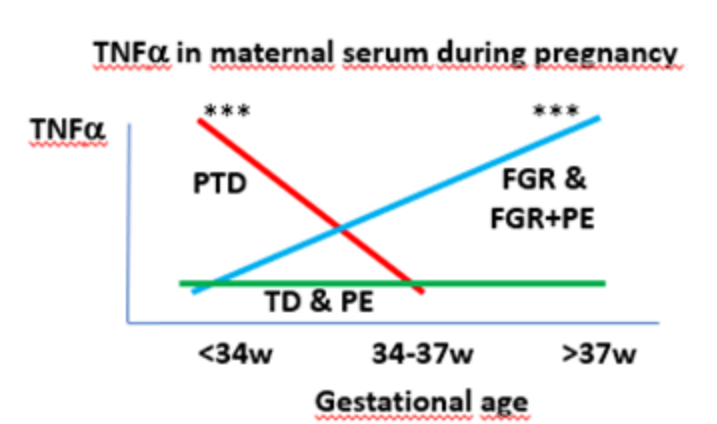

Figure 5: Graphical model of TNFα in pregnancy complications

FGR – fetal growth restriction

PE – preeclampsia

PTD – preterm delivery

TD – term delivery

TNFa – tumor necrosis factor alpha

(A) The different slopes of TNF□ changes are displayed versus gestational age for preterm delivery (PTD) (red) going downward and for FGR (blue) going upward. There are no changes of TNF□ for term deliveries (TD) and pure preeclampsia cases (PE) (green).

(B) Left - Maternal side with the spiral arteries entering the placenta, middle – the placenta with the villous trees, right – the fetal side with the umbilical cord. The arrows represent normal (green) and increased TNF□ (red for PTD, coming from maternal tissues, and blue for FGR, coming from placental tissues). The colors of mother, spiral arteries, intervillous space and villous trees of the placenta, umbilical cord and the fetus represent the different oxygen levels. Normally, there are decreasing levels of oxygen from mother to placenta to fetus as depicted for PTD, TD and PE without FGR. All these different levels of oxygen are normal oxygen levels and thus are referred to as physioxia. 27 By contrast, in cases with FGR (with and without PE), less oxygen is extracted from the placenta leading to a double effect with more oxygen remaining in maternal blood in the intervillous space of the placenta (hyperoxia) and with less oxygen being transferred to fetal blood and the fetus (hypoxia). 27-28.

Supplementary Table 1: The frequency of anti-pregnancy complications taken by the patients according to the number (N) of users and proportion (%) from in the cohort.

Type of drug	TD	PTD	PE	FGR	FGR+PE
Anti-Hypertensive	0 (0)	0 (0)	23 (74.2)	2 (12.5)	31 (73.8)
$MgSO_4$	0 (0)	0 (0)	10 (32.3)	0 (0)	13 (31.0)
Aspirin*	0 (0)	0 (0)	0 (0)	6 (37.5)	6 (14.3)
Corticosteroids	1 (4.8)	7 (46.7)	3 (9.7)	5 (31.3)	19 (45.2)
Progesterone**	0 (0)	0 (0)	1 (3.2)	1 (6.3)	7 (16.7)

The various drugs administrated to the patients in the various clinical groups are presented according to the number of treated patients and their relative proportion (%).

*Aspirin was administrated daily from before <16 wks' gestation, ** Progesterone was given at admission or delivery but not to prevent PTD due to measurements of short cervix at 19-25 wks' gestation. Corticosteroids were given at admission for suspected preterm birth for any reason. Most women in the FGR and FGR+PE groups were treated with more than one drug.

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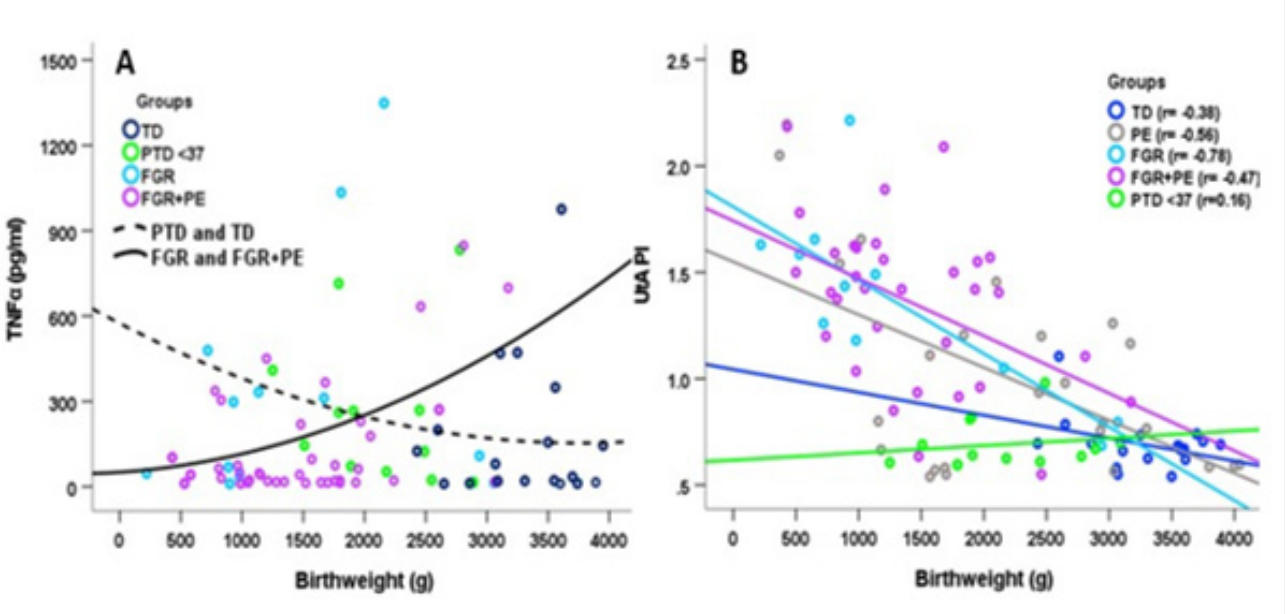
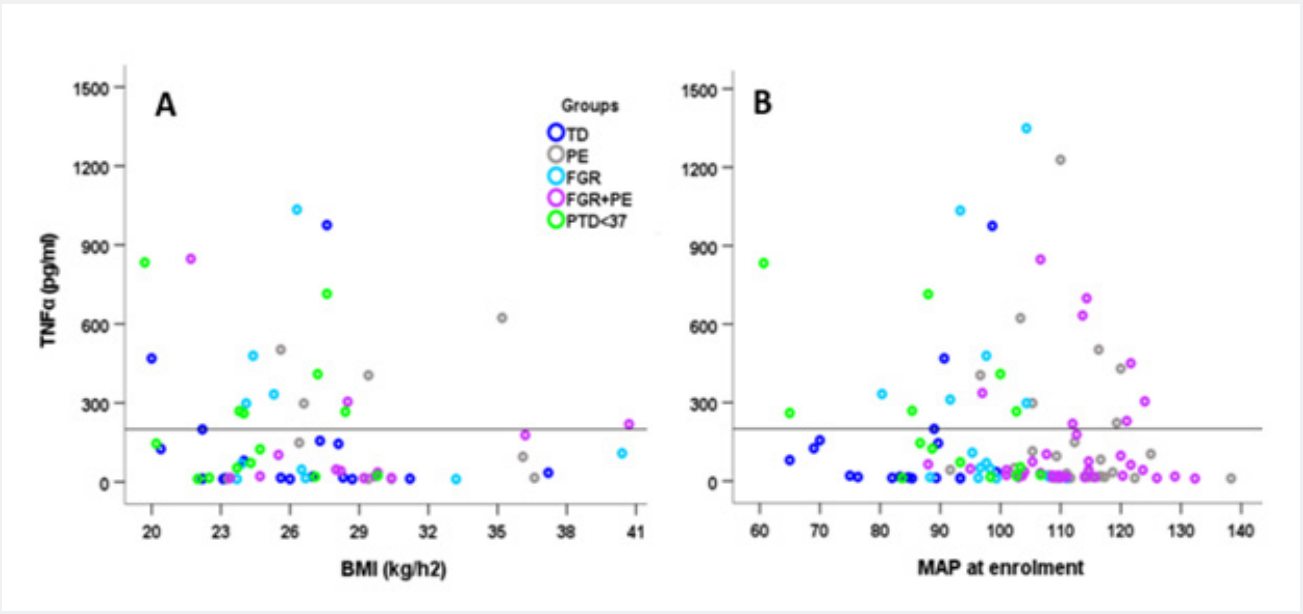


Supplementary Figure 1: TNFα by birthweight and uterine artery pulsatility index (UtA-PI) and gestational age at enrolment

A. TNFα plotted against birthweight.

B. UtA-PI is plotted against the birthweight.

PTD- preterm delivery, FGR- fetal growth restriction, PE - preeclampsia, TD - term delivery control.



Supplementary Figure 2: TNFα, MAP and BMI

Changes in TNFα versus BMI (A) and MAP (B).

PTD- preterm delivery, FGR- fetal growth restriction, PE - preeclampsia, TD- term delivery control.

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