HHS Public Access

Author manuscript

Pregnancy Hypertens. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Pregnancy Hypertens. 2018 April ; 12: 53–57. doi:10.1016/j.preghy.2018.02.009.

The impact of female fetal sex on preeclampsia and the maternal immune milieu

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Abstract

Objective—Small studies suggest that fetal sex alters maternal inflammation. We examined the association between fetal sex, preeclampsia and circulating maternal immune markers.

Methods—This was a secondary data analysis within a nested case-control study of 216 preeclamptic women and 432 randomly selected normotensive controls from the Collaborative Perinatal Project. All women had singleton, primiparous pregnancies without chronic health conditions. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for associations between female fetal sex and preeclampsia. Outcomes included preeclampsia, preterm preeclampsia (<37 and <34 weeks), and normotensive preterm birth <37 weeks. Associations between female fetal sex and immune markers [interleukin (IL)-6, IL4, IL5, IL12, IL10, IL8, IL1-beta, interferon (IFN)-gamma, tumor necrosis factor (TNF)-beta, and transforming growth factor-beta] were examined using a statistical method developed for large

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Conflicts of interest

The authors have no conflict of interests to disclose

proportions of censored biomarker data. Models were adjusted for maternal age, race, body mass index, and smoking.

Results—Women with early preterm preeclampsia (<34 weeks) had higher odds of having a female fetus ($OR_{adj.}$ 3.1, 95% CI 1.2-8.0) and women with normotensive preterm birth had lower odds ($OR_{adj.}$ 0.5, 95% CI 0.3-0.9). Female fetal sex was associated with lower first trimester pro-inflammatory IFNγ and IL-12 but higher second trimester pro-inflammatory IL1β and TNFβ, anti-inflammatory IL4r, and regulatory cytokines IL5 and IL10. Female fetal sex was associated with higher postpartum IL10 in preeclamptic women only.

Conclusions—We identified sexual dimorphism in maternal inflammation. Longitudinal studies are needed to determine if fetal sex impacts the maternal immune milieu across pregnancy.

Keywords

Fetal; inflammation; preeclampsia; postpartum

Introduction

Sexual dimorphism is present in several pregnancy complications. Observational studies consistently report associations between male fetal sex and preterm birth, fetal loss, and infant mortality [1–3]. A meta-analysis found that male fetal sex increased the risk of preeclampsia/eclampsia (relative risk = 1.1) in non-Asian populations [4]. Another meta-analysis reports that female fetal sex is associated with preterm preeclampsia <37 weeks (odds ratio 1.1) and <34 weeks (odds ratio 1.4) of gestation [5]. Thus, the literature remains conflicting.

Despite evidence that sexual dimorphism may influence pregnancy outcomes the mechanisms are not elucidated. In a study of 80 women, stimulated production of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL1β, but not circulating cytokines, were higher in women with female fetuses [6]. In 38 healthy women, women with female fetuses had higher serum regulatory cytokines (IL-5, IL-9, IL-17, and IL-25) while women with male fetuses had higher pro-inflammatory markers (G-CSF, IL-12p70, IL-21, and IL-33) across pregnancy [7]. Clifton et al. reports sexual dimorphism in placental adaptation. Female placentas have significant changes (e.g. immune gene expression) in response to maternal asthma while male placentas display little change [8, 9].

The hypothesis that fetal sex affects the maternal immune milieu is interesting and aligned with sex differences in immune function across the life-course (e.g. males are susceptible to some infectious diseases and females are biased towards auto-immunity) [10]. However, investigations of fetal sex, maternal immunity and pregnancy outcomes are limited to a handful of small studies. Only one study has examined fetal sex and maternal immune markers postpartum [7], finding no differences. Exploring the postpartum period among complicated pregnancies may be warranted as preeclampsia increases the risk of cardiovascular disease by 3-fold [11]. Additionally, maternal immune markers differ by race/ethnicity and future investigations should account for this potential confounding factor [12].

This study examined associations between fetal sex, preeclampsia and the systemic maternal immune milieu during pregnancy and postpartum. We utilized a sandwich immunoassay which is appealing for large investigations as they are cost saving, measure multiple biomarkers and required smaller sample volume. However, immunoassays rely on identifying epitopes which can be blocked by interactions between cytokines and host blood proteins [13] resulting in subjects measuring outside of the limit of detection (LOD) [14]. To increase the validity of our analysis to handle data below the LOD, we utilized a statistical method developed for large proportions of censored biomarker data [15].

Methods

Population and study design

This was a secondary data analysis of a nested case control study within the Collaborative Perinatal Project (CPP), a longitudinal study of 55,908 pregnancies [16] enrolled between 1959 and 1965 from 12 university-affiliated medical centers in the United States. Oral consent (standard at the time) was obtained [17]. The nested case control study examined associations between immune biomarkers and preeclampsia [18] and included a random sample of 216 preeclamptic women who had primiparous and singleton pregnancies. Exclusions included a history of diabetes, cardiovascular disease or hypertension or no stored first study visit serum samples (collected prior to 27 weeks of gestation with no recorded thaws). Two normotensive controls (1:2) were randomly selected for each case using the above inclusion/exclusion criteria. Matching was not used in this study. The Texas A&M University Institutional Review Board approved the current investigation.

Outcome measurements

Preeclampsia was the primary outcome and in the CPP was based on chart abstraction of blood pressure and protein levels and defined as new gestational hypertension after 20 weeks of gestation (≥two measurements of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) and proteinuria (two random urine dipsticks of 1+ protein or one dipstick of 2+ protein). In the intrapartum period, the first five pressures obtained after hospital admission for delivery were averaged.

Secondary outcomes were included in this study. It is accepted that preeclampsia is a heterogeneous disease with subtypes that have different pathophysiological pathways [19, 20]. Preeclampsia can be classified as term preeclampsia (birth ≥37 weeks gestation) or preterm preeclampsia (birth < 37 weeks gestation or <34 weeks gestation). Cases of preeclampsia can also be considered severe if they had at least one of the following symptoms: systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥110 mmHg, proteinuria of 5g/24 hours, proteinuria of 3+ or more, oliguria, pulmonary edema, or convulsions/eclampsia. The HELLP syndrome had not yet been described at the time of the CPP, and liver function tests and platelet counts were not included in the database. Small for gestational age (SGA) was defined previously in the CPP as a birth weight <10th percentile for gender, race, and gestational age using birth weight distributions. As fetal sex is implicated in preterm birth, we examined preterm birth <37 weeks of gestation in

normotensive controls only. Gestational age was determined by the date of delivery minus the date of last menstrual period.

Cytokine measurements

In the CPP, first study visit fasting blood samples were collected in glass vacutainers. After separation, maternal serum was stored at −20°C. Serum samples were monitored continuously from the time of collection and had no recorded thaws. Immune markers were measured in duplicate with an in-house multiplex flow cytometric assay system (LabMap, Luminex Corporation, Austin, Texas) at the Statens Serum Institute in Copenhagen [13]. Calibration curves for analytes were calculated by the Bio-Plex 3.0 software (BioRad, US). Mean intra- and inter-assay CVs were 6.2% and 16%, and ranged from 6.7 to 13 (IL4 and TNF-α) and 10 to 25 (IL-4 and TNF-α) [13]. We acknowledge that the long-term storage of CPP samples raises concerns. Our assay was validated using ten anonymously collected residual dried blood spot specimens stored for 23 years at −24°C in the Danish biological bio-bank [13]. In a CPP study, cytokines were measured and compared to fresh samples finding consistent results [21]. Furthermore, the same immune markers measured using CPP samples had similar proportions of non-detectable levels compared to the more contemporary Danish National Birth Cohort (DNBC) [22] [23]. As IL2, IL1α, and TNF-α measured below the LOD in 75% of patients, they were excluded. Table 1 shows the median, interquartile range and proportion below the limit of detection for each marker. Associations between immune markers and preeclampsia in the CPP have been described previously [24].

Statistical analyses

Potential covariates included self-reported maternal age, marital status, family income, occupation, education, race (white, non-white), maternal smoking (yes/no), and pre-pregnancy body mass index (BMI) determined by weight (kg)/height $(m)^2$. Socioeconomic status was determined using a composite score of education, occupation and family income [24]. Maternal age, BMI, race, and smoking were included in all final models described below. All analyses were performed using SAS V9.2 (Cary, NC).

We examined associations between female fetal sex and preeclampsia. Secondary outcomes included term preeclampsia, preterm (<37 weeks) preeclampsia, early preterm (<34 weeks) preeclampsia and severe preeclampsia. Separate models were run with normotensive controls as the reference group (outcomes were binary). Other binary outcomes included SGA and SGA with preeclampsia (reference: women without SGA). Among normotensive controls we examined associations between fetal sex and preterm birth (<37 weeks of gestation) using normotensive women who delivered ≥ 37 weeks of gestation as the reference [5]. Logistic regression calculated adjusted odds ratios (OR) and 95% confidence intervals.

We examined associations between immune markers and female fetal sex. As immune marker levels can vary by gestational age at sampling, we stratified by the blood sampling time-frame. There were 242 women with first trimester serum samples (mean gestation age 10.7±1.9) and 392 women with second trimester serum samples (mean gestation age 16.5±1.7). In total, 591 women had postpartum samples collected 6-8 weeks after delivery

available for analysis. To reduce the number of analyses, cases and controls were combined. It is possible that the relationship between fetal sex and immune markers may be modified by pathological conditions. We added a preeclampsia*fetal sex interaction term to the models and if significant the analysis was stratified by normotensive and preeclamptic women to explore differences in effect estimates. Common approaches to handling data below the LOD, such as replacing with a set value (0 or LOD/2) or multiple imputation, are well recognized to be prone to biases [15, 25]. We used a statistical method described by Dinse et al., to handle biomarkers with a high proportion below the LOD [15]. For each biomarker, a value M is used so that the outcome for such analyses is M minus the biomarker value (i.e. the time variable is the designated upper bound minus the marker value). This transformation makes observations under the LOD become administratively censored data (at M-LOD) and allows comparing the distribution of biomarkers among groups without exclusion or imputation of values. Cox proportional hazard models are then applied to examine associations between biomarkers and binary variables where the hazard ratio can be interpreted as an odds ratio between the biomarkers and those binary variables [15]. Deviance residuals were plotted, as cox models assume that hazard functions for different covariate (i) values are proportional for all values of t, finding some aberration from randomness.

Results

Compared to normotensive women, preeclamptic women had a lower maternal age [median 19.0 (5.0) vs. 20.0 (IQR 5.0)], were more likely to be single (33.8% vs. 22.5%), more likely to have a low SES (42.5% vs. 26.9%), have a higher BMI [median 21.3 (4.0) vs. 20.8 (3.2)], and less likely to smoke (31.9% vs. 42.8%).

Female fetal sex was not associated with preeclampsia overall ($OR_{adj.}$ 1.2, 95% CI 0.8-1.7) but was associated with preterm preeclampsia <34 weeks ($OR_{adj.}$ 3.2, 95% CI 1.1-9.6). Among normotensive controls, female fetal sex was inversely associated with preterm birth ($OR_{adj.}$ 0.5, 95% CI 0.2-0.9) (Table 2). No other associations were observed.

In first trimester samples, female fetal sex was inversely associated with pro-inflammatory IFNγ ($OR_{adj.}$ 0.5, 95% CI 0.3-0.8) and IL-12 ($OR_{adj.}$ 0.7, 0.5-1.0) (Table 3). There were no other associations found. There were no differences in the relationship between fetal sex and immune markers by preeclampsia/normotensive status.

In second trimester samples, female fetal sex was associated with pro-inflammatory IL1β ($OR_{adj.}$ 1.5, 95% CI 1.1-2.0) and TNFβ ($OR_{adj.}$ 1.7, 95% CI 1.1-2.5), anti-inflammatory IL4r ($OR_{adj.}$ 1.3, 95% CI 1.1-1.6), and regulatory IL5 ($OR_{adj.}$ 1.4, 95% CI 1.1-1.8) and IL10 ($OR_{adj.}$ 1.3, 95% CI 1.1-1.7) (Table 3). There was a significant interaction between female fetal sex and preeclampsia for IL1β (p=0.0005) and marginally for IL12 (p=0.07). When stratified by normotensive ($OR_{adj.}$ 2.1, 95% CI 1.5-2.9) and preeclamptic women ($OR_{adj.}$ 0.8, 95% CI 0.5-1.1) there were differences observed in the direction of the association between fetal sex and IL1β. Results were similar for IL12 (normotensive: $OR_{adj.}$ 1.7, 95% CI 1.1-2.5; preeclampsia: $OR_{adj.}$ 0.8, 95% CI 0.5-1.5). There were no other differences observed by preeclampsia status.

In postpartum samples, female fetal sex was associated with regulatory IL10 (OR_{adj}. 1.2, 95% CI 1.0-1.4) (Table 3). Female fetal sex significantly interacted with preeclampsia for IL10 (p=0.01). Female fetal sex was associated with IL10 in preeclamptic women (OR_{adj}. 1.7, 95% CI 1.2-2.4) but not normotensive women (OR_{adj}. 1.0, 95% CI 0.7-1.2).

Discussion

We found that female fetal sex was associated with early preterm preeclampsia (<34 weeks) but inversely associated with normotensive preterm birth <37 weeks. The association with early preterm preeclampsia is consistent with a recent meta-analysis of 219,575 independent live born singleton pregnancies from 11 studies [5]. Observational studies have found similar results [1–3, 26, 27]. Our finding that male fetal sex is associated with preterm birth in normotensive women is consistent with large Scandinavian studies [2, 3, 28]. In over 1 million singleton pregnancies in Norway, women with female fetuses were more likely to have preterm preeclampsia (< 37 weeks) and but less likely to have term preeclampsia and preterm birth [3]. A population based study of 574,353 Australian women found similar results [26]. Still, the effect of fetal sex on preterm preeclampsia may be minimal as reported by the meta-analysis (OR 1.4) [5]. While our study found an effect size of 3.2, our smaller sample size resulted in lower precision. Thus, we cannot make any definitive conclusions regarding the magnitude of effect that female fetal sex may have on preterm preeclampsia. Nor are we able to establish a cause-effect relationship.

As combining heterogeneous subtypes of preeclampsia may mask associations [19], it may not be surprising to find an association with preterm preeclampsia but no association among all preeclamptic women, as most women delivered at term. Preeclampsia is considered a heterogeneous syndrome consisting of subtypes (e.g. early vs. late onset) with different underlying pathologies [19, 20]. In earlier onset disease, implantation abnormalities and placental dysfunction may be more common [20]. Inadequate placental perfusion is hypothesized to lead to the release of materials into the maternal circulation. Maternal/fetal interactions may influence placental perfusion or the response to this stimulus resulting in excessive inflammation, endothelial dysfunction and the clinical symptoms of preeclampsia [29]. However, preeclampsia can occur with a normally functioning placenta (often late onset preeclampsia) [20]. Male fetal sex is associated with implantation failure, spontaneous abortion, stillbirth, preterm birth [2, 26] and increased perinatal death following preeclampsia [3]. Vatten et al. proposed that women with male fetuses have a higher risk of severe implantation failure resulting in early fetal loss [3, 28] leading to biased data (i.e. these pregnancies are excluded from analyses of fetal sex and preeclampsia).

Fetal sex may influence maternal immune system function which could be a plausible mediator between fetal sex and preeclampsia. The role of the immune system in preeclampsia has been described previously [30, 31]. The pathogenesis likely begins very early in pregnancy for which uterine natural killer cells, dendritic cells and T regulatory (Tregs) are critical for implantation, placentation and maternal-fetal tolerance [31]. An altered immune response, beyond that in normal pregnancy, is observed in preeclampsia along with lower Tregs, increased Th17 cells and circulating pro-inflammatory cytokines (e.g. IL-6, TNF-α and IL-17) [30]. Female fetal sex was inversely associated with first

trimester IFNγ and IL-12. Previous studies report that male fetal sex is associated with increased maternal inflammation and may explain the increased risk of preterm birth [7, 32, 33]. As first trimester IFNγ is linked to preterm preeclampsia [23], perhaps the observed decrease of these markers in women with female fetuses supports the hypothesis by Vatten et al., described above.

In contrast, female fetal sex was associated with increased second trimester pro-inflammatory (TNFβ and IL1β), anti-inflammatory (IL4r) and regulatory cytokines (IL5 and IL10). A small study of healthy pregnant women also reported associations between female fetal sex and regulatory cytokines but also increased anti-angiogenic markers [7]. Second trimester TNFβ was associated with preeclampsia in the Danish National Birth Cohort [22]. However, in two of our previous studies, increased second trimester IL1β was associated with reduced odds of preeclampsia [22, 23]. We did observe lower IL1β levels among preeclamptic women with female fetuses but higher levels among normotensive women with female fetuses.

Our results were not consistent by trimester. Perhaps sexual dimorphism in second trimester immune markers is a reflection of a differential response to early clinical symptoms of preeclampsia. Clifton et al., report that male fetuses subjected to inflammatory conditions remain on their growth trajectory while female fetuses respond by reducing growth [34]. The male fetal response to adverse maternal stimuli may increase the risk of fetal loss or early spontaneous delivery. Perhaps female fetuses respond in a way that reduces growth and alters severity of symptoms prompting induction of labor. The female placenta increases immune gene expression (JAK1, IL2RB, IL1RTL, TNFr) [35] and cytokine expression (TNF-α, IL-1β, IL-6, IL-5, IL-8) in response to maternal asthma [34]. Others report that male placenta has higher expression of TLR4 and subsequent TNF-α in response to LPS [33], perhaps explaining the risk of preterm birth [34]. Placental cytokine expression in preeclamptic women (n=5) is reported to be higher compared to normotensive women (n=5) in male fetuses only [36].

Preeclamptic women with female fetuses had higher levels of IL10 in the postpartum period. Preeclampsia is associated with an increased risk of cardiovascular disease later in life [11], particularly for early onset disease (<34 weeks gestation) [11]. IL10 can have anti-inflammatory effects and is protective against heart failure in patients with previous cardiovascular events [37]. However, other studies have found opposite results [38, 39]. Future research should examine whether fetal sex modifies the risk of long-term health consequences in preeclamptic women.

Compared to other studies examining fetal sex and maternal systemic markers, we had a large sample size and were able to examine complicated pregnancies. We were limited by a single time point during pregnancy for each subject. The age of the CPP cohort is a limitation and although this may affect the stability of immune markers it would be similar between groups limiting bias. The proportion of non-detectable levels for each immune marker is similar to those in the more contemporary DNBC [22]. Furthermore, the statistical analyses used to handle data with large proportions below the LOD is a strength over substitution methods [15]. Multiple comparisons lead to an increase in type I error and

results need to be interpreted with caution. We did not have data on anti-angiogenic markers which are associated with preeclampsia and female fetal sex [7, 40]. Lastly, our cohort is not representative of contemporary American women. Maternal risk exposures can change over time such as BMI which is associated with placental dysfunction and preeclampsia [41, 42]. Future investigations examining interactions between fetal sex and BMI would be interesting. Lean women with male fetuses have higher antioxidant activity which is lost during obesity [42], suggesting that male antioxidant defense mechanism are disrupted.

Female fetal sex may be associated with early preterm preeclampsia. However, it remains unclear if this is due to a physiologic difference in the maternal response to male and female fetuses or bias in the data. Our results may suggest that female fetuses respond differently to subclinical preeclampsia resulting in an altered systemic maternal immunological response in the second trimester. However, longitudinal studies across pregnancy in diverse populations should be conducted to make any conclusions regarding the effect of fetal sex on maternal immunity.

Acknowledgments

Sources of funding

R01HD048669 from the National Institute of Allergy and Infectious Diseases (CH) and a grant from the Discovery Foundation (BT).

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Highlights

- Female fetal sex was associated with early preterm preeclampsia (<34 weeks)
- Male fetal sex was associated with normotensive preterm birth
- Male fetal sex was associated with first trimester pro-inflammatory cytokines
- Female fetal sex was associated with anti-inflammatory and regulatory cytokines
- Women with preeclampsia and a female fetus had higher IL-10 postpartum

Table 1

Medians, interquartile range (IQR) and proportion below the limit of detection (LOD) for each serum immune marker

Immune marker	First Trimester N=250	Second Trimester N=392	Postpartum N=591
	Median (IQR) % below LOD	Median (IQR) % below LOD	Median (IQR) % below LOD
IFNγ pg/ml	4 (6) 50% < LOD	4 (4) 50% < LOD	4 (0) 75% < LOD
IL1β pg/ml	9 (12) 25% < LOD	10 (13) 25% < LOD	9 (13) 25% < LOD
IL4 pg/ml	21.5(70) 25% < LOD	20 (71) 25% < LOD	18 (67) 25% < LOD
IL4r pg/ml	239.5 (327) 10% < LOD	250 (302) 5% < LOD	198 (278) 5% < LOD
IL5 pg/ml	13 (16) 25% < LOD	11 (15) 25% < LOD	12 (18) 25% < LOD
IL8 pg/ml	19 (101) 25% < LOD	18 (113) 25% < LOD	4 (18) 50% < LOD
IL6 pg/ml	73.5(197) 10% < LOD	4 (209) 25% < LOD	96 (229) 10% < LOD
IL10 pg/ml	42(86) 25% < LOD	43 (97) 25% < LOD	18 (109) 10% < LOD
IL12 pg/ml	4 (11) 50% < LOD	4 (10) 50% < LOD	4 (9) 50% < LOD
MIF pg/ml	16.9(28.5) 10% < LOD	17.4 (32.9) 10% < LOD	23 (42) 5% < LOD
MIP pg/ml	193 (259) 10% < LOD	201 (283) 10% < LOD	195 (272) 10% < LOD
TGFβ pg/ml	665 (1030) 10% < LOD	225 (1083) 10% < LOD	706 (1148) 10% < LOD
TNFβ pg/ml	10(20) 50% < LOD	10 (15) 50% < LOD	10 (0) 75% < LOD
TNFr pg/ml	0.52 (0.59) 10% < LOD	0.52 (53) 5% < LOD	0.96 (0.95) 5% < LOD

Table 2

Pregnancy outcomes by fetal sex (N=648)

	Male fetal sex n=323	Female fetal sex n=325	[a] OR 95% CI	[b] Adj. OR 95% CI
[c] *Preeclampsia*				
Preeclampsia	102 (31.6)	114 (35.1)	1.1 (0.8–1.6)	1.2 (0.8–1.7)
[c] *Preeclampsia defined by gestational age at delivery and severity*				
Term preeclampsia	86 (28.0)	92 (30.4)	1.1 (0.8–1.6)	1.2 (0.8–1.7)
Delivery <37 weeks gestation	16 (6.8)	22 (9.4)	1.4 (0.8–2.8)	1.6 (0.8–3.4)
Delivery <34 weeks gestation	5 (2.2)	13 (5.8)	2.7 (1.0–7.7)	3.2 (1.1–9.6)
Severe preeclampsia	22 (9.1)	31 (12.8)	1.5 (0.8–2.6)	1.5 (0.8–2.7)
[d] *Small for gestational age*				
Small for gestational age				
SGA only	42 (13.6)	37 (11.9)	0.9 (0.6–1.4)	0.9 (0.6–1.4)
SGA and preeclampsia	24 (7.7)	25 (8.0)	1.0 (0.6–7.8)	1.1 (0.8–1.5)
[e] *Preterm birth among normotensive controls*				
Normotensive preterm birth	37 (11.5)	19 (5.9)	0.5 (0.3–0.9)	0.5 (0.2–0.9)

[a] Odds ratios were estimated using logistic regression

[b] Adjusted for race, maternal age, body mass index and smoking

[c] Reference group normotensive controls

[d] Reference group pregnancies without SGA

[e] Reference group term (≥37 weeks) control deliveries

Table 3

Associations between female fetal sex and immune markers in the first, second and postpartum periods

	First Trimester N=250	Second Trimester N=392	Postpartum N=591
	[ab] Adj. OR (95% CI)	[ab] Adj. OR (95% CI)	[ab] Adj. OR (95% CI)
IFNγ	0.5, 0.3–0.8	1.0, 0.7–1.5	[c] NA
IL1β	0.9, 0.6–1.3	1.5, 1.1–2.0	1.1, 0.9–1.4
IL4	1.0, 0.7–1.4	1.2, 0.9–1.6	1.1, 0.9–1.4
IL4r	0.9, 0.7–1.2	1.3, 1.1–1.6	1.1, 0.9–1.3
IL5	0.8, 0.5–1.1	1.4, 1.1–1.8	1.1, 0.9–1.4
IL8	0.8, 0.5–1.1	1.2, 0.9–1.5	1.0, 0.8–1.2
IL6	1.0, 0.8–1.4	1.1, 0.8–1.4	1.1, 0.9–1.3
IL10	0.9, 0.7–1.3	1.3, 1.1–1.7	1.2, 1.0–1.4
IL12	0.7, 0.5–1.0	1.3, 1.0–1.8	1.2, 0.9–1.6
MIF	0.9, 0.7–1.2	1.2, 0.9–1.5	0.8, 0.7–1.0
MIP	0.8, 0.6–1.1	1.2, 0.9–1.5	1.1, 0.9–1.3
TGFβ	1.0, 0.7–1.3	1.1, 0.9–1.4	1.1, 0.9–1.3
TNFβ	0.9, 0.5–1.5	1.7, 1.1–2.5	[c] NA
TNFr	0.8, 0.6–1.1	1.1, 0.8–1.3	0.9, 0.8–1.1

[a] Cox regression was used to estimate effect size using a method described by Dinse et al. Details are in the methods section.

[b] Adjustments were made for race, age, gestational age at blood draw, smoking and BMI

[c] Excluded as >50% were below the LOD