Second and third trimester serum levels of growth-differentiation factor-15 in the prediction of pre-eclampsia

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What are the novel findings of this work?

It has been suggested that serum levels GDF-15 could be predictive of pre-eclampsia. In this case-control study, in asymptomatic pregnant women who later developed pre-eclampsia, GDF-15 levels were higher at 30-34 weeks but not significantly different at 19-24 weeks and at 35-37 weeks, when compared to unaffected pregnancies.

What are the clinical implications of this work?

GDF-15 seems to increase a few weeks prior to pre-eclampsia development. Leading to significantly higher levels at 30-34 weeks in patients who will later develop preterm pre-eclampsia. However, this marker may not be as good as previously described markers, and the high variability in the population may preclude its utility in clinical practice.

ABSTRACT

Background: Pre-eclampsia (PE) is a significant contributor to maternal and perinatal adverse outcome, however accurate prediction and early diagnosis of this condition remain a challenge.

Objectives: To compare serum levels of growth-differentiation factor 15 (GDF-15) at three different gestational ages between asymptomatic women who subsequently developed preterm or term PE and healthy controls.

Methods: This was a case-control study drawn from a prospective observational study for adverse pregnancy outcomes in women attending for their routine second and third trimester hospital visits. Serum GDF-15 was determined in 300 samples using a commercial GDF-15 enzyme-linked immunosorbent assay: 120 samples at 19-24 weeks of gestation, 120 samples at 30-34 weeks and 60 samples at 35-37 weeks. Multiple linear regression was applied on logarithmically transformed GDF-15 control values to evaluate the influence of gestational age at blood sampling and maternal characteristics on GDF-15 results. Multiples of the normal median (MoM) GDF-15 values, adjusted for gestational age and maternal characteristics, were compared between the PE group and healthy controls.

Results: Values of GDF-15 increased with gestational age. There were no significant differences in GDF-15 values between cases of preterm or term PE and normotensive pregnancies at 19-24 or 35-37 weeks of gestation. At 30-34 weeks, GDF-15 MoM values were significantly increased in cases of preterm PE,

but not in those who later developed term PE. Elevated GDF-15 MoM values were significantly associated with a shorter interval between sampling at 30-34 weeks and delivery with PE (P=0.005).

Conclusion: Serum GDF-15 levels at 19-24 or 35-37 weeks of gestation are not predictive of preterm or term PE. At 30-34 weeks, GDF-15 levels are higher in women who subsequently develop pre-eclampsia; however, this difference is small and GDF-15 is unlikely to be useful in clinical practice when used in isolation.

INTRODUCTION

Pre-eclampsia (PE) is a serious pregnancy complication associated with increased short- and long-term risks of death and morbidity for both the mother and her child. Such risks can be reduced by screening during the first trimester of pregnancy and use of low dose aspirin in the high-risk group and screening during the second and third trimester to stratify the population into risk categories in need of different intensities of subsequent monitoring, thereby minimizing unexpected adverse perinatal events.[1-6] Several studies have investigated the value of potential biomarkers for the prediction of PE. Those found to be useful at 11-13 and 19-24 weeks' gestation are mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and placental growth factor (PLGF), in the early third trimester MAP, UtA-PI, PLGF and serum soluble fms-like tyrosine kinase-1 (sFLT-1) and in late third trimester MAP, PLGF and sFLT-1.[7-10] Screening at 11-13 weeks by a combination of maternal factors, MAP, UtA-PI and PLGF identifies, at 10% screen-positive rate (SPR) about 75% of preterm PE with delivery at <37 weeks' gestation but only about 45% of term-PE.[2,11] Improved prediction of term-PE to 70% is achieved through screening at 35-37 weeks by a combination of maternal factors, MAP, PlGF and sFLT-1.[12] Consequently, there is need for further research to identify new biomarkers that could improve the prediction of both preterm and term PE.

One potential new biomarker for PE is growth-differentiation factor 15 (GDF-15), which is also known as macrophage inhibitory cytokine-1 (MIC-1), a member of the transforming growth factor beta superfamily.[13,14] In healthy adults, GDF-15 expression in normal tissues is low, but its concentrations are higher in pathways of injury, inflammation and repair processes.[13,15] During pregnancy, GDF-15 is highly expressed in the placenta and can be detected in maternal serum throughout gestation with a significant increase from the first to the third trimester.[16-18] During the first trimester of normal pregnancies, GDF-15 levels are about 15-fold higher compared to non-pregnant women,[16] and GDF-15 is thought to be involved in trophoblast invasion during placentation to suppress the maternal immune response.[17] Lower levels of GDF-15 during early stages of pregnancy have been associated with miscarriage and ectopic pregnancy.[19,20] Previous studies have demonstrated no differences in GDF-15 levels at 11-13 weeks' gestation between women who later developed PE and controls,[18] and the possible role of this marker at PE diagnosis is unclear, with conflicting reports suggesting decreased, unchanged or increased GDF-15 levels in pregnancies following PE diagnosis.[18,21-23]

The objective of this study is to compare serum levels of GDF-15 serum levels in asymptomatic women who later developed preterm or term PE compared to healthy controls at three different gestational ages (GA) prior to diagnosis.

METHODS

Study population

This was a case-control study drawn from a prospective observational study for adverse pregnancy outcomes in women attending for their routine second and third trimester hospital visits at the Harris Birthright Unit for Fetal Medicine, King's College Hospital, London, between July 2011 and January 2016.

Patient characteristics recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or *in vitro* fertilization), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), systemic lupus erythematosus (yes or no), antiphospholipid syndrome (yes or no), pre-gestational diabetes (yes – type 1 or type 2 – or no), family history of PE in the mother of the patient (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks), previous pregnancy with PE (yes or no) and maternal weight and height.

PE was defined according to the criteria established by the International Society for the Study of Hypertension in Pregnancy,[24] as systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure 90 mmHg or more developing after 20 weeks of gestation together with significant proteinuria or other maternal organ dysfunction in a previously normotensive woman. Significant proteinuria is

defined by 300 mg or more in 24 hours or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available. In PE superimposed on chronic hypertension, significant proteinuria (as defined earlier or worsening proteinuria compared to baseline levels) should develop after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

Blood collection and analysis

Written informed consent was obtained from all pregnant women agreeing to participate in the study, which was approved by the UK National Research Ethics Committee (reference number: 02-03-033). Overall, 300 samples were obtained: 120 samples at 19-24 weeks (60 controls, 30 cases of preterm PE and 30 cases of term PE), 120 samples at 30-34 weeks (60 controls, 30 cases of preterm PE and 30 cases of term PE), and 60 samples at 35-37 weeks of gestation (40 controls and 20 cases of term PE). Maternal blood was collected, and the resulting serum was stored at -80°C until analysis.

Serum GDF-15 was determined using a commercial GDF-15 enzyme-linked immunosorbent assay (ELISA) kit (R&D System, Minneapolis, USA) according to the manufacturer's protocol. The assay was performed by a laboratory professional who was blinded to the outcomes of the pregnancies. Serum

samples were diluted 1/100, and the standard was serially diluted from a starting concentration of 3000 pg/mL down to 46.88 pg/mL, in the sample diluent supplied with the kit.

Statistical analysis

Qualitative data are presented as absolute numbers and proportions and quantitative variables are summarized as means and standard deviations. Comparison of baseline characteristics between cases and controls used the Mann-Whitney U test and Chi-square or Fisher's exact test, as appropriate. Multiple linear regression with stepwise backward elimination was applied on logarithmically transformed GDF-15 control values to determine the influence of gestational age at blood sampling and maternal characteristics that significantly impact on GDF-15 results. GDF-15 values in the study groups were presented in multiples of the normal median (MoM) and adjusted for gestational age and maternal characteristics (variables with significance level below 0.1 were kept in the model). The distributions of GDF-15 MoM values were then compared between the groups at each gestational age interval with one-way analysis of variance (ANOVA) using logarithmically transformed values, with Dunnett's post-hoc adjustment for multiple comparisons within each gestational age period and using the control group as the reference category. To investigate the significance of the relationship between GDF-15 MoM values and the interval between blood sampling and delivery with PE, Cox proportional-hazards models were applied at

each gestational censoring deliveries that occurred without PE. Finally, the ability of GDF-15 MoM values to predict PE at each gestational window was assessed by analysis of the proportion of cases with GDF-15 MoM levels exceeding the upper quartile of controls and with the use of receiver-operating characteristics (ROC) curves.

Between-group comparisons were considered statistically significant at a 0.05 significance level. Statistical analysis was performed with SPSS Statistics (IBM Corp. Released 2019. IBM SPSS Statistics for Macintosh, Version 26.0 Armonk, NY: IBM Corp).

RESULTS

Maternal characteristics according to pregnancy outcome are presented in Table 1. Women who developed PE, compared to healthy controls, had significantly higher maternal weight, incidence of PE in a previous pregnancy and chronic hypertension.

GDF-15 was detectable in all 300 samples tested, and the values increased with gestation in both the cases of PE and the controls (Figure 1). Values of GDF-15 increased with maternal age and gestational age and decreased with increasing maternal weight. The median GDF-15 values in each group and at the different blood sampling intervals are summarized in Table 2.

Amongst PE cases, GDF-15 levels exceeded the upper quartile of the control group in 33.3% (20/60) at 19-24 weeks, in 43.3% (26/60) at 30-34 weeks and in 25% (5/20) at 35-37 weeks of gestational age.

There were no significant differences in GDF-15 values between cases of preterm or term PE and healthy controls at 19-24 or 35-37 weeks of GA. At 30-34 weeks, GDF-15 MoM values were significantly increased in those who developed preterm PE, but not in those who developed term PE (Table 2). A statistically significant association between increased GDF-15 values and time-to-delivery with PE was found at 30-34 weeks of gestation ($P = 0.005$), while no significant association was found at 19-24 weeks and 35-37 weeks of gestation ($P= 0.259$ and $P=0.832$, respectively, Figure 2).

ROC curve analysis using GDF-15 MoM values in isolation for the prediction of pre-eclampsia at each gestational window are presented in Figure 3 and demonstrated areas under the curve (AUC) of 0.54 (95% CI 0.44 to 0.65) at 19-24 weeks, 0.66 (95% CI 0.57 to 0.76) at 30-34 weeks and 0.59 (95% CI 0.44-0.74) at 35-37 weeks' gestation.

DISCUSSION

Main findings

GDF-15 levels in maternal serum increased throughout gestation in healthy pregnancies as well as in patients who later developed PE. GDF-15 levels were significantly higher in the PE group at 30-34 weeks, due to the differences between preterm PE and normotensive pregnancies. We also identified a significant negative association between elevated GDF-15 levels at 30-34 weeks of gestation and the interval from sampling to delivery with PE. No significant differences were identified at 19-24 and 35-37 weeks. It is plausible that, analogous to sFLT-1, levels of GDF-15 increase only within a few weeks prior to the development of PE.

Results of previous studies

In healthy non-pregnant adults, GDF-15 levels are low, whereas during pathologic processes including inflammation and injury increased concentrations have been detected.[13-15] Although the reason for this GDF-15 elevation under certain pathological circumstances is not clearly understood, it could be partly explained by the pleomorphic effects of GDF-15 on different cell types.[25] GDF-15 seems to be closely related to tissue remodeling processes and may regulate cell growth as well as apoptosis, which are typical events observed in neoplastic and cardiovascular diseases.[25] As a consequence, GDF-15 can be used as a

prognostic marker in arteriosclerotic diseases and in oncology, indicating poor prognosis when the levels are increased.[25,26] Reproductive organs, especially the endometrium with its monthly recurrence of menstruation, proliferation, decidualization and the possible implantation represents actions that are typically associated with remodeling, programmed cell death and differentiation.[27] In case of a pregnancy, these appropriately working processes are crucial for normal placental development in the early developmental stages. Although the pathophysiology of hypertensive disorders, including PE, is not entirely elucidated, abnormal placentation is considered to be the central problem. It has been suggested that successful suppression of pro-inflammatory cytokines is necessary to allow normal placentation.[21] Noteworthy, placental inflammation might be the result of incomplete placentation with impaired trophoblast invasion in the first half of pregnancy in women who will later develop PE.[21,28] Besides, increased apoptosis of placental cells is seen in pregnancies complicated by PE and fetal growth restriction, resulting in an imbalance between cell growth and cell fall.[21,28]

GDF-15 has been found in placental tissue, namely in syncytiotrophoblast and other trophoblast cells, and also in membranes and amniotic fluid.[21] Previous studies on serum levels of GDF-15 in pregnancy have reported conflicting results. Moore *et al.* and Chen *et al.* reported that in normal pregnancies serum GDF-15 increases with advancing gestation,[16,18] whereas Marjono *et al.* found that serum GDF-15 was stable for the first 24 weeks, but increased thereafter and peaked at

33-35 weeks.[21] Similarly, there are conflicting reports on the relation to serum levels in patients with established PE. Chen *et al.* found decreased levels of GDF-15 in both early- and late-onset PE.[18] However, when tested in the first trimester, controls and those who later developed PE did not display different levels of GDF-15, and the authors speculated that the decreased levels of GDF-15 at the time of clinical PE presentation might be a maternal reaction to the disease [18]. Other research groups reported increased levels of GDF-15 in patients with PE and suggested this is the consequence of endothelial dysfunction. [22,23]

Limitations and strengths

The main limitation of this study is its case-control design with a relatively small number of cases included. PE is a heterogeneous disorder in terms of maternal phenotype, pathophysiology and severity. Consequently, the total number of normal and pathological pregnancies examined may be inadequate to conclude that GDF-15 levels are not altered in different gestational ages and in preterm and term PE. This limitation is particularly marked at 35-37 weeks of gestation, where the small number of cases may preclude meaningful conclusions.

To avoid the introduction of bias, blood samples for this study were prospectively collected, and the assays were performed by an investigator who was blinded to pregnancy outcomes. In addition, we adjusted the GDF-15 results for possible confounders and accounted for the changes in biomarker values throughout gestation. The lack of adjustment for gestational age and maternal characteristics

in previous studies may partly explain the inconsistencies reported in the literature.

Clinical Implications and future research

The results of this study suggest that GDF-15 MoM levels rise within a few weeks (from about three to four weeks) prior to delivery with PE. Consequently, the best window of screening seems to be 30-34 weeks of gestation. Given that very early-onset PE is rare, screening at 20-24 weeks may be too early to demonstrate significant differences in GDF-15 levels. However, the small differences in distribution with large variability in the population, as well as the possible overlap with well evaluated and established markers that show better discrimination in predicting PE, such as PLGF and sFLT-1 (which were not tested in this study), may limit the potential use of this test in clinical practice for PE prediction. Future research should focus on the interaction between GDF-15 levels with those of other biomarkers, and the possible role of GDF-15 levels in the prediction of PE when incorporated in multimarker predictive algorithms.

CONCLUSION

Serum GDF-15 serum levels are not predictive of preterm or term PE at 19-24 weeks or 35-37 weeks. At 30-34 weeks, serum GDF-15 is increased in pregnancies that develop preterm PE. The poor predictive capacity of GDF-15, however, makes it unlikely that this biomarker will be clinically useful in isolation.

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CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest.

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FIGURE LEGENDS

Figure 1. GDF-15 levels at 19-24, 30-34 and 35-37 weeks of gestation in controls (open circles and dashed regression line) and PE cases (closed circles and solid regression line).

Figure 2. Relationship between serum growth-differentiation factor 15 multiples of the median at 19-24 (a), 30-34 (b), and 35-37 (c) weeks of gestational age and the interval between sampling and delivery in pregnancies with PE. Regression lines are shown (P values 0.259 at 19-24 weeks, 0.005 at 30-34 weeks and 0.832 at 35-37 weeks of gestational age).

Figure 3. Receiver-operating characteristics (ROC) curves for the use of GDF-15 MoM values in the prediction of pre-eclampsia at 19-24 (a), 30-34 (b), and 35-37 (c) weeks of gestational age.

TABLE LEGENDS

Table 1: Baseline characteristics of the study population.

Table 2. Comparison of median GDF-15 values according to different outcome groups at 19-24, 30-34 and 35-37 weeks.

TABLES

Table 1: Baseline characteristics of the study population.

Characteristic	Controls (n=160)	PE (n=140)
Maternal age in years, mean ± SD	32.13 ± 5.88	31.82 ± 5.19
Maternal weight in kg, mean ± SD	69.25 ± 14.57	77.74 ± 18.78*
Maternal height in cm, mean ± SD	163.96 ± 6.75	164.52 ± 5.99
Racial origin		
Caucasian, n (%)	78 (26)	60 (20)
Afro-Caribbean, n (%)	59 (19.7)	68 (22.7)
South Asian, n (%)	11 (3.7)	9 (3.0)
East Asian, n (%)	7 (2.3)	1 (0.3)
Mixed, n (%)	5 (1.7)	2 (0.7)
Parity		
Nulliparous, n (%)	70 (23.3)	79 (26.3)
Parous with no previous PE, n (%)	85 (28.3)	40 (13.3)*
Parous with previous PE, n (%)	5 (1.7)	21(7.0)*
Cigarette smoker, n (%)	11 (3.7)	7 (2.3)
Family history of PE, n (%)	10 (3.4)	6 (2.0)
Conception		
Spontaneous, n (%)	153 (51.0)	132 (44.0)
Assisted, n (%)	7 (2.4)	8 (2.6)
Chronic hypertension, n (%)	3 (1.0)	22 (7.3)*
History of SLE/APS	1 (0.3)	1 (0.3)
History of Diabetes (Type 1 or 2)	5 (1.6)	1 (0.3)

PE: pre-eclampsia; SD: standard deviation; SLE: Systemic Lupus Erythematosus; APS: antiphospholipid syndrome; * $P < 0.05$.

Table 2. Comparison of median GDF-15 values according to different outcome groups at 19-24, 30-34 and 35-37 weeks.

	GDF-15 (pg/mL), Median (IQR)	**P value**	**GDF-15 MoM,** Median (IQR)	**P value**
19-24 weeks				
Control (n=60)	15,084.0 (11,375.5 to 17,867.0)	–	1.015 (0.757 to 1.18.3)	–
Preterm PE (n=30)	17,288.0 (12,065.8 to 20,870.5)	0.450	1.105 (0.869 to 1.441)	0.254
Term PE (n=30)	14,672.5 (12,615.0 to 20,070.5)	0.992	0.978 (0.792 to 1.268)	0.997
30-34 weeks				
Control (n=60)	35,110.0 (26,349.8 to 44,680.5)	–	0.976 (0.775 to 1.260)	–
Preterm PE (n=30)	41,129.5 (30,748.5 to 62,849.8)	0.020	1.362 (0.861 to 1.845)	0.006
Term PE (n=30)	37,594.5 (30,267.8 to 55,313.0)	0.285	1.202 (0.887 to 1.602)	0.082
35-37 weeks				
Control (n=40)	49,729.5 (36,085.8 to 66,124.8)	–	0.966 (0.703 to 1.441)	–

Term PE (n=20)	51,456.0 (41,782.8 to 66,913.5)	0.890	1.091 (0.963 to 1.654)	0.416

Comparisons between groups were performed with one-way ANOVA on logarithmically transformed values with post-hoc Dunnett's adjustment for multiple comparisons, using the control group as the reference category.

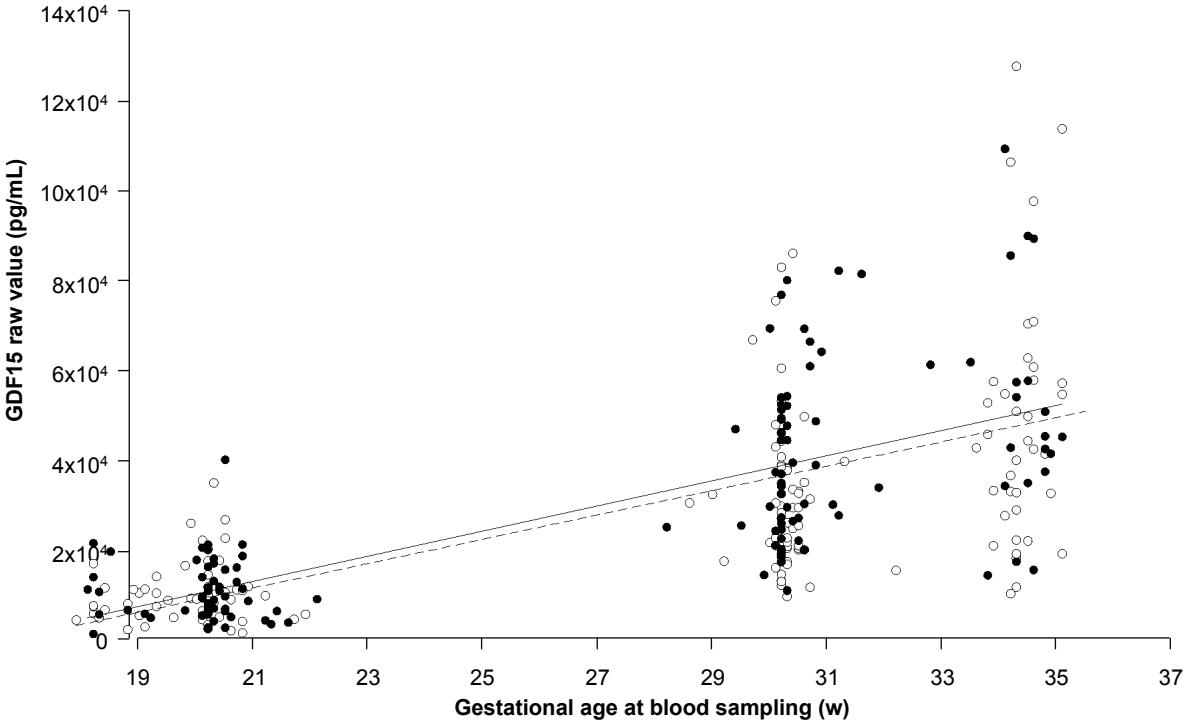

Figure 1

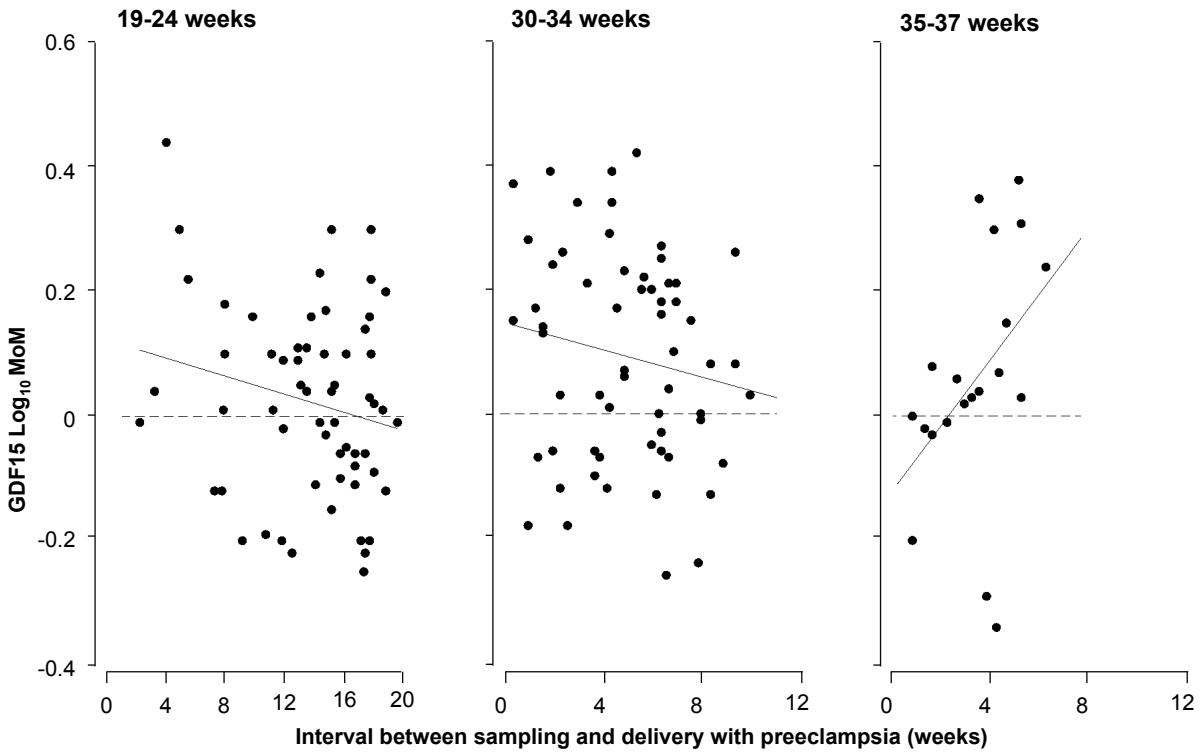

Figure 2

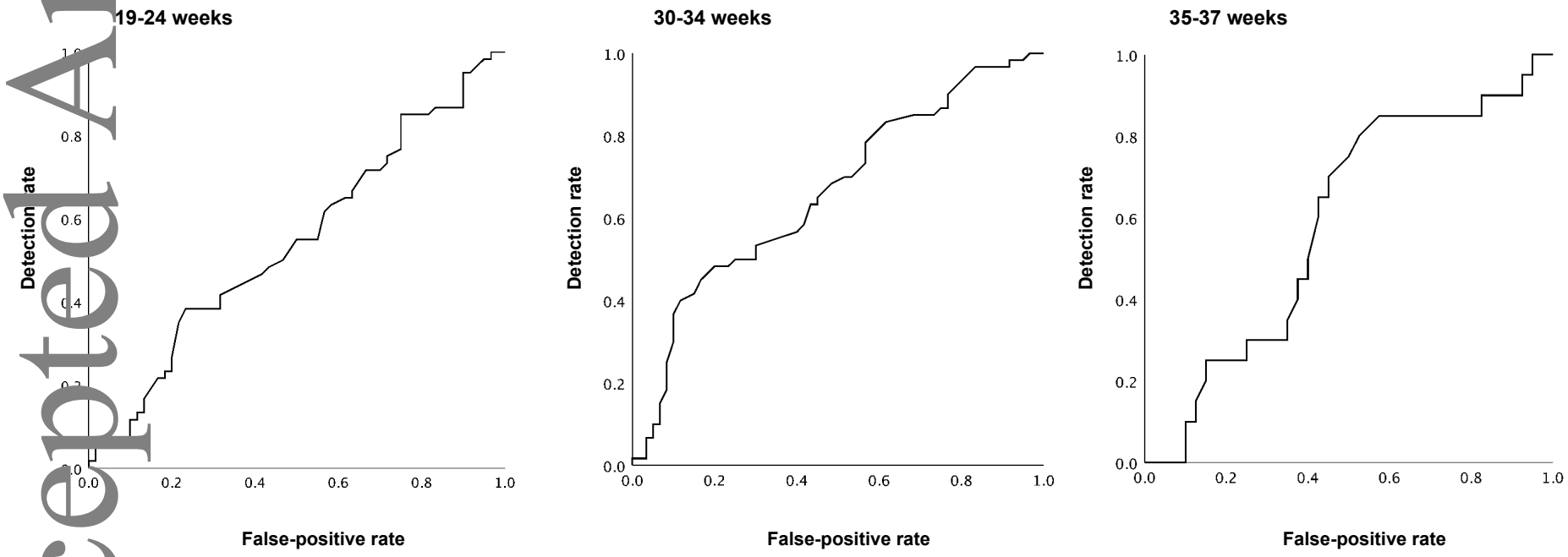

Figure 3