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Screening for pre-eclampsia by maternal serum glycosylated fibronectin at 11–13 weeks' gestation

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KEYWORDS: competing-risks model; first-trimester screening; mean arterial pressure; pre-eclampsia; survival model; uterine artery Doppler

CONTRIBUTION

What are the novel findings of this work?
In a case–control study, we found that maternal serum glycosylated fibronectin (GlyFn) at 11–13 weeks' gestation is a potentially useful biomarker for preterm pre-eclampsia (PE). The detection rate, at 10% false-positive rate, of screening by the triple test, comprising mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF), is similar to that of screening by MAP, UtA-PI and GlyFn or by MAP, PlGF and GlyFn. The performance of screening for term PE or gestational hypertension by any combination of biomarkers is poor.

What are the clinical implications of this work?
GlyFn is a potentially useful biomarker in first-trimester screening for preterm PE, but the findings of this case–control study need to be validated by prospective screening studies.

ABSTRACT

Objective *To examine the performance of screening for preterm and term pre-eclampsia (PE) at 11–13 weeks' gestation by maternal factors and combinations of maternal serum glycosylated fibronectin (GlyFn), mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF).*

Methods *This was a case–control study in which maternal serum GlyFn was measured using a point-of-care device in stored samples from a non-intervention screening study of singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation. In the same samples, PlGF was measured by time-resolved fluorometry. We used samples from women who delivered with PE at < 37 weeks' gestation (n = 100), PE at ≥ 37 weeks (n = 100), gestational hypertension (GH) at < 37 weeks (n = 100), GH at ≥ 37 weeks (n = 100) and 1000 normotensive controls with no pregnancy complications. In all cases, MAP and UtA-PI had been measured during the routine 11–13-week visit. Levels of GlyFn were transformed to multiples of the expected median (MoM) values after adjusting for maternal demographic characteristics and elements of medical history. Similarly, the measured values of MAP, UtA-PI and PlGF were converted to MoMs. The competing-risks model was used to combine the prior distribution of gestational age at delivery with PE, obtained from maternal characteristics, with various combinations of biomarker MoM values to derive the patient-specific risks of delivery with PE or GH at < 37 and ≥ 37 weeks' gestation. Screening performance was estimated by examining the area under the receiver-operating-characteristics curve (AUC) and detection rate (DR) at 10% fixed false-positive rate (FPR).*

Results *The maternal characteristics and elements of medical history with a significant effect on the measurement of GlyFn were maternal age, weight, height, race, smoking status and history of PE. In pregnancies that developed PE, GlyFn MoM was increased and the deviation from normal decreased with increasing gestational age at delivery. The DR and AUC of screening for delivery with PE at < 37 weeks' gestation by maternal factors alone were 50% and 0.834, respectively, and these increased to 80% and 0.949, respectively, when maternal risk factors were combined with MAP, UtA-PI and PlGF (triple test). The performance of the triple test was similar to that of screening by a combination of maternal factors, MAP, UtA-PI and GlyFn (DR, 79%; AUC, 0.946) and that of screening by a combination of maternal*

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factors, MAP, PlGF and GlyFn (DR, 81%; AUC, 0.932). The performance of screening for delivery with PE at ≥ 37 weeks' gestation was poor; the DR for screening by maternal factors alone was 35% and increased to only 39% with use of the triple test. Similar results were obtained when GlyFn replaced PlGF or UtA-PI in the triple test. The DR of screening for GH with delivery at < 37 and ≥ 37 weeks' gestation by maternal factors alone was 34% and 25%, respectively, and increased to 54% and 31%, respectively, with use of the triple test. Similar results were obtained when GlyFn replaced PlGF or UtA-PI in the triple test.

Conclusions *GlyFn is a potentially useful biomarker in first-trimester screening for preterm PE, but the findings of this case–control study need to be validated by prospective screening studies. The performance of screening for term PE or GH at 11 + 0 to 13 + 6 weeks' gestation by any combination of biomarkers is poor.* © *2023 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

The ASPRE trial showed that, in pregnancies identified at 11–13 weeks' gestation by screening with maternal factors and biomarkers as being at high-risk for pre-eclampsia (PE), administration of aspirin (150 mg/day from 11–14 to 36 weeks' gestation) reduces the rate of preterm PE with delivery at < 37 weeks' gestation by about 60%; there was little evidence of a reduction in the incidence of PE with delivery at term[1]. Effective first-trimester screening for PE, which allows estimation of individual patient-specific risk of PE requiring delivery before a specified gestational age, is provided by the competing-risks model, which combines the prior distribution of gestational age at delivery with PE, obtained from maternal characteristics and medical history, with the results of various combinations of biophysical and biochemical measurements[2–7]. Useful biomarkers at 11–13 weeks' gestation are mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF)[7,8]. Screening by the triple test (maternal factors plus MAP, UtA-PI and PlGF) in the first trimester has a detection rate (DR) for preterm and term PE of about 75% and 40%, respectively, at a 10% false-positive rate (FPR)[7].

Another potentially useful biomarker for PE is glycosylated fibronectin (GlyFn)[9–11]. A small study of 11 women that developed PE and 26 normotensive controls reported that serum GlyFn was increased significantly in the PE group and that the increase was apparent from 7–11 weeks' gestation[9]. It was postulated that the increased serum levels of GlyFn may be the consequence of first-trimester inflammation and endothelial dysfunction related to disrupted spiral artery remodeling. Three prospective observational studies reported that serum GlyFn is a potentially useful biomarker in the detection of PE in women presenting with signs and symptoms of the disease at any stage of pregnancy[9–11]. However, a panel of experts at the National Institute of Health and Care Excellence reported that there is insufficient evidence at present to determine the accuracy of GlyFn testing compared with standard care in the UK and that larger studies are needed[12].

The objective of this study was to examine the performance of screening for preterm and term PE and GH at 11–13 weeks' gestation by maternal factors and combinations of maternal serum GlyFn, MAP, UtA-PI and serum PlGF. Specifically, we wanted to address the following points: first, whether GlyFn at 11–13 weeks is a useful biomarker of preterm or term PE or GH; second, whether inclusion of GlyFn improves the prediction of preterm PE beyond that provided by the triple test; and third, whether GlyFn can replace PlGF or UtA-PI in the triple test for the prediction of preterm PE.

METHODS

Study population

This was a case–control study in which maternal serum GlyFn and PlGF were measured in stored samples obtained from non-intervention screening studies of women with a singleton pregnancy attending for a routine visit at 11 + 0 to 13 + 6 weeks' gestation at Kings' College Hospital, London, UK, between January 2011 and September 2022. This visit included recording of maternal characteristics and medical history[4], measurement of left and right UtA-PI on transabdominal color Doppler ultrasound and calculation of mean UtA-PI[13], measurement of MAP using validated automated devices and a standardized protocol[14], and storage of serum at −80°C for subsequent research. Gestational age was determined by measurement of fetal crown–rump length at 11–13 weeks' gestation[15]. All participants gave written informed consent to participate. The study was approved by the NHS Research Ethics Committee (REC reference: 02-03-033).

The participant characteristics recorded included maternal age, self-declared ethnicity (white, black, South Asian, East Asian or mixed), method of conception (natural or assisted by *in-vitro* fertilization (IVF) or ovulation induction), cigarette smoking during pregnancy, medical history of chronic hypertension or diabetes mellitus, family history of PE (woman's mother affected) and obstetric history, including parity (parous or nulliparous if no previous pregnancy at ≥ 24 weeks' gestation) and, for parous women, previous pregnancy with PE and interpregnancy interval.

Included were singleton pregnancies examined at 11 + 0 to 13 + 6 weeks' gestation delivering a non-malformed liveborn or stillborn fetus at ≥ 24 weeks' gestation. Excluded were pregnancies with aneuploidy or major fetal abnormality.

Outcome measures

Outcome measures were delivery with PE or GH at < 37 and ≥ 37 weeks' gestation. PE was defined according to the 2019 American College of Obstetricians and

Gynecologists criteria as chronic hypertension or GH, with development of one or more of the following: new-onset proteinuria, serum creatinine > 97 µmol/L in the absence of underlying renal disease, serum transaminases more than twice the normal level (≥ 65 IU/L for our laboratory), platelet count < 100 000/µL, headache or visual symptoms, or pulmonary edema[16]. Chronic hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, at least twice, 4 h apart, documented before pregnancy or at < 20 weeks' gestation[17]. GH was defined as new-onset hypertension at ≥ 20 weeks' gestation in a previously normotensive woman[16].

Data on pregnancy outcome were collected from participants' hospital maternity records or those of their general medical practitioners. The maternity records of all women with chronic hypertension or GH were examined to determine the diagnosis of PE and GH.

Case–control study

All data from the assessment at 11–13 weeks' gestation and details on pregnancy outcome were recorded in a Fetal Medicine database (Viewpoint 5.6; GE Healthcare, Munich, Germany). We searched the database to identify singleton pregnancies examined at 11 + 0 to 13 + 6 weeks' gestation with available measurements of MAP and UtA-PI and stored serum samples. We randomly selected 100 cases that delivered with each of the following outcomes: PE at < 37 weeks, PE at ≥ 37 weeks, GH at < 37 weeks and GH at ≥ 37 weeks. Each case of PE or GH was matched to two or three controls that were sampled on the same or subsequent day as the case. In total, we included 1000 controls without PE, GH, gestational diabetes or cholestasis, who delivered at ≥ 37 weeks' gestation a neonate with birth weight above the 10th percentile and below the 90th percentile[18].

The frozen serum samples were thawed and then analyzed for GlyFn using a point-of-care test (Lumella™; DiabetOmics, Inc., Hillsboro, OR, USA). Briefly, 5 µL of serum was diluted 1:350 in running buffer and 120 µL of diluted serum was added to a test strip and inserted into a hand-held reader system. Test strips were configured with monoclonal antibodies against GlyFn labeled with gold particles. The GlyFn concentration was displayed on the reader after 10 min. According to the manufacturer, the measurable range of the Lumella™ assay is 50–800 µg/mL and the intra- and interassay coefficients of variation at mean concentrations of 50–800 µg/mL are 5–10% and 6–10%, respectively.

The concentration of PlGF was measured by time-resolved fluorometry (DELFIA Xpress system; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA).

Statistical analysis

Levels of GlyFn were transformed to multiples of the expected median (MoM) value after adjusting for maternal demographic characteristics and elements of medical history. Similarly, the measured values of MAP, UtA-PI and PlGF were converted to MoMs as reported previously[7]. The competing-risks model was used to combine the prior distribution of gestational age at delivery with PE, obtained from maternal characteristics, with various combinations of biomarker MoM values to derive the patient-specific risks of delivery with PE or GH at < 37 and ≥ 37 weeks' gestation[6]. The performance of screening was estimated by examining the area under the receiver-operating-characteristics curve (AUC) and DR at 10% FPR.

McNemar's test and bootstrap sampling were used to compare the predictive performance for preterm PE of the following screening strategies: first, maternal factors plus GlyFn *vs* maternal factors alone; second, maternal factors plus MAP, UtA-PI and PlGF *vs* maternal factors plus MAP, UtA-PI and GlyFn; third, maternal factors plus MAP, UtA-PI and PlGF *vs* maternal factors plus MAP, PlGF and GlyFn; and fourth, maternal factors plus MAP, UtA-PI and PlGF *vs* maternal factors plus MAP, UtA-PI, PlGF and GlyFn. The statistical software package R was used for data analysis[19]. *P*-values of < 0.05 were considered statistically significant.

RESULTS

Study participants

Maternal and pregnancy characteristics of the study population are summarized in Table 1. Compared with the control group, women who developed PE were heavier, with higher median maternal weight and body mass index (BMI), and were more likely to be black, have a history of chronic hypertension, diabetes mellitus and/or a family history of PE, have conceived by IVF, and be nulliparous or, if parous, have a history of PE. Compared with controls, women in the GH group were heavier, with higher median weight and BMI, and were more likely to have a history of diabetes mellitus and/or a family history of PE and be nulliparous or, if parous, have a history of PE. In both the PE and GH groups, compared with the control group, the median MAP MoM, UtA-PI MoM and GlyFn MoM were higher and median PlGF MoM was lower, and the differences were most marked in those delivering at < 37 weeks' gestation.

Distribution of glycosylated fibronectin

Maternal characteristics and elements of medical history with a significant effect on the measurement of serum GlyFn are shown in Table 2. These variables were used for standardization into MoM values. Serum GlyFn increased with weight and age and decreased with height. Compared with levels in white women, the level was 20.0% higher in black women, 19.7% higher in East Asian women and 9.3% higher in South Asian women. The level of serum GlyFn was 13.1% lower in smokers than that in non-smokers, and 7.0% lower in parous women

Table 1 Maternal and pregnancy characteristics of study population, according to development of pre-eclampsia (PE) or gestational hypertension (GH)

Characteristic	*Controls (n = 1000)*	*PE (n = 200)*	P*	*GH (n = 200)*	P*
Age (years)	33.3 (29.4–36.4)	32.8 (29.7–36.4)	0.537	33.4 (29.6–37.7)	0.547
Weight (kg)	67.0 (60.0–77.5)	74.1 (64.6–86.8)	< 0.0001	71.6 (63.0–83.8)	< 0.0001
Height (cm)	165 (161–170)	165 (160–170)	0.414	165 (159–170)	0.324
Body mass index (kg/m^2)	24.5 (21.9–28.4)	27.3 (23.7–32.8)	< 0.0001	26.4 (23.3–30.9)	0.0001
Gestational age (weeks)	12.7 (12.4–13.1)	12.7 (12.4–13.1)	0.632	12.7 (12.1–13.1)	0.632
Ethnicity			< 0.0001		0.627
White	738 (73.8)	118 (59.0)		147 (73.5)	
Black	153 (15.3)	66 (33.0)		30 (15.0)	
South Asian	53 (5.3)	9 (4.5)		15 (7.5)	
East Asian	21 (2.1)	5 (2.5)		4 (2.0)	
Mixed	35 (3.5)	2 (1.0)		4 (2.0)	
Medical history					
Chronic hypertension	3 (0.3)	25 (12.5)	< 0.0001	0 (0)	1
Diabetes mellitus	9 (0.9)	7 (3.5)	0.013	7 (3.5)	0.002
Smoker	56 (5.6)	4 (2.0)	0.051	6 (3.0)	0.180
Family history of PE	37 (3.7)	15 (7.5)	0.0003	15 (7.5)	0.004
Method of conception			0.018		0.560
Natural	944 (94.4)	179 (89.5)		185 (92.5)	
In-vitro fertilization	49 (4.9)	20 (10.0)		13 (6.5)	
Ovulation drugs	7 (0.7)	1 (0.5)		2 (1.0)	
Parity			< 0.0001		< 0.0001
Nulliparous	399 (39.9)	129 (64.5)		123 (61.5)	
Parous, no previous PE	572 (57.2)	48 (24.0)		55 (27.5)	
Parous, previous PE	29 (2.9)	23 (11.5)		22 (11.0)	
Interpregnancy interval (years)	2.8 (1.7–4.5)	3.3 (2.0–5.4)	0.117	3.0 (1.9–5.6)	0.383
MAP MoM	1.00 (0.95–1.05)	1.06 (1.02–1.10)	< 0.0001	1.06 (1.02–1.11)	< 0.0001
Delivery < 37 weeks		1.06 (1.03–1.12)	< 0.0001	1.06 (1.03–1.13)	< 0.0001
Delivery ≥ 37 weeks		1.05 (1.00–1.10)	< 0.0001	1.06 (1.01–1.09)	< 0.0001
UtA-PI MoM	1.00 (0.81–1.26)	1.17 (0.92–1.52)	< 0.0001	1.09 (0.83–1.34)	0.039
Delivery < 37 weeks		1.37 (1.16–1.62)	< 0.0001	1.14 (0.87–1.43)	0.018
Delivery ≥ 37 weeks		0.96 (0.76–1.28)	0.45	1.06 (0.81–1.31)	0.574
PlGF MoM	1.01 (0.79–1.26)	0.74 (0.54–1.02)	< 0.0001	0.81 (0.64–1.02)	< 0.0001
Delivery < 37 weeks		0.63 (0.46–0.87)	< 0.0001	0.74 (0.55–0.95)	< 0.0001
Delivery ≥ 37 weeks		0.84 (0.67–1.13)	0.0006	0.88 (0.72–1.04)	0.0002
GlyFn MoM	1.02 (0.84–1.22)	1.13 (0.94–1.38)	< 0.0001	1.06 (0.85–1.32)	0.028
Delivery < 37 weeks		1.18 (0.99–1.44)	< 0.0001	1.07 (0.86–1.35)	0.032
Delivery ≥ 37 weeks		1.09 (0.93–1.28)	0.008	1.05 (0.83–1.27)	0.321

Values are given as median (interquartile range) or *n* (%). **vs* controls. GlyFn, glycosylated fibronectin; MAP, mean arterial pressure; MoM, multiples of the median; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

with no previous history of PE than that in nulliparous women.

The model that defines GlyFn MoM in pregnancies that deliver with PE is summarized in Table S1. MoM values of GlyFn in the PE group and the fitted regression relationship with gestational age at delivery are shown in Figure 1. There was more separation between the PE and control groups at earlier compared with later gestational ages, and this is reflected in the superior performance of screening for preterm compared with term PE. The correlations of $\log_{10}$ MoM values of GlyFn with those of MAP, UtA-PI and PlGF are shown in Table S2.

Performance of screening for pre-eclampsia and gestational hypertension

The DR, at a fixed FPR of 10%, and AUC of screening for PE with delivery at < 37 and ≥ 37 weeks' gestation by maternal factors and combinations of biomarkers are shown in Table 3 and the respective values for GH in Table 4.

Table 2 Maternal characteristics and elements of medical history with significant effect on measurement of serum glycosylated fibronectin at 11–13 weeks' gestation

Variable	*Estimate (95% CI)*	P
Intercept	2.4847 (2.4729 to 2.4966)	< 0.0001
Maternal weight (in kg – 69)	0.0014 (0.0009 to 0.0018)	< 0.0001
Maternal height (in cm – 164)	−0.0025 (−0.0036 to −0.0014)	0.0001
Maternal age (in years – 35)	0.0033 (0.0020 to 0.0046)	< 0.0001
Black ethnicity	0.0809 (0.0616 to 0.1002)	< 0.0001
East Asian ethnicity	0.0781 (0.0295 to 0.1266)	0.002
South Asian ethnicity	0.0386 (0.0076 to 0.0697)	0.015
Smoker	−0.0609 (−0.0943 to −0.0275)	0.0004
Parous, no previous PE	−0.0316 (−0.0460 to −0.0171)	0.00002

PE, pre-eclampsia.

The DR and AUC of screening for delivery with PE at < 37 weeks' gestation by maternal factors alone were 50% and 0.834, respectively, and these increased to 80% and 0.949, respectively, when maternal risk factors were combined with MAP, UtA-PI and PlGF (triple test). The performance of the triple test was similar to that of screening by a combination of maternal factors, MAP, UtA-PI and GlyFn (DR, 79%; AUC, 0.946) and that of screening by a combination of maternal factors, MAP, PlGF and GlyFn (DR, 81%; AUC, 0.932). The performance of screening for delivery with PE at ≥ 37 weeks' gestation was poor: the DR, at 10% FPR, of screening by maternal factors alone was 35% and increased to only 39% with use of the triple test. The DR of screening by a combination of maternal factors, MAP, UtA-PI and GlyFn was also 39% and that of screening by a combination of maternal factors, MAP, PlGF and GlyFn was 45%.

The DR of screening for GH with delivery at < 37 and ≥ 37 weeks' gestation by maternal factors alone was 34% and 25%, respectively. These values were not increased appreciably by the addition of GlyFn, but rose to 54% and 31%, respectively, with use of the triple test. However, there was overlap in the 95% CI of the predictive performance for screening by the triple test *vs* screening by maternal factors alone (Table 4). Similar results were obtained when GlyFn replaced PlGF or UtA-PI in the triple test.

McNemar's test demonstrated that screening by maternal factors plus GlyFn had superior predictive performance for preterm PE compared with screening by maternal factors alone (Table 5). There was no significant difference in predictive performance for preterm PE between the triple test and screening methods in which GlyFn replaces PlGF or UtA-PI in the triple test or all four biomarkers are combined.

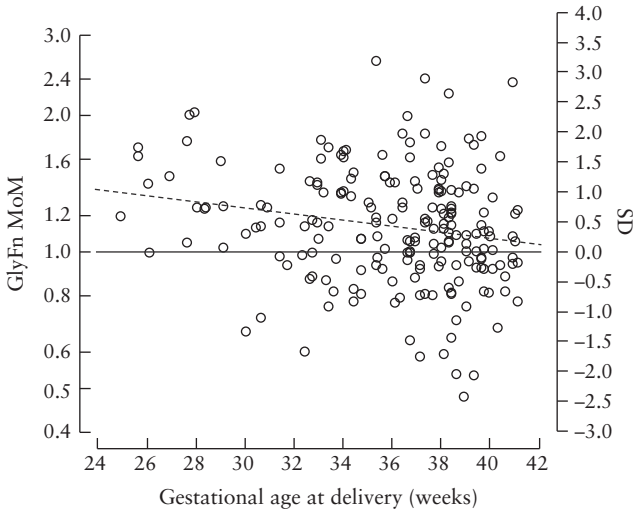

Figure 1 Scatter diagram and regression line (dashed line) for relationship between maternal serum glycosylated fibronectin (GlyFn) multiples of the median (MoM) and gestational age at delivery in pregnancies with pre-eclampsia.

DISCUSSION

Principal findings

There are four main findings of this nested case–control study in women with a singleton pregnancy undergoing routine assessment at 11–13 weeks' gestation. First, serum GlyFn concentration is affected by a number of maternal characteristics, including age, weight, ethnicity and smoking, and therefore, when comparing normal and pathological pregnancies, measured values should be converted to MoMs after adjustment for these characteristics. Second, in pregnancies that develop PE, GlyFn MoM is significantly increased and the deviation from normal decreases with gestational age at delivery; consequently, the performance of screening with GlyFn is superior for preterm compared with term PE.

Table 3 Detection rate (DR), at fixed false-positive rate of 10%, and area under receiver-operating-characteristics curve (AUC) of screening for pre-eclampsia (PE) with delivery at < 37 and ≥ 37 weeks' gestation by maternal factors (MF) and combinations of biomarkers

	PE with delivery < 37 weeks		*PE with delivery ≥ 37 weeks*	
Method of screening	*DR (95% CI) (%)*	*AUC (95% CI)*	*DR (95% CI) (%)*	*AUC (95% CI)*
MF	50 (39.8–60.2)	0.834 (0.796–0.872)	35 (25.7–45.2)	0.724 (0.673–0.775)
MF + GlyFn	58 (47.7–67.8)	0.863 (0.829–0.897)	33 (23.9–43.1)	0.739 (0.688–0.789)
MF + MAP	61 (50.7–70.6)	0.887 (0.859–0.915)	39 (29.4–49.3)	0.768 (0.722–0.814)
MF + MAP + GlyFn	65 (54.8–74.3)	0.902 (0.876–0.927)	42 (32.2–52.3)	0.775 (0.729–0.821)
MF + UtA-PI	63 (52.8–72.4)	0.901 (0.873–0.928)	30 (21.2–40.0)	0.705 (0.653–0.758)
MF + UtA-PI + GlyFn	72 (62.1–80.5)	0.920 (0.896–0.945)	34 (24.8–44.2)	0.720 (0.668–0.771)
MF + PlGF	67 (56.9–76.1)	0.896 (0.865–0.928)	36 (26.6–46.2)	0.743 (0.693–0.794)
MF + PlGF + GlyFn	74 (64.3–82.3)	0.909 (0.878–0.940)	46 (36.0–56.3)	0.759 (0.709–0.808)
MF + MAP + UtA-PI	77 (67.5–84.8)	0.936 (0.916–0.955)	36 (26.6–46.2)	0.756 (0.708–0.803)
MF + MAP + UtA-PI + GlyFn	79 (69.7–86.5)	0.946 (0.930–0.963)	39 (29.4–49.3)	0.762 (0.715–0.810)
MF + MAP + PlGF	73 (63.2–81.4)	0.926 (0.902–0.950)	44 (34.1–54.3)	0.791 (0.748–0.834)
MF + MAP + PlGF + GlyFn	81 (71.9–88.2)	0.932 (0.909–0.956)	45 (35.0–55.3)	0.800 (0.757–0.843)
MF + MAP + UtA-PI + PlGF	80 (70.8–87.3)	0.949 (0.931–0.967)	39 (29.4–49.3)	0.781 (0.737–0.825)
MF + MAP + UtA-PI + PlGF + GlyFn	83 (74.2–89.8)	0.954 (0.937–0.971)	42 (32.2–52.3)	0.789 (0.746–0.833)

GlyFn, glycosylated fibronectin; MAP, mean arterial pressure; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

Table 4 Detection rate (DR), at fixed false-positive rate of 10%, and area under receiver-operating-characteristics curve (AUC) of screening for gestational hypertension (GH) with delivery at < 37 and ≥ 37 weeks' gestation by maternal factors (MF) and combinations of biomarkers

	GH with delivery < 37 weeks		*GH with delivery ≥ 37 weeks*	
Method of screening	*DR (95% CI) (%)*	*AUC (95% CI)*	*DR (95% CI) (%)*	*AUC (95% CI)*
MF	34 (24.8–44.2)	0.760 (0.715–0.805)	25 (16.9–34.7)	0.663 (0.608–0.718)
MF + GlyFn	34 (24.8–44.2)	0.751 (0.705–0.797)	29 (20.4–38.9)	0.663 (0.607–0.718)
MF + MAP	44 (34.1–54.3)	0.818 (0.779–0.856)	28 (19.5–37.9)	0.719 (0.667–0.770)
MF + MAP + GlyFn	40 (30.3–50.3)	0.809 (0.769–0.849)	36 (26.6–46.2)	0.716 (0.664–0.768)
MF + UtA-PI	34 (24.8–44.2)	0.753 (0.704–0.803)	23 (15.2–32.5)	0.664 (0.609–0.719)
MF + UtA-PI + GlyFn	36 (26.6–46.2)	0.750 (0.700–0.801)	24 (16.0–33.6)	0.663 (0.607–0.718)
MF + PlGF	45 (35.0–55.3)	0.816 (0.775–0.858)	22 (14.3–31.4)	0.691 (0.639–0.744)
MF + PlGF + GlyFn	47 (36.9–57.2)	0.815 (0.772–0.858)	29 (20.4–38.9)	0.690 (0.637–0.743)
MF + MAP + UtA-PI	45 (35.0–55.3)	0.809 (0.766–0.852)	29 (20.4–38.9)	0.719 (0.667–0.771)
MF + MAP + UtA-PI + GlyFn	42 (32.2–52.3)	0.804 (0.761–0.847)	32 (23.0–42.1)	0.716 (0.664–0.769)
MF + MAP + PlGF	54 (43.7–64.0)	0.855 (0.819–0.891)	34 (24.8–44.2)	0.742 (0.693–0.792)
MF + MAP + PlGF + GlyFn	60 (49.7–69.7)	0.851 (0.814–0.888)	31 (22.1–41.0)	0.740 (0.691–0.789)
MF + MAP + UtA-PI + PlGF	54 (43.7–64.0)	0.843 (0.803–0.884)	31 (22.1–41.0)	0.741 (0.692–0.791)
MF + MAP + UtA-PI + PlGF + GlyFn	54 (43.7–64.0)	0.840 (0.799–0.882)	34 (24.8–44.2)	0.739 (0.689–0.789)

GlyFn, glycosylated fibronectin; MAP, mean arterial pressure; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

Table 5 Results of McNemar's test for comparison of performance of different screening methods for prediction of preterm pre-eclampsia, at 10% false-positive rate

Comparison of screening methods	*Difference in DR (95% CI) (%)*	P
MF + GlyFn *vs* MF alone	8.0 (0.0 to 13.0)	0.024
MF + MAP + UtA-PI + GlyFn *vs* triple test	−1.0 (−10.0 to 6.0)	1.000
MF + MAP + PlGF + GlyFn *vs* triple test	1.0 (−8.0 to 3.0)	1.000
MF + MAP + UtA-PI + PlGF + GlyFn *vs* triple test	3.0 (−2.0 to 9.0)	0.146

Triple test refers to maternal factors (MF) plus mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and placental growth factor (PlGF). DR, detection rate; GlyFn, glycosylated fibronectin.

Third, effective screening for preterm PE is provided by the triple test, which combines maternal factors with MoM values of MAP, UtA-PI and PlGF; similar performance was achieved if either PlGF or UtA-PI was replaced by GlyFn in the triple test. Fourth, the performance of first-trimester screening for term PE and both preterm and term GH by any combination of biomarkers is poor.

Comparison with findings of previous studies

The finding of this study that the triple test is among the best-performing screening strategies for preterm PE is consistent with the results of previous studies[5,7,20–25]. Likewise, it was shown previously that the predictive performance of the triple test for term PE was poor. The median DR, at 10% FPR, for preterm PE of the triple test in this study was 80%, which is higher than the 75% DR observed previously[7]. The most likely explanation is that, in the present case–control study, the control group was 'super normal'.

Our study has demonstrated that GlyFn is a good first-trimester biomarker for preterm PE, which supports the findings of Rasanen *et al.*[9] obtained in a much smaller number of subjects. Our study is novel in that it formalizes the inclusion of GlyFn in an effective first-trimester screening algorithm for preterm PE.

Implications for clinical practice

This study provides details on the performance of first-trimester screening for PE by various combinations of biomarkers, including GlyFn. Screening for preterm PE is important because treatment of high-risk women substantially reduces the incidence of the disease. In contrast, aspirin does not reduce the incidence of term PE or GH[1].

There are various considerations and implications, in terms of general applicability and cost, to the components of a first-trimester screening test for preterm PE. The choice of which biomarkers should be used in a particular setting will ultimately depend not only on performance, but also on the feasibility of implementation and health economic considerations. Recording of maternal characteristics and medical history, measurement of blood pressure and hospital attendance at 11–13 weeks' gestation for an ultrasound scan are an integral part of routine antenatal care in many countries. Measurement of UtA-PI can be carried out by the same sonographers using the same ultrasound machines as in the routine 11–13-week scan; however, sonographers require training to carry out this test, and the measurement would add 2–3 min to the current 20–30 min duration of the scan. We have demonstrated that, in centers in which measurement of UtA-PI is difficult or not possible to implement, a triple test composed of MAP, PlGF and GlyFn may be an alternative screening strategy with high performance. Similarly, if measurement of PlGF is not possible to implement in a center, then GlyFn may be a welcome alternative, because the test can be carried out in any clinical setting and does not require use of a laboratory. However, some sort of quality assurance

on measurements taken from these devices would be necessary.

Screening for term PE is best performed at 35 + 0 to 36 + 6 weeks' gestation by a combination of maternal factors, MAP, PlGF and serum soluble fms-like tyrosine kinase-1, which can identify approximately 70% of subsequent disease[26,27]. The rationale for such late third-trimester screening is identification of a high-risk group that would benefit from close monitoring to minimize adverse perinatal events for those that develop PE, by determining the appropriate time and place for delivery. A randomized trial is currently evaluating timed birth based on personalized risk of PE (reference: ISRCTN41632964), given the potential of this strategy to decrease the rate of term PE by about 60%[28].

Strengths and limitations

The strengths of this first-trimester study are: first, examination of a large number of cases that subsequently developed PE or GH; second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE and define prior risk; third, use of a specific methodology and appropriately trained doctors to measure UtA-PI and MAP; fourth, use of an automated machine to provide accurate measurement of serum PlGF; fifth, expression of biomarker values as MoMs after adjustment for factors that affect the measurements; and sixth, use of Bayes' theorem to combine the prior risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for both preterm and term PE and GH.

The main limitation of the study is its case–control design, based on stored serum samples rather than prospective screening. This was necessary to allow adequate assessment of the value of GlyFn as a potential biomarker of PE and GH; in a prospective screening study, it would have been necessary to recruit more than 15 000 women in order to include 100 who would have delivered with preterm PE. Now that the potential value of GlyFn has been established, our findings require validation in prospective screening studies.

Conclusion

GlyFn is a potentially useful biomarker in first-trimester screening for preterm PE, but the results of this case–control study need to be validated by prospective screening studies.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Model to define multiples of the median value of glycosylated fibronectin in pregnancies that deliver with pre-eclampsia

Table S2 Correlation between $\log_{10}$-transformed multiples of the median values of glycosylated fibronectin and those of other biomarkers of pre-eclampsia

