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Midpregnancy prediction of pre-eclampsia using serum biomarkers sFlt-1 and PlGF

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A B S T R A C T

Objectives: Pre-eclampsia remains a significant cause of morbidity and mortality. Placental biomarkers soluble Fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) have been investigated previously for their ability to predict pre-eclampsia. We compared the performance of these biomarkers for midpregnancy pre-eclampsia prediction using three different immunoassay platforms.

Study design: Prospective study including singleton pregnancies 19–22 weeks' gestation. Maternal bloods were collected at recruitment. Screening performances using receiver operating characteristic (ROC) curves for PlGF and sFlt-1/PlGF ratio raw data and MoM values in isolation were evaluated for three immunoassay platforms using selected cut-off values.

Main outcome measures: Pre-eclampsia was defined as early-onset (< 34 weeks' at delivery) and preterm (< 37 weeks' at delivery).

Results: For prediction of preterm pre-eclampsia, PlGF MoM and sFlt-1/PlGF ratio MoM performed similarly, with areas under the curve (AUC), detection rates (DR) and false positive rates (FPR) for PlGF MoM and sFlt-1/PlGF ratio MoM being 0.77–0.79 and 0.71–0.74, 62.5% for both and 9.7–14.9 and 10.7–17.7, respectively. For the prediction of early-onset pre-eclampsia, sFlt-1/PlGF ratio raw data and MoM values performed similarly, with AUC, DR and FPR being 0.92–0.97 and 0.93–0.96, 100% for both, and 4.13–16.9 and 9.4–12.2, respectively.

Conclusions: For midpregnancy prediction of preterm pre-eclampsia, PlGF MoM for all three platforms and sFlt-1/PlGF ratio MoM for the two platforms that tested sFlt-1 performed similarly. For midpregnancy prediction of early-onset pre-eclampsia at midpregnancy, sFlt-1/PlGF ratio raw data and MoM values using the early-onset cut-off for the two platforms that tested sFlt-1 gave similar performance from a clinical perspective.

1. Introduction

Pre-eclampsia is a multisystem disorder which affects 3% of pregnancies in Australia [1] and is responsible for a substantial degree of maternal and perinatal morbidity and mortality [2]. Although a complete understanding of the pathophysiological basis for this condition remains elusive, dysregulation of circulating angiogenic biomarkers arising from deficient placentation [3] is thought to play a central role. In 2004, it was demonstrated that increased levels of angiogenic biomarkers soluble fms-like tyrosine kinase 1 (sFlt-1) and decreased levels of placental growth factor (PlGF) can predict the subsequent onset of pre-eclampsia [4].

As a result of increased sFlt-1 levels, reduced PlGF and vascular endothelial growth factor (VEGF) levels may halt placental vascular growth much earlier in pregnancies affected by pre-eclampsia than in normal pregnancy. This proposed mechanism for the development of pre-eclampsia has been supported by previous research [4–16]. PlGF concentrations have been demonstrated to be decreased in women with pre-eclampsia as early as 13 weeks' gestation, with significantly decreased levels of PlGF and increased levels of sFlt-1 recorded from 9 to

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11 weeks before the onset of symptoms, with deviations in biomarker values becoming more pronounced 5 weeks prior to symptom onset [4].

Current evidence indicates that screening for pre-eclampsia during the second trimester may be less useful than screening during the first trimester, as the window of opportunity for women considered high risk for pre-eclampsia to benefit from aspirin administration will have diminished [17,18]. However, given that around one-third of Australian women do not begin their antenatal care until after 14 weeks' gestation [19], screening beyond this gestation would prove useful, to avoid unnecessary interventions in low risk women and ensure that those at high risk are triaged to appropriate models of care.

PlGF has been clinically validated in the PELICAN study [20] to confirm the diagnosis of pre-eclampsia in symptomatic patients and to stratify their risk of requiring delivery within a fortnight. The PROGNOSIS study [21] successfully derived and validated an sFlt-1/PlGF ratio cut-off to predict the presence or absence of pre-eclampsia in the short term in women with features of pre-eclampsia, concluding that an sFlt-1/PlGF ratio of 38 or lower can be used to reliably exclude pre-eclampsia within the next 7 days in women in whom the syndrome is clinically suspected. The COMPARE study [22], published in 2018, compared the performance of three PlGF-based kits (DELFIA Xpress PlGF 1-2-3™ test, Triage PlGF test and Elecsys immunoassay sFlt-1/PlGF ratio) in prediction of delivery within a fortnight of testing in symptomatic patients prior to 35 weeks' gestation. The study demonstrated similar test results between platforms and established PlGF < 150 pg/mL as a cut-off to rule out suspected pre-eclampsia for the DELFIA Xpress PlGF 1-2-3™ test.

Despite these compelling findings, it has taken a long time for PlGF and sFlt-1/PlGF ratio testing to be implemented into clinical practice [23–25]. The aim of this study was to determine how well serum PlGF and/or the sFlt-1/PlGF ratio, or both, when tested at midpregnancy using three different immunoassay platforms, perform for the prediction of subsequent pre-eclampsia.

2. Methods

2.1. Study population

This was a prospective observational study in singleton pregnancies. Women booking for antenatal care between 19 and 22 weeks' gestation at The Royal Women's Hospital in Melbourne, Australia, between June 2012 and January 2015 were eligible. Study participants represented the general pregnant population, but were required to be able to give written consent in English. Women with multiple pregnancies, major fetal anomalies, fetal aneuploidy, fetal death or pregnancy loss prior to 24 weeks gestation were excluded, as were women with substantial missing outcome data.

Study data were collected and managed using REDCap electronic data capture tools hosted at The University of Melbourne [26]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Baseline characteristics and confirmation of eligibility were obtained through participant interview. Pregnancy outcomes were determined and the development of pre-eclampsia was confirmed or excluded by review of individual medical records by two independent adjudicators.

Pre-eclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) research definition, as *de novo* hypertension (systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg) after 20 weeks gestation, plus proteinuria (> 300 mg/day of urinary protein) [27]. While the ISSHP guidelines have since been revised [25,28], the earlier guidelines, published in 2001, were most applicable at the time this study was designed and conducted. Pre-eclampsia was classified according to gestation at delivery as 1) early-onset pre-eclampsia (< 34 weeks); 2) preterm pre-eclampsia (< 37 weeks) or 3) term pre-eclampsia (≥ 37 weeks). Pregnancies that went on to develop pre-eclampsia were compared with pregnancies within the cohort that did not go on to develop pre-eclampsia. This "unaffected group" included patients that developed gestational hypertension, defined as new onset hypertension in the absence of proteinuria or other features of pre-eclampsia.

To assess the performance of these tests in predicting outcomes other than pre-eclampsia, secondary outcomes including clinically suspected placental abruption, stillbirths greater than 24 weeks gestation and fetal growth restriction (FGR), defined as having one of doppler cerebroplacental ratio (CPR) less than the 5th centile, umbilical artery pulsatility index greater than the 95th centile or EFW less than the 3rd centile [29], were also analysed.

The study was approved by the Royal Women's Hospital Research and Ethics Committee (project approval number 11/23). All participants gave written informed consent.

2.2. Blood sample collection and storage

A single blood sample was collected from each participant at recruitment. A volume of 10 mL of maternal blood was drawn and divided between non-heparinised, silicone coated tubes for serum samples and ethylenediaminetetraacetic acid (EDTA) tubes for plasma samples. Samples were centrifuged and transferred into plain polypropylene tubes. Samples were stored as serum or plasma at −80 °C until the time of analysis. Sample analysis was undertaken by one technician blinded to clinical outcomes.

2.3. Immunoassay platforms

Single measurements for each patient were performed on each of three immunoassay platforms between April 2015 and February 2017 in the following order:

1) **DELFIA® Xpress** (Perkin Elmer, Inc. Wallac Oy, Turku, Finland): PlGF only,
2) **cobas® e 411** (Roche Diagnostics, GmbH): PlGF and sFlt-1, and
3) **B.R.A.H.M.S KRYPTOR compact PLUS** (ThermoScientific, GmbH): PlGF and sFlt-1.

Sample analysis was performed according to the manufacturer's instructions for each platform.

2.4. Statistical analysis

Patient baseline characteristics were compared using the Mann-Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Data were assessed for normality and found to be non-parametric. After logarithmic transformation, the results for PlGF, sFlt-1 and the sFlt-1/PlGF ratio from each immunoassay platform were adjusted for maternal characteristics that were found to significantly influence the logarithmic results on backward stepwise linear regression analysis and expressed as multiples of the expected median (MoM). Median raw data and MoM values for PlGF and the sFlt-1/PlGF ratio were compared between patients with pre-eclampsia and unaffected pregnancies using the Mann-Whitney *U* test. P-values < 0.05 were deemed to be statistically significant. Screening performances for PlGF and sFlt-1/PlGF ratio raw data and MoM values were then evaluated using receiver operating characteristic (ROC) curves to calculate the

area under the curve (AUC). Curve coordinates were used to select cut-off values for PlGF and the sFlt-1/PlGF ratio that gave greatest sensitivity and specificity for the prediction of term, preterm and early-onset pre-eclampsia for each immunoassay platform. Two cut-off points were determined for each platform; one for prediction of patients with preterm pre-eclampsia, referred to as the *preterm PE cut-off*; the second for prediction of early-onset pre-eclampsia, referred to as the *early PE cut-off*. Data analysis was performed using Microsoft® Excel™ 2016 (Redmond, Washington, USA) and IBM Statistical Package for the Social Sciences (SPSS) Version 24 (Armonk, New York, USA).

3. Results

There were 600 patients recruited, of whom 88 were excluded from analysis. Seven exclusions occurred due to aneuploidy or major fetal anomaly, and 81 exclusions were due to incomplete data, leaving 512 patients for inclusion in the analysis. There were 25 patients (4.9%) with pre-eclampsia, including 17 patients (3.3%) with term pre-eclampsia (≥37 weeks), 8 patients (1.6%) with preterm pre-eclampsia (< 37 weeks) and 3 patients (0.6%) with early-onset pre-eclampsia (< 34 weeks).

3.1. Descriptive statistics

The characteristics of the study population and descriptive statistics are summarised in Table 1.

Patients who developed preterm or early-onset pre-eclampsia had significantly lower PlGF levels, both raw data and MoM, at midpregnancy across all three platforms ($p < 0.05$) compared with unaffected pregnancies or those that developed term pre-eclampsia (Table 2). There was no significant difference in sFlt-1 raw data or MoM values between patients who developed early-onset, preterm or term pre-eclampsia compared with unaffected pregnancies.

Patients who developed early-onset pre-eclampsia had significantly higher sFlt-1/PlGF values, both raw data and MoM at midpregnancy across all three platforms ($p < 0.05$) (Table 2), while patients who developed preterm pre-eclampsia had significantly higher sFlt-1/PlGF ratio MoM values, with no significant difference in sFlt-1/PlGF raw data values between patients who developed preterm pre-eclampsia and unaffected pregnancies (Table 2). While patients who developed early-onset pre-eclampsia were observed to have increased sFlt-1 levels at midpregnancy (Supplementary Table S1), these levels were not significantly different between patients who developed pre-eclampsia and unaffected pregnancies (Supplementary Table S1). Hence, the difference in sFlt-1/PlGF ratio values at midpregnancy between patients who subsequently develop pre-eclampsia and those with unaffected pregnancies can be attributed mainly to the PlGF component of the test.

The predictive properties of PlGF and sFlt-1/PlGF ratio raw data and MoM at midpregnancy were compared using ROC curve analysis, displayed in Figs. 1 and 2. Test performance was compared using cut-offs derived from these ROC curves (Table 3). Using these cut-offs, tests with higher sensitivity for preterm pre-eclampsia (**cobas® e 411** raw data values and **B.R.A.H.M.S KRYPTOR compact PLUS** raw data values) had lower specificities, while those with higher specificities (**DELFIA® Xpress** PlGF, **DELFIA® Xpress** PlGF MoM, **cobas® e 411** PlGF MoM and **B.R.A.H.M.S KRYPTOR compact PLUS** PlGF MoM) had lower sensitivities (Table 3).

Given there was no significant difference in either PlGF or the sFlt-1/PlGF ratio between unaffected patients and those who developed term pre-eclampsia, further analysis of the predictive value of these tests for this outcome was not performed. Similarly, given there was no significant difference in sFlt-1 raw data or MoM values between patients who developed early-onset, preterm or term pre-eclampsia compared with unaffected pregnancies, further analysis of the predictive value of these tests for this outcome was not performed.

The number of women who actually developed pre-eclampsia

Table 1
Characteristics of the study population for midpregnancy prediction of pre-eclampsia.

Maternal characteristics	Unaffected (n = 487)	PE < 34 weeks (n = 3)	PE < 37 weeks (n = 8)	PE ≥ 37 weeks (n = 17)
Maternal age in years	35.4 (31.2–38.3)	35.7 (33.3–36.9)	33.8 (30.0–36.0)	33.1 (29.5–35.4)
Maternal weight in kg	68.2 (62.0–78.0)*	96.0 (84.0–97.0)	85.5 (70.5–97.0)*	76.0 (64.0–93.0)
Maternal height in cm	164.8 (160.0–169.0)	164.0 (160.0–167.3)	163.3 (157.8–169.3)	164 (163–167)
BMI	25.2 (23.0–28.4)*	33.7 (30.3–36.6)*	32.1 (27.3–34.8)*	28.7 (23.8–34.6)
GA in weeks	20.3 (20.0–20.9)	20.7 (20.4–21.0)	20.3 (20.1–21.0)	20.6 (20.1–21.3)
GA at delivery	39.3 (38.1–40.4)*	30.1 (29.2–31.1)*	35.9 (31.1–36.3)*	38.14 (37.7–39.1)
Racial origin, n (%)				
Caucasian	366 (75.2)	2 (66.6)	5.0 (62.5)	15.0 (88.2)
Afro-Caribbean	17.0 (3.5)	0.0	1.0 (12.5)	0.0
South Asian	40.0 (8.2)	0.0	1.0 (12.5)	0.0
East Asian	41.0 (8.4)	1 (33.3)	1.0 (12.5)	2.0 (11.8)
Mixed	23.0 (4.7)	0.0	0.0	0.0
Medical History, n (%)				
Chronic hypertension	17 (3.5)	1 (33.3)	3 (37.5)*	0.0
Diabetes Mellitus, n (%)				
Type 1	14 (2.9)	1 (33.3)	1 (12.5)	3 (17.6)*
Type 2	8 (1.6)	0.0	1 (12.5)	0.0
Gestational Diabetes	22 (4.5)	0.0	2 (25.0)	0.0
SLE	2 (0.4)	0.0	0.0	0.0
APS	3 (0.6)	0.0	0.0	1 (5.9)
Cigarette smokers, n (%)	33 (6.8)	0.0	1 (12.5)	2 (11.8)
Family history of PE, n (%)	33 (6.8)	0.0	0.0	1 (5.9)
Parity, n (%)				
Nulliparous	206 (42.3)	1 (33.3)	1 (12.5)	11 (64.7)
Parous with no previous PE	248 (50.9)	2 (66.6)	4 (50.0)	4 (23.5)
Parous with previous PE	33 (6.8)	0.0	3 (37.5)*	2 (11.8)
Infant Characteristics				
Birthweight (g)*	3351.0 (3028.0–3690.0)	1193.0 (1088.0–1244.0)*	2114.0 (1244.0–3161.5)*	3420.0 (3192.0–3680.0)

PE = pre-eclampsia; GA = gestational age; BMI = body mass index; SLE = systemic lupus erythematosus; APS = antiphospholipid syndrome. Values reported in median (interquartile range) unless otherwise stated.

* $p < 0.05$ when compared to the unaffected group.

Table 2
Comparison of PlGF and sFlt-1/PlGF raw data and MoM values between patients with term, preterm and early-onset pre-eclampsia and unaffected pregnancies.

Angiogenic Markers	Unaffected (n = 487)	PE ≥ 37 weeks (n = 17)	p-value	PE < 37 weeks (n = 8)	p-value	PE < 34 weeks (n = 3)	p-value
Delfia PlGF							
Raw data	166.9 (125.1–231.0)	183.7 (142.6–266.0)	0.457	95.5 (42.2–171.7)*	0.018	47.9 (34.2–81.9)*	0.010
MoM	1.0 (0.9–1.1)	1.1 (0.9–1.2)	0.385	0.8 (0.6–1.0)*	0.010	0.5 (0.4–0.7)*	0.020
Cobas PlGF							
Raw data	248.5 (183.9–332.8)	273.7 (196.7–339.7)	0.310	161.3 (60.9–215.6)*	0.008	54.7 (52.2–116.4)*	0.012
MoM	1.0 (0.9–1.1)	1.1 (0.9–1.1)	0.215	0.8 (0.6–1.0)*	0.006	0.6 (0.5–0.8)*	0.023
Kryptor PlGF							
Raw data	201.4 (152.3–276.1)	204.2 (160.2–316.3)	0.638	127.9 (54.3–206.0)*	0.015	48.8 (47.4–96.7)*	0.010
MoM	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.495	0.8 (0.7–0.9)*	0.008	0.6 (0.5–0.8)*	0.020
Cobas sFlt-1/PlGF ratio							
Raw data	5.8 (4.0–8.1)	5.2 (3.2–10.1)	0.506	9.9 (5.3–13.8)	0.067	14.0 (13.8–29.2)*	0.005
MoM	1.0 (0.9–1.2)	0.9 (0.8–1.2)	0.497	1.3 (1.0–1.5)*	0.021	1.6 (1.5–2.0)*	0.006
Kryptor sFlt-1/PlGF ratio							
Raw data	6.4 (4.1–9.3)	5.8 (3.0–11.6)	0.329	9.3 (5.6–14.3)	0.121	15.9 (13.2–36.2)*	0.012
MoM	1.0 (0.8–1.2)	0.9 (0.8–1.2)	0.410	1.3 (1.0–1.4)*	0.046	1.4 (1.3–2.1)*	0.011

Units for sFlt-1 and PlGF are in pg/mL. All values are reported as median (interquartile ranges).
* $p < 0.05$ when compared to the unaffected group. PE = pre-eclampsia. MoM = multiples of the median.

following a positive screen result using the preterm PE cutoff was one in between 11 and 16 women for PlGF MoM and one in between 12 and 18 women using sFlt-1/PlGF ratio MoM values. The number of women who actually developed pre-eclampsia following a positive screen result using the early-onset PE cutoff was one in 8 for sFlt-1/PlGF ratio raw data values from the Cobas platform, one in 30 women for the Kryptor platform, and one in between 17 and 21 women using sFlt-1/PlGF ratio MoM values.

4. Discussion

The significant reduction in maternal PlGF levels reported in this study at midpregnancy in women who subsequently developed pre-eclampsia, when compared with unaffected pregnancies, is comparable with findings from previous studies [4–7,30–35]. Other studies similarly report limited clinical utility of PlGF testing alone in this context due to a high proportion of false positive results (around 21.7%) and low PPV (around 5.8%) [36]. Clinical characteristics obtained for preterm pre-eclampsia in this study appear comparable to those of Tidwell et al, who reported sensitivity and specificity for prediction of pre-eclampsia when testing for PlGF at 16- to 20-weeks' gestation as 0.667 and 0.889, respectively [5]. Predictive performance of PlGF for pre-eclampsia when tested at midpregnancy in this patient cohort appeared superior to results from Polliotti et al, who reported an AUC of 0.799 for prediction of early-onset pre-eclampsia [6].

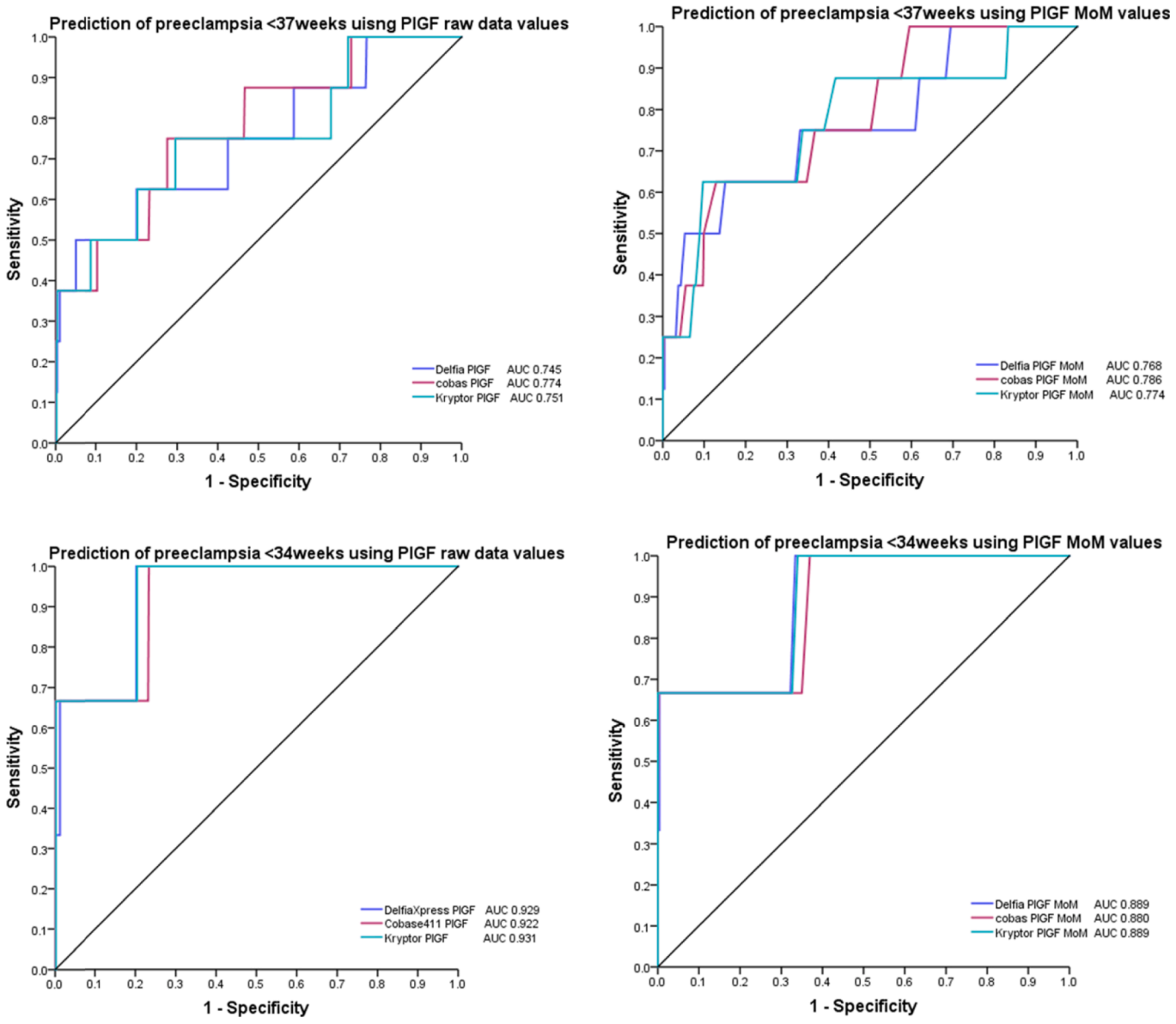
As with previous studies, addition of sFlt-1 in a ratio with PlGF slightly improved predictive performance for early-onset pre-eclampsia compared with use of PlGF alone [9,10,12–16], however this was not observed for preterm pre-eclampsia (Table 3). Though midpregnancy sFlt-1 levels were not significantly different between patients with pre-eclampsia and unaffected pregnancies (Supplementary Table S1), patients who developed early-onset pre-eclampsia were observed to have increased sFlt-1 levels at midpregnancy (Supplementary Table S1), which may account for the slight improvement in performance over testing with PlGF alone.

Tests with higher sensitivity for preterm pre-eclampsia (**cobas® e 411** raw data values and **B.R.A.H.M.S KRYPTOR compact PLUS** raw data values) had slightly lower specificities, translating to higher FPR's (Table 3 and Supplementary Fig. S1). Tests with higher specificities (**DELFIA® Xpress** PlGF, **DELFIA® Xpress** PlGF MoM, **cobas® e 411** PlGF MoM and **B.R.A.H.M.S KRYPTOR compact PLUS** PlGF MoM) displayed lower FPR's and lower sensitivities (Table 3 and Supplementary Fig. S1). When using sFlt-1/PlGF MoM for prediction of preterm pre-eclampsia, specificities remained higher compared with sFlt-1/PlGF ratio raw data values, with lower FPR's at 10.7 for **cobas® e 411** sFlt-1/PlGF MoM and 17.7 for **B.R.A.H.M.S KRYPTOR compact PLUS** sFlt-1/PlGF MoM. Comparing PlGF MoM with sFlt-1/PlGF ratio MoM for prediction of preterm pre-eclampsia, following clinical correlation, their performance in our patient population was actually very similar (Table 3 and Supplementary Fig. S1).

For the prediction of early-onset pre-eclampsia, DR's were consistently 100%. **Cobas® e 411** sFlt-1/PlGF raw data values had the lowest FPR of all the tests at 4.13% (Supplementary Fig. S1), reflecting the higher PPV observed with the **cobas® e 411** platform compared with the **B.R.A.H.M.S KRYPTOR compact PLUS**. While the **cobas® e 411** sFlt-1/PlGF ratio raw data values appeared to display superior AUC, FPR and a higher PPV than other tests, the very small number of patients in the cohort that developed early-onset pre-eclampsia (n = 3) necessitates confirmation of this finding with further research. It would be expected that with a larger sample size, the tests would perform very similarly. As demonstrated in the COMPARE study [22], further optimisation of cut-off values for midpregnancy PlGF and sFlt-1/PlGF ratio testing using a larger sample size is required, to determine recommended test thresholds applicable to different immunoassay platforms.

In most studies, the majority of patients with an imbalance between angiogenic and antiangiogenic factors do not subsequently develop pre-eclampsia [37–40]. The low PPV's for these biomarkers when used in isolation for prediction of pre-eclampsia at midpregnancy in our population translates to significant false positive rates. The addition of PlGF or sFlt-1 values to a multivariable logistic regression model may increase PPV, reducing FPR and further optimising the performance of screening for preterm pre-eclampsia in this patient population, as has been demonstrated in other studies [41].

The relatively high false positive rates associated with midpregnancy PlGF and sFlt-1/PlGF ratio testing may promote patient anxiety and result in unnecessary utilisation of healthcare resources due to increased pregnancy surveillance, despite a significant number of patients who screen positive having normal outcomes. Interestingly, in this study, a significant number of patients who had false positive PlGF or sFlt-1/PlGF ratio tests at midpregnancy for the outcome of pre-eclampsia, actually went on to have either a preterm delivery in the absence of pre-eclampsia, or another adverse perinatal outcome. The rates of these adverse outcomes were higher in patients who had a



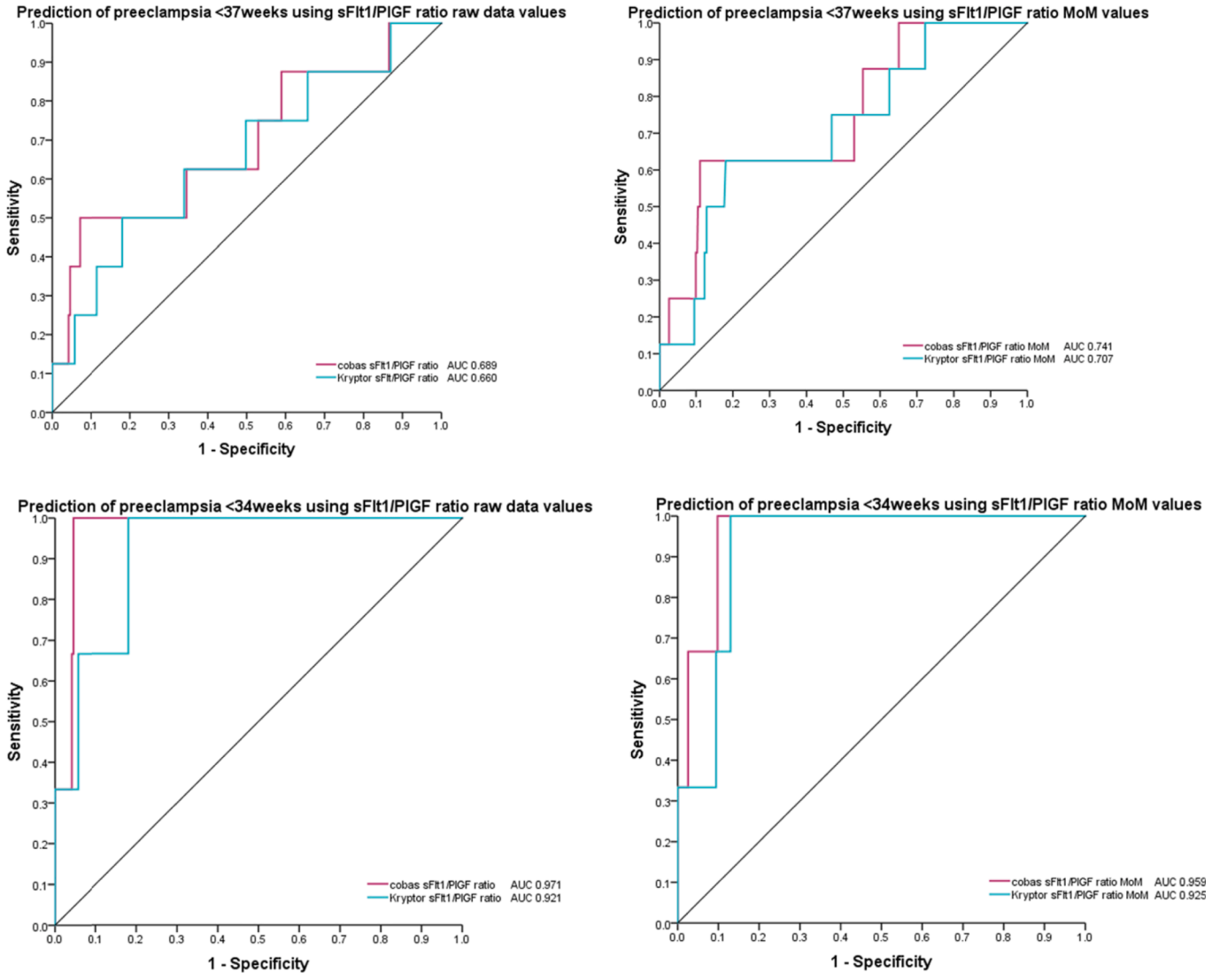
Pregnancies that developed PE		
25 (4.9%) overall PE		
17 (3.3%) term (≥37 weeks) PE	8 (1.6%) preterm (<37 weeks) PE	3 (0.6%) early onset (<34 weeks) PE

Fig. 1. ROC curves for prediction of preterm (< 37 weeks) and early-onset (< 34 weeks) pre-eclampsia using PlGF raw data and MoM values.

positive PlGF or sFlt-1/PlGF ratio screening test at midpregnancy when compared with those who had a negative screening test, as displayed in Supplementary Table S2.

Whilst identifying excess false positives reflects poorly on a screening test, if a significant proportion of patients with false positive results develop other adverse outcomes and would potentially have benefitted from increased surveillance antenatally, the intervention stemming from screening may improve outcomes for these patients. Additionally, these adverse outcomes detected by screening may reflect the ability of PlGF and sFlt-1/PlGF ratio testing at midpregnancy to detect a broader range of placental pathology. Once the clinical utility of these tests is better established, these patients may end up being identified as true positives, rather than false positives.

So how could successful screening for pre-eclampsia at midpregnancy improve current management? From a research perspective, accurate prediction of preterm pre-eclampsia aids recruitment for future studies investigating prophylactic or therapeutic agents for pre-eclampsia, or the validation of additional predictive markers [42–48]. Clinically, prophylactic agents shown to reduce the risk of pre-eclampsia include low dose aspirin and calcium commenced in the first or early second trimester [49–55]. Other benefits in stratifying pre-eclampsia risk in midpregnancy include triaging to appropriate models of care, enhanced surveillance for those at high risk, and reassurance for those who screen negative. PlGF and the sFlt-1/PlGF ratio, using both raw data and MoM values and cut-offs for preterm and early-onset pre-eclampsia all had very high negative predictive values (NPV), exceeding 99%, making these tests useful for ruling out the development of pre-eclampsia at < 37 weeks, providing reassurance for the majority



Pregnancies that developed PE		
25 (4.9%) overall PE		
17 (3.3%) term (≥37 weeks) PE	8 (1.6%) preterm (<37 weeks) PE	3 (0.6%) early onset (<34 weeks) PE

Fig. 2. ROC curves for prediction of preterm (< 37 weeks) and early-onset (< 34 weeks) pre-eclampsia using sFlt-1/PlGF ratio raw data and MoM values.

of women tested.

Given that these tests used in isolation are limited by low PPV and high FPR's, including them in a multivariable regression model should further enhance the clinical validity of screening for preterm pre-eclampsia in this patient population. Ultimately, randomised trials comparing patients who are screened with placental biomarkers, either in isolation or as part of a multivariable algorithm, with those undergoing usual care are required in order to determine the extent to which they may be capable of optimising clinical outcomes.

The strengths of this study included: 1) two independent adjudicators checking patient outcomes; 2) use of fully automated platforms to optimise accuracy of results; 3) one technician for blood sample analysis, blinded to outcomes throughout testing and 4) conversion of biomarker levels to MoM. Limitations included: 1) small sample size, particularly for preterm (8 patients) and early-onset (3 patients) pre-eclampsia. Another potential limitation was the increasing proportion of previously thawed samples when using the second (**cobas® e 411**) and third (**B.R.A.H.M.S KRYPTOR compact PLUS**) analysers, however, in a previous paper published by our group (submitted for publication), there did not appear to be any marked difference in results obtained for samples that had undergone up to five freeze–thaw cycles.

5. Conclusion

For the midpregnancy prediction of preterm pre-eclampsia, PlGF MoM values among all three platforms and sFlt-1/PlGF MoM values between the **cobas® e 411** and **B.R.A.H.M.S KRYPTOR compact PLUS** platforms performed similarly. For the midpregnancy prediction of early-onset pre-eclampsia, while **cobas® e 411** sFlt1/PlGF ratio using raw data values appeared to perform best, this finding requires confirmation with further research. The **cobas® e 411** and **B.R.A.H.M.S KRYPTOR compact PLUS** sFlt-1/PlGF ratio using MoM values and the early-onset PE cut-off had relatively similar performances from a

Table 3
Screening performance for PlGF raw data and MoM values andsFlt-1/PlGF ratio raw data and MoM values for preeclampsia delivering before 34 weeks (early onset PE) and before 37 weeks (preterm PE).

Assay	AUC	Cut-off value	DR (%)	FPR (%)	PPV (95% CI)	NPV (95% CI)	FPR 5% DR (%)	FPR 10% DR (%)
Delfia PlGF								
Early Onset PE	0.93	115.9	100 (29.2–100)	19.3	2.97 (2.50–3.53)	100	66.7	66.7
Preterm PE	0.75	115.9	62.5 (24.5–91.5)	19.4	4.85 (2.82–8.24)	99.27 (98.22–99.70)	37.5	50.0
Delfia PlGF MoM								
Early Onset PE	0.89	0.925	100 (29.2–100)	31.8	1.82 (1.60–2.06)	100	66.7	66.7
Preterm PE	0.77	0.815	62.50 (24.49–91.48)	14.9	6.25 (3.61–10.60)	99.31 (98.32–99.72)	50.0	50.0
Cobas PlGF								
Early Onset PE	0.92	178.2	100 (29.2–100)	22.2	2.59 (2.21–3.03)	100	66.7	66.7
Preterm PE	0.77	191.2	75 (34.91–96.81)	26.8	4.26 (2.82–6.37)	99.46 (98.23–99.84)	37.5	37.5
Cobas PlGF MoM								
Early Onset PE	0.88	0.945	100 (29.2–100)	35.4	1.64 (1.46–1.70)	100	66.7	66.7
Preterm PE	0.79	0.815	62.5 (24.49–91.48)	12.9	7.14 (4.12–12.11)	99.32 (98.35–99.72)	37.5	50.0
Kryptor PlGF								
Early Onset PE	0.93	144.8	100 (29.24–100)	19.45	2.94 (2.48–3.49)	100	66.7	66.7
Preterm PE	0.75	161.2	75 (34.9–96.8)	28.6	4.00 (2.66–5.98)	99.45 (98.19–99.83)	37.5	50.0
Kryptor PlGF MoM								
Early Onset PE	0.89	0.935	100 (29.2–100)	32.2	1.80 (1.59–2.03)	100	66.7	66.7
Preterm PE	0.77	0.795	62.5 (24.5–91.5)	9.7	9.26 (5.31–15.67)	99.34 (98.41–92.32)	25.0	62.5
Cobas sFlt-1/PlGF ratio								
Early Onset PE	0.97	13.58	100 (29.24–100)	4.13	12.50 (8.59–17.48)	100	100	100
Preterm PE	0.69	7.2	62.5 (24.49–91.48)	33.53	2.87 (1.68–4.88)	99.11 (97.85–99.64)	37.5	50.0
Cobas sFlt-1/PlGF ratio MoM								
Early Onset PE	0.96	1.35	100 (29.24–100)	9.4	5.88 (4.56–7.56)	100	66.7	100
Preterm PE	0.74	1.327	62.5 (24.49–91.48)	10.7	8.47 (4.87–14.35)	99.34 (98.39–99.73)	25.0	37.5
Kryptor sFlt-1/PlGF ratio								
Early Onset PE	0.92	10.57	100 (29.24–100)	16.9	3.37 (2.80–4.06)	100	33.3	66.7
Preterm PE	0.66	7.98	62.5 (24.49–91.48)	32.94	2.92 (1.71–4.97)	99.12 (97.87–99.64)	12.5	25.0
Kryptor sFlt-1/PlGF ratio MoM								
Early Onset PE	0.93	1.352	100 (29.24–100)	12.2	4.62 (3.69–5.76)	100	33.3	66.7
Preterm PE	0.71	1.274	62.5 (24.49–91.48)	17.7	5.32 (3.08–9.03)	99.28 (98.26–99.71)	12.5	25.0

clinical perspective. Given the limitations of using these tests in isolation, results following inclusion of PlGF and/or sFlt-1 values from this patient population in a multivariable regression model are awaited to determine whether multifaceted testing is more effective for prediction of pre-eclampsia within this patient population.

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Conflicts of interest

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.03.009.

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