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First and second trimester immune biomarkers in preeclamptic and normotensive women

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Abstract

Introduction—Circulating immune markers may be associated with preeclampsia but further investigations in early pregnancy and among preeclampsia subtypes are warranted. We examined immune markers in 208 preeclamptic women and 411 normotensive controls.

Methods—Our study was nested within the Collaborative Perinatal Project. A total of 242 women had first trimester serum samples and 392 had second trimester serum samples. Preeclampsia was defined as hypertension >20 weeks of gestation with proteinuria or pulmonary edema, oliguria, or convulsions. Preterm preeclampsia was defined as preeclampsia with delivery less than 37 weeks of gestation. Associations between immune markers RANTES, interleukin (IL)-6, IL4, IL5, IL12, IL10, IL8, IL1-beta, interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha and beta, transforming growth factor (TGF)-beta and preeclampsia were explored using a modified version of cox regression developed to address data with non-detectable levels. Models were adjusted for body mass index, gestational age of blood sampling, fetal sex, smoking, socioeconomic status and maternal age.

Results—In first trimester samples, IL-12 was associated with preeclampsia (p=0.0255). IFN-gamma (p=0.0063), IL1-beta (p=0.0006), IL5 (p=0.0422) and TNFr (p=0.0460) were associated with preterm preeclampsia only. In second trimester samples, IL1-beta was associated with

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preeclampsia (p=0.0180) and term preeclampsia (p=0.0454). After correction for multiple comparisons, only IL1-beta remained associated with preterm preeclampsia in the first trimester (p=0.0288).

Discussion—Elevated first trimester IL1-beta appears to be associated with preterm preeclampsia. However, few associations were observed in the second trimester. Systemic immune markers alone may not be useful for preeclampsia prediction.

Keywords

Inflammation; preeclampsia; pregnancy

INTRODUCTION

Despite progress in understanding preeclampsia pathogenesis, useful prediction models, treatments and specific diagnostic tests have been limited. Preeclampsia is a systemic maternal syndrome that affects 3–10% of pregnancies and is a leading cause of maternal mortality [1]. Clinically, preeclampsia is diagnosed as the new onset of hypertension (≥ 140/90 mm Hg) and proteinuria (≥ 300 mg per 24 hour urine collection) or end-stage organ failure after 20 weeks of gestation and the only treatment is delivery of the placenta [2]. The exact mechanisms that lead to preeclampsia are not completely elucidated. Furthermore, preeclampsia is hypothesized to have several subtypes which can complicate prediction. Thus, identifying biomarkers to better understand pathogenesis and differentiate subtypes will improve clinical management.

In normal pregnancy, the placenta secretes immune stimulating placental derived factors into the maternal circulation [3]. Balanced secretion of these factors may play a role in maternal immune tolerance towards the fetal allograft [4]. However, abnormal placentation could lead to increased placental shedding, exaggerated systemic inflammation and subsequent endothelial dysfunction, the key characteristics of preeclampsia [5, 6]. This is consistent with third trimester studies reporting increased circulating pro-inflammatory markers [tumor necrosis factor alpha (TNFα), interleukin (IL)-8, IL-1β and interferon (IFN)-γ] in preeclampsia [7, 8]. Similar studies have reported associations between pleiotropic or immune-modulatory markers including IL-6, IL-2, and IL-4, as well as anti-inflammatory marker IL10 [7–9]. However, studies conducted prior to the third trimester are conflicting. A second trimester study has reported lower circulating IL10, TNF-α and IFN-γ among women who develop preeclampsia [10]. While others report no significant differences in circulating immune markers [11, 12]. First trimester investigations are limited. Circulating IP-10, a chemokine induced by IFN-γ, is increased in preeclampsia [13]. Additionally, first trimester elevated IL1β was shown to predict early onset preeclampsia [14].

Our own research has shown that mid-trimester systemic immune markers including IL6 and TNFβ are increased in preeclamptic women while IL1β is decreased [15]. This study conducted among 707 women from the Danish National Birth Cohort was unable to fully examine first trimester systemic immune markers. First trimester investigations are important as preeclampsia pathogenesis may begin early in pregnancy. The objective of this paper is to examine the association between first and second trimester circulating immune

markers and preeclampsia. Serum immune markers including IL-6, IL-6 receptor, IL-4, IL-4 receptor, IL-5, IL-12, IL-2, TNF-α, TNF-β, TNF-receptor, IL-1β, IL-1α, IL-8, IL-10, IFN-γ, macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MIP), transforming growth factor-beta (TGF-β), and RANTES were included in our study based on previous associations with preeclampsia or their involvement in systemic inflammation or the Th1/Th17 paradigm [16–18].

METHODS

We conducted a nested case control study of 208 singleton and primiparous preeclamptic women and 411 singleton and primiparous normotensive controls within the Collaborative Perinatal Project (CPP) [19]. Both cases and controls had no history of diabetes, cardiovascular disease or hypertension. The CPP is a longitudinal study of 55,908 pregnancies [20]. Women were enrolled between 1959 and 1965 from 12 university-affiliated medical centers in the United States (Baltimore, MD; Boston, MA; Buffalo, NY; Memphis, TN; Minneapolis, MN; New Orleans, LA; two sites in New York, NY; Philadelphia, PA; Portland, OR; Providence, RI; and Richmond, VA). Oral consent, as was standard at the time of the CPP study, was obtained from all women in the study [21]. We analyzed a total of 242 women who had first serum samples collected in the first trimester (mean gestation age 10.7±1.9; range 5–13 weeks) and 392 women who had first serum samples collected in the second trimester (mean gestation age 16.5±1.7; range 14–19 weeks). The study was approved by the University of Pittsburgh Institutional Review Board.

Data collection

At the first prenatal visit, all women were interviewed in person to obtain data on maternal characteristics, behavior, medical and pregnancy history. Delivery data was recorded by the attending physician. For our analysis, we considered self-reported maternal age (years), marital status (married, single), socioeconomic status, race (white, non-white), maternal smoking (yes/no), and pre-pregnancy body mass index (BMI) as potential covariates. Socioeconomic status was previously determined using a composite score that combined education, occupation and family income [22]. Pre-pregnancy BMI was determined by (weight (kg)/height $(m)^2$ which was reported at enrollment. Gestational week was determined by the date of delivery minus the date of last menstrual period.

Cytokine measurements

Non-fasting blood samples were obtained at the first study visit and stored at −20°C in glass vials and monitored continuously from the time of collection. There were no recorded thaws from collection time until they were aliquoted for analysis. Each sample was labeled with a study ID and name and checked against a pull sheet at the time the samples were pulled. Our subset of samples was mailed to the Statens Serum Institute in Copenhagen where immune biomarkers were measured in duplicate with an in house multiplex flow cytometric assay system Luminex MultiAnalyte Profiling Technology (LabMap, Luminex Corporation, Austin Texas) [23]. The calibration curves for each analyte were calculated by the Bio-Plex 3.0 software (BioRad, US). Mean intra- and interassay CVs (CV %) were 6.2% and 16%, and ranged from 6.7 (IL-4) to 13 (IL-10 and TNF-α) and 10 (IL-4) to 25 (TNF-α) [23].

Variation in precision profiles among analytes is similar to other studies [24–26]. We acknowledge that the long-term storage of CPP samples raises concerns about the measurement of the analytes. This multiplex assay has been demonstrated to be valid for the measurement from specimens obtained from long-term storage using10 anonymously collected residual dried blood spot specimens stored for 23 years at −24°C in a national, Danish biological specimen bank [23]. In that study the measurable amounts of most cytokines were constant. Additionally, analytes measured in serum from the CPP cohort have shown to be stable in other studies [27–29]. In another CPP study, cytokines measured in the CPP were compared to fresh samples and found to be consistent across groups [30]. As IL2 and IL1α measured below the LOD for greater than 75% of patients, these biomarkers were not analyzed.

Preeclampsia definition

Preeclampsia was based on chart abstraction of blood pressure and protein levels and defined as gestational hypertension (2 or more measurements of systolic blood pressure >=140 mmHg and/or diastolic blood pressure >=90 mmHg for the first time after 20 weeks of gestation) and proteinuria (2 random urine dipsticks of 1+ protein or one dipstick of 2+ protein). In the intrapartum period, the first 5 pressures obtained after hospital admission for delivery were averaged. It is accepted that preeclampsia is heterogeneous disease with subtypes (early/late onset) that have different pathophysiological pathways [31]. For our study, we classified preeclampsia resulting in either a term birth (≥37 weeks gestation) or a preterm birth (< 37 weeks gestation) as separate outcomes. Preeclampsia with preterm birth is a valid proxy for disease severity and early onset of disease. Preeclampsia with preterm birth <34 weeks of gestation was not used as the sample size was too small for analysis.

Statistical analyses

For all biomarkers, raw median levels and ranges were calculated for preeclamptic and normotensive women. Our primary analysis examined the association between immune biomarkers and preeclampsia. As circulating immune biomarkers display heterogeneity by gestational age, we stratified our analysis by first and second trimester samples. In multiplex assays, subjects frequently measure outside of the limit of detection (LOD) [32]. In general, the frequency of women who measure beyond the LOD varies and those who are outside the detectable range may have very low or undetectable levels. Common approaches to handling data below the LOD such as replacing with a set value (0 or LOD/2), dichotomizing (above LOD vs. below LOD) or multiple imputation are well recognized to be prone to biases [24]. Specifically, many of these methods perform poorly when there are a high proportion of individuals with data below the LOD for a particular analyte. In addition, methods which rely on parametric models (i.e. multiple imputations) result in overly narrow confidence intervals when the model is miss-specified [33]. We used a new method based on cox regression to identify associations between immune biomarkers and preeclampsia [33]. This method developed by Dinse et al., was shown to be a valid approach to handle measurements below the LOD especially when analytes with a high proportion of non-detects were present. First, measurements which are left censored (lower detection limits) are reversed to right censored data. Cox regression was then utilized treating the reversed scale for each analyte as an outcome and preeclampsia as the independent variable. The biomarker is treated as

censored data and the hazard ratio for a binary health outcome is interpreted as an adjusted odds ratio. We examined associations between baseline demographics (maternal age, socioeconomic status, body mass index, race, and smoking status), pregnancy outcome data (preterm birth, small for gestational age) and preeclampsia using logistic regression. Maternal age, marital status, socioeconomic status (SES), race, smoking, BMI and gestational age at blood draw were considered as potential covariates. Maternal age, SES, BMI, fetal sex, gestational age at blood draw and smoking were included in the final model as they changed the effect size by more than 10%. All analyses were repeated for term and preterm preeclampsia. To account for multiple comparisons, we used a resampling-style step-down method that incorporates correlation and distributional characteristics of the dataset (as is done with permutations) while maintaining control of the family-wise error rate [34]. SAS V9.2 (Cary, NC) was used for analyses.

Results

Table 1 shows that compared to normotensive women, preeclamptic women had a lower maternal age (β=−0.07, p=0.0009), were more likely to be single [odds ratio (OR) 1.8, 95% confidence interval (CI) 1.2–2.5], more likely to have a mid (OR 2.3, 95% CI 1.3–3.8) or low (OR 3.7, 95% CI 2.2–6.4) SES, less likely to smoke during pregnancy (OR 0.6, 95% CI 0.4–0.9), have a higher BMI (β=0.09, p=0.0035) and more likely to have a small for gestational age baby (OR 1.9, 95% CI 1.2–3.2).

Among women sampled in the first trimester, preeclamptic women have higher median levels of RANTES, IL4r, IL6, IL12, IL1β, IL10 and TGFβ compared to normotensive women (Table 2). Among women sampled in the second trimester, preeclamptic women had lower median values of IL4r, MIP, IL1β, IL10, and TGFβ compared to normotensive women. In contrast, women with preeclampsia had higher median levels of MIF, IL6, and IL8.

In first trimester samples, only IL-12 was significantly associated with preeclampsia (HR 1.6, 95% CI 1.1–2.6; p=0.0255) after adjustments (Table 3). IFNγ (HR 3.5, 95% CI 1.4–8.5; p=0.0063), IL1β (HR 3.6, 95% CI 1.7–7.6; p=0.0006), IL5 (HR 2.1, 95% CI 1.0–4.2; p=0.0422), and TNFr (HR 2.0, 95% CI 1.0–3.6; p=0.0460) were significantly associated with preterm preeclampsia. The association between IL1β and preterm preeclampsia remained significant after correcting for multiple comparisons.

We observed different trends in second trimester samples (Table 4). Decreased IL1β (HR 0.7, 95% CI 0.5–0.9; p=0.0180) and TGFβ (HR 0.8, 95% CI 0.6–0.9; p=0.0292) were significantly associated with all preeclampsia cases. No associations were found with preterm preeclampsia. No other significant associations were observed. Results were not significant after correction for multiple comparisons.

As our second trimester results differed from the first trimester, we compared maternal characteristics between women with first trimester samples and women with second trimester samples. Compared to first trimester controls, second trimester controls were younger, more likely to smoke, had a lower SES, and were more likely to be African American (Chi-square test p<0.05 for all). Second trimester cases were similar to first

trimester cases except that they were more likely to be African American and less likely to have a SGA baby (10^{th} percentile based on maternal characteristics) (Chi-square test $p<0.05$).

As an exploratory analysis we stratified analyses by race to determine if potential effect modification exist. We also explored differences between preeclampsia with and without SGA. In the second trimester, TNFβ (HR 0.6, 95% CI 0.3–0.9) was associated with preeclampsia without SGA and there was no difference by race. Similarly, IL1β was associated with preeclampsia without SGA (HR 0.6, 95% CI 0.5–0.8) and effect estimates were similar among races. There was some effect modification by race present for IL6 which was associated with preeclampsia in white (HR 1.7, 95% CI 1.1–2.9) but not African American women (HR 0.7, 95% CI 0.5–1.3).

Discussion

We found that IL1β was associated with preterm preeclampsia in the first trimester. Our results are similar to a study of 70 women reporting that first trimester IL1β is a significant predictor of early onset preeclampsia [14]. IL1β has been suggested to be necessary for immune-tolerance at the maternal-fetal interface through modulation of the nuclear factor kappa-B pathway [35]. Alteration in immune-tolerance could adversely effect placentation. Indeed, a recent placental gene expression study identified an immunological preeclampsia subtype that correlates with placental dysfunction and poor fetal growth [36]. Our study measured systemic markers which may not represent placental immune dysfunction. Still, severe subtypes of preeclampsia (i.e. early-onset, poor fetal growth) are hypothesized to be associated with abnormal placentation while “maternal” preeclampsia may occur despite normal placental function [36, 37]. Placental dysfunction increases syncytiotrophoblast microvesicle production leading to exaggerated systemic inflammation [6]. In fact, treatment of peripheral blood mononuclear cells by first trimester microvesicles has been shown to increase IL1β secretion [38]. Thus, increased systemic IL1β in the first trimester may indicate an immunological preeclampsia subtype perhaps mediated by abnormal placentation and increased placental microvesicles.

Our results were conflicting in second trimester samples where IL1β was lower in preeclamptic women. However, these findings replicate our previous study where mid-pregnancy IL1β was associated with decreased odds of preeclampsia in the Danish National Birth Cohort [15]. The results are also consistent with another study of serum IL1β at 34 weeks gestation [18]. Still, the reasons for these differing results in first and second trimester samples are not entirely clear. Placental microvesicles are hypothesized to drive systemic inflammation. In healthy pregnancy, microvesicle production of IL1β from peripheral blood mononuclear cells is not observed at term [38]. In fact, IL1β is downregulated by macrophages following phagocytosis of placental microvesicles at term [39]. This may suggest that cytokine production via placental microvesicles is increased in early pregnancy but not in later gestations. Conversely, microvesicles from preeclamptic pregnancies are reported to increase IL1β production in the first trimester and at term [38]. However, investigations among term preeclamptic women may be biased by MgS04 treatment which increases IL1β [40]. Longitudinal profiles of systemic immune markers during pregnancy

are limited. IL1β has been shown to significantly decrease from the first to second trimester before increasing again at delivery [41, 42]. One longitudinal study which began sampling at 18 weeks gestation until term, found no associations between IL1β and preeclampsia [43]. Thus, systemic IL1β levels and associations with preeclampsia are not consistent across pregnancy. Our results may suggest that increased first trimester systemic IL1β is downregulated later in pregnancy possible due to a compensatory mechanism.

We observed differences in maternal characteristics between women sampled in the first and second trimesters. Controls sampled in the second trimester were more likely to be young African Americans, smoke and have a low SES compared to first trimester controls. Second trimester cases were also more likely to be African American but less likely to have a SGA baby compared to first trimester cases. Maternal characteristics may impact systemic immune markers [44]. Thus, second trimester controls may have been more likely to have elevated immune markers via unmeasured maternal factors biasing results towards the null. Lastly, it is also possible that a decrease in IL1β in the second trimester is capturing a different preeclampsia subtype [36]. Our exploratory analyses did show that in the second trimester IL1β was significantly decreased in preeclampsia without SGA but displayed trends towards increased levels in preeclampsia with SGA.

We found no other significant associations after correction for multiple comparisons. In a previous investigation, we found that mid-trimester IL-6 and TNFβ were associated with term preeclampsia [15]. However, the current investigation could not replicate these findings. The conflicting results may be due to differences in the study populations (contemporary Danish Cohort vs. older American cohort). In our exploratory analysis, IL6 was associated with preeclampsia but only in white women. Overall, we found few associations between systemic immune markers and preeclampsia in second trimester samples which is consistent with other mid-trimester investigations [11, 12]. Thus, second trimester serum immune markers may have little use for prediction of preeclampsia.

Our study included a large sample size and blood sample collection prior to the third trimester of pregnancy. We were able to investigate both first and second trimester samples but were limited by a single time point for each subject. We did not have data on time of diagnosis of preeclampsia. However, samples were collected prior to 20 weeks of gestation (range 5–18 weeks). Still, we cannot rule out subclinical disease prior to sampling. We acknowledge that the age of the CPP cohort is a limitation and may affect the stability of our immune markers. Analytes measured using CPP data, including cytokines, have shown to be stable in previous studies. Compared to our study within the DNBC [15], most immune markers measured in the CPP cohort have similar proportions of non-detectable levels. IL1β levels do appear to be lower in the CPP compared to the DNBC. However, we observed the same association with second trimester IL1β in both studies and these cohorts are not comparable in many ways due to differences in demographics.

We found that IL-1β was significantly associated with preterm preeclampsia in the first trimester. Elevated IL-1β in early pregnancy may indicate a subtype of preeclampsia. However, these associations were not observed in the second trimester. Longitudinal changes in IL1β in relation to preeclampsia subtypes may be warranted. Overall, no single immune

biomarker is likely a strong predictor for preeclampsia or preeclampsia subtypes, particularly in the second trimester. Investigations which combine several immune markers, biomarkers from pathways which may induce inflammation and clinical data may be useful to define an immunological subtype of preeclampsia.

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Highlights

- Serum IL1β measured in the first trimester was associated with preterm preeclampsia after correction for multiple comparisons.
- IFNγ, IL5, and TNFr measured in the first trimester were also associated with preterm preeclampsia but not after correction for multiple comparisons.
- Analysis among second trimester samples revealed no significant associations after adjustments.

Table 1

Baseline characteristics and pregnancy outcomes among preeclamptic cases and normotensive controls

Variable	Controls N=432	All Cases N=216	[a] Effect estimates, 95% confidence intervals and p-values
	%(n)	%(n)	
Maternal Age			
Median (IQR)	20.0 (5.0)	19.0 (5.0)	β = −0.07, p=0.0009
Marital Status			
Married	335 (77.5)	143 (66.2)	Reference
Single	97 (22.5)	73 (33.8)	1.8 (1.2 – 2.5)
Socio - economic status			
Low	115 (26.9)	91 (42.5)	3.7 (2.2 – 6.4)
Mid	213 (49.9)	102 (47.7)	2.3 (1.3 – 3.8)
High	99 (23.2)	21 (9.8)	Reference
Maternal race			
White	274 (63.6)	125 (57.9)	Reference
Non-white	157 (36.4)	91 (42.1)	1.3 (0.9 – 1.8)
Smoking status			
Non-smoker	247 (57.2)	147 (68.1)	Reference
Smoker	185 (42.8)	69 (31.9)	0.6 (0.4 – 0.9)
Body mass index			
Median (IQR)	20.8 (3.2)	21.3 (4.0)	β = 0.09, p=0.0035
PTB <37 wks			
No	376 (87.7)	178 (84.4)	Reference
Yes	53 (12.3)	33 (15.6)	1.3 (0.8 – 2.1)
PTB <34 wks			
No	408 (95.1)	198 (93.8)	Reference
Yes	21 (4.9)	13 (6.2)	1.3 (0.6 – 2.6)
Fetal Sex			
Male	221 (51.2)	102 (47.2)	1.2 (0.8 – 1.6)
Female	211 (48.8)	114 (52.8)	Reference
SGA 5^{th} percentile			
No	382 (91.4)	171 (84.7)	Reference
Yes	36 (8.6)	31 (15.3)	1.9 (1.2 – 3.2)
SGA 10^{th} percentile			
No	339 (81.1)	153 (75.7)	Reference
Yes	79 (18.9)	49 (24.3)	1.4 (0.9 – 2.1)

[a]Determined by logistic regression. Continuous variables display β, and binary or categorical variables odds ratios.

Table 2

Raw mean, median and range of immune biomarkers in first and second trimester samples from preeclamptic cases and normotensive controls

	First Trimester N=242						Second Trimester N=392					
	Normotensive N=178			Preeclampsia N=64			Normotensive N=233			Preeclampsia N=144		
Biomarker	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**
RANTES	47.4	44.0	18.3–160.0	52.4	45.9	26.0–160.0	46.6	43.0	2.0–160.0	49.4	43.4	23.6–160.0
IL4r	338.9	235.0	4.0–4000.0	507.2	247.0	4.0–4000.0	385.6	266.0	4.0–4000.0	338.3	211.0	4.0–4000.0
MIF	23.5	17.0	0.1–100.0	21.8	16.3	0.1–100.0	25.8	15.4	0.05–101.0	27.6	19.2	0.1–100.0
MIP	336.4	193.0	10.0–7792	234.8	195.5	10.0–1343	306.1	219.0	10.0–4223	288.9	172.0	10.0–3300
TNFR1	0.78	0.51	0.08–27.4	0.6	0.5	0.1–2.5	0.68	0.53	0.08–9.3	0.86	0.5	0.1–25.2
TNFα	48.5	4.0	4.0–754.0	40.2	4.0	4.0–467.0	53.4	4.0	4.0–930.0	50.8	4.0	4.0–599.0
IL6	190.1	63.0	4.0–4000.0	413.5	88.5	4.0–4000.0	213.1	71.0	4.0–4000.0	182.8	73.0	4.0–4000.0
IL4	67.1	21.0	4.0–2063.0	72.7	25.0	4.0–1064.0	77.6	21.0	4.0–2538.0	70.8	19.0	4.0–1694.0
IL5	15.3	12.0	4.0–78.0	15.6	13.5	4.0–65.0	17.8	11.0	4.0–244.0	15.2	11.0	4.0–163.0
IL12	18.6	4.0	4.0–1218.0	55.9	8.5	4.0–1508.0	16.2	4.0	4.0–276.0	11.9	4.0	4.0–298.0
IL1α	4.4	4.0	4.0–29.0	4.0	4.0	4.0–4.0	7.3	4.0	4.0–705.0	9.5	4.0	4.0–745.0
IL1β	21.0	9.0	4.0–1489.0	15.1	10.0	4.0–98.0	19.8	10.0	4.0–373.0	23.3	9.0	4.0–1634.0
IL8	201.7	21.5	4.0–4000.0	180.8	13.0	4.0–1458.0	188.0	15.0	4.0–4000.0	190.6	20.5	4.0–4000.0
IL10	83.7	40.0	4.0–801.0	92.0	44.5	4.0–743.0	87.4	46.0	4.0–891.0	79.4	41.5	3.0–836.0
IFNγ	36.3	4.0	4.0–4000.0	33.7	4.0	4.0–902.0	27.3	4.0	4.0–2117.0	36.8	4.0	4.0–2450.0
TGFβ	804.0	604.0	39.0–5501	897.8	718.0	39.0–3468	952.4	872.0	39.0–3765	814.9	682.5	39.0–4882
TFNβ	66.4	10.0	10.0–2364	65.2	10.0	10.0–1658	94.7	10.0	10.0–6821	114.5	10.0	10.0–10000

Table 3

Association between first trimester circulating immune biomarkers and preeclampsia

	All PE (n=64) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value	Preterm PE (n=10) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value	Term PE (n=54) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value
IFNγ	1.2 (0.7–2.2)	0.4853	0.9493	**3.5 (1.4–8.5)**	**0.0063**	0.1134	0.9 (0.5–1.8)	0.8645	0.9493
IL1β	1.2 (0.8–1.8)	0.4928	0.9493	**3.6 (1.7–7.6)**	**0.0006**	**0.0216**	0.9 (0.6–1.5)	0.8240	0.9493
IL4	1.0 (0.7–1.5)	0.8036	0.9493	1.2 (0.6–2.6)	0.4880	0.9493	1.0 (0.7–1.5)	0.9493	0.9493
IL4r	1.1 (0.8–1.6)	0.4645	0.9493	1.5 (0.7–2.8)	0.2754	0.8019	1.1 (0.8–1.5)	0.6458	0.9493
IL5	1.0 (0.7–1.6)	0.6725	0.9493	**2.1 (1.0–4.2)**	**0.0422**	0.5064	0.9 (0.6–1.5)	0.8217	0.9493
IL8	1.0 (0.8–1.4)	0.8410	0.9493	0.8 (0.4–1.8)	0.6351	0.5885	1.0 (0.8–1.4)	0.8977	0.9493
IL6	1.3 (0.9–1.9)	0.1431	0.6244	1.5 (0.8–3.0)	0.1750	0.6886	1.3 (0.9–1.8)	0.2543	0.7629
IL6r	0.9 (0.7–1.4)	0.9445	0.9493	1.0 (0.5–2.0)	0.8894	0.9493	1.0 (0.5–2.0)	0.8981	0.9493
IL10	1.1 (0.8–1.7)	0.4894	0.9493	1.6 (0.8–3.2)	0.2060	0.7063	1.1 (0.7–1.6)	0.7499	0.9493
IL12	**1.6 (1.1–2.6)**	**0.0255**	0.5064	**2.2 (1.0–5.0)**	**0.0589**	0.5774	**1.6 (1.0–2.5)**	**0.0655**	0.5648
MIF	1.0 (0.7–1.4)	0.8925	0.9493	0.8 (0.4–1.7)	0.5518	0.9493	1.0 (0.7–1.5)	0.9201	0.9493
MIP	1.2 (0.8–1.7)	0.3866	0.9278	1.7 (0.9–3.4)	0.0973	0.5838	1.1 (0.7–1.6)	0.6814	0.9493
TGFβ	1.2 (0.9–1.7)	0.2741	0.7739	1.5 (0.8–3.1)	0.2361	0.7555	1.2 (0.8–1.7)	0.4155	0.9493
TNFα	0.9 (0.6–1.5)	0.7802	0.9493	0.9 (0.4–2.5)	0.8184	0.9493	0.9 (0.6–1.6)	0.8230	0.9493
TNFβ	1.1 (0.6–2.1)	0.7305	0.9493	2.1 (0.7–5.9)	0.1786	0.6886	1.0 (0.5–1.9)	0.9341	0.9493
TNFr	0.9 (0.6–1.2)	0.3470	0.8766	**2.0 (1.0–3.6)**	**0.0460**	0.5462	0.8 (0.5–1.1)	0.1138	0.5885

[a] Hazard ratio (HR) and 95% confidence intervals (CI) were calculated using cox regression. Hazard ratios can be interpreted as adjusted odds ratios (Dinse et al.) All models were adjusted for maternal age, fetal sex, BMI, SES, gestational age at blood draw and smoking

[b] Adjusted for multiple comparisons

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Table 4

Association between second trimester immune biomarkers and preeclampsia

	All PE (n=144) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value	Preterm PE (n=27) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value	Term PE (n=117) [a] HR (95% CI)	Raw p-value	[b] Adjusted p-value
IFNγ	0.9 (0.6–1.4)	0.5997	0.9687	0.8 (0.3–1.9)	0.5965	0.9687	0.9 (0.6–1.4)	0.7395	0.9687
IL1β	**0.7 (0.5–0.9)**	**0.0180**	0.5350	0.6 (0.4–1.1)	0.0860	0.7559	**0.7 (0.5–0.9)**	**0.0454**	0.5350
IL4	0.8 (0.6–1.1)	0.2394	0.6760	1.0 (0.6–1.6)	0.9968	0.9968	0.8 (0.6–1.1)	0.1743	0.6436
IL4r	1.0 (0.6–1.1)	0.1089	0.5350	0.9 (0.6–1.4)	0.6518	0.9687	0.8 (0.6–1.0)	0.0962	0.5350
IL5	1.1 (0.8–1.4)	0.5383	0.9687	1.5 (0.9–2.3)	0.1226	0.5350	1.0 (0.8–1.4)	0.8977	0.9968
IL8	1.0 (0.7–1.3)	0.9674	0.9968	0.6 (0.4–1.2)	0.2218	0.6760	1.1 (0.8–1.4)	0.6390	0.9687
IL6	1.1 (0.8–1.3)	0.9510	0.9968	1.1 (0.7–1.7)	0.7011	0.9687	1.0 (0.7–1.3)	0.9292	0.9968
IL6r	1.0 (0.8–1.3)	0.8437	0.9968	1.1 (0.7–1.8)	0.5325	0.9687	1.0 (0.8–1.3)	0.9915	0.9968
IL10	0.8 (0.6–1.1)	0.1582	0.6328	1.0 (0.6–1.6)	0.9877	0.9968	0.8 (0.6–1.1)	0.1074	0.5350
IL12	0.8 (0.6–1.2)	0.2260	0.6760	1.1 (0.6–1.9)	0.8394	0.9968	0.9 (0.6–1.2)	0.4044	0.9243
MIF	1.1 (0.8–1.3)	0.6853	0.9687	0.9 (0.6–1.4)	0.5684	0.9687	1.1 (0.8–1.4)	0.5944	0.9687
MIP	0.9 (0.7–1.1)	0.3212	0.7709	0.8 (0.5–1.3)	0.2384	0.6760	0.9 (0.7–1.2)	0.4647	0.9687
TGFβ	**0.8 (0.6–0.9)**	**0.0292**	0.5350	0.7 (0.4–1.1)	0.1173	0.5350	0.8 (0.6–1.0)	0.0642	0.5350
TNFα	1.1 (0.8–1.5)	0.7669	0.9687	0.9 (0.5–1.7)	0.7224	0.9687	1.1 (0.8–1.5)	0.6249	0.9687
TNFβ	0.7 (0.4–1.1)	0.1158	0.5350	0.4 (0.1–1.2)	0.1028	0.5350	0.8 (0.5–1.2)	0.2711	0.7229
TNFr	1.0 (0.8–1.3)	0.7468	0.9687	1.0 (0.6–1.6)	0.9493	0.9968	1.0 (0.8–1.3)	0.7300	0.9687

[a] Hazard ratio (HR) and 95% confidence intervals (CI) were calculated using cox regression. Hazard ratios can be interpreted as adjusted odds ratios (Dinse et al.) All models were adjusted for maternal age, BMI, SES, gestational age at blood draw and smoking

[b] Adjusted for multiple comparisons