

Maternal Serum Placental Growth Factor Isoforms 1 and 2 at 11–13, 20–24 and 30–34 Weeks' Gestation in Late-Onset Pre-Eclampsia and Small for Gestational Age Neonates

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Key Words

Pyramid · Placental growth factor · Isoforms · Small for gestational age · Pre-eclampsia

Abstract

Objective: To compare the maternal serum concentrations of placental growth factor (PIGF)-1 and PIGF-2 in the first, second and third trimesters in normal pregnancies and in those complicated by pre-eclampsia (PE) or the delivery of small for gestational age (SGA) neonates after 37 weeks.

Methods: Serum PIGF-1 and PIGF-2 were measured at 11–13, 20–24 and 30–34 weeks' gestation in 50 cases of PE, 99 cases of SGA and 298 controls. The values of PIGF-1 and PIGF-2 at 11–13 weeks were expressed as multiples of the median (MoM) after adjustment for maternal characteristics. The distributions of PIGF-1 and PIGF-2 in cases and controls at 20–24 and 30–34 weeks were converted to MoM of the values at 11–13 weeks and compared. **Results:** Serum PIGF-1 and PIGF-2 levels were highly correlated and both increased with gestational age. At 30–34 weeks, the median MoM values for PIGF-1 and PIGF-2 in the late PE (4.2 and 4.3) and late SGA (7.2 and 6.0) groups were significantly lower than in the con-

trols (12.8 and 9.9). Combining the two isoforms did not improve the prediction of late PE and late SGA provided by PIGF-1 alone. **Conclusions:** The performances of serum PIGF-1 and PIGF-2 in the prediction of late PE and late SGA are similar.

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Introduction

Placenta growth factor (PIGF) exists in at least 4 isoforms due to alternative mRNA splicing of the PIGF primary transcript, but the major ones are thought to be PIGF-1 and PIGF-2 [1]. The main difference between the 4 isoforms is that PIGF-1 and PIGF-3 are non-heparin binding and can potentially affect targets in a paracrine manner, whereas PIGF-2 and PIGF-4 have additional heparin-binding domains and most likely work in an autocrine way [1].

In pregnancy, PIGF is believed to be implicated in placental development and several studies have reported that the maternal serum PIGF concentration is reduced in pregnancies that subsequently develop pre-eclampsia

(PE) and in those that deliver small for gestational age (SGA) neonates [2–5]. The underlying cause for this decrease has been attributed to early and persisting placental hypoxia [6]. Although in these studies it has been assumed that PIGF-1 was measured, in the various commercially available assays there is considerable cross-reactivity with other PIGF isoforms. For example, the method instructions of the PIGF-1 assays report a cross-reactivity to PIGF-2 of 13–50% (BRAHMS KRYPTOR, Thermo Fisher Scientific, Hennigsdorf, Germany; DELFIA Xpress, Perkin Elmer Inc., Waltham, Mass., USA; Roche Cobas and Elecsys Systems, Roche Diagnostics, Mannheim, Germany, and R&D Systems Inc., Minneapolis, Minn., USA). A study examining the levels of serum PIGF-1 and PIGF-2 at 11–13 weeks' gestation in normal pregnancies and in those complicated by PE with delivery at 24–42 weeks, SGA neonates with delivery at 24–42 weeks and fetal trisomies 21, 18 and 13 reported that in each pathological group both PIGF isoforms were reduced but the deviation from normal was greater for PIGF-1 than for PIGF-2 [7]. It was therefore concluded that the performance of screening for these conditions by serum PIGF at 11–13 weeks' gestation was likely to be inversely related to the percentage of cross-reactivity of the assay to PIGF-2.

The objective of this study was to compare the maternal serum concentrations of PIGF-1 and PIGF-2 at 11–13, 20–24 and 30–34 weeks' gestation in normal pregnancies and in those complicated by PE and the delivery of SGA neonates after 37 weeks and to analyse whether the combination of the two isoforms could improve the prediction rate of late PE and late SGA provided by PIGF-1 alone.

Methods

Study Population

This is a case-control study drawn from a prospective observational study of adverse pregnancy outcomes in women attending for their routine first-, second- and third-trimester ultrasound scans in pregnancy at King's College Hospital (London, UK). The first-trimester visit, at 11–13 weeks' gestation, included recording of the maternal characteristics and medical history and an ultrasound scan to, firstly, confirm the gestational age based on the measurement of the fetal crown-rump length [8], secondly, diagnose any major fetal abnormalities and, thirdly, measure the fetal nuchal translucency thickness as part of a combined screening for aneuploidies [9]. The second- and third-trimester visits, at 20–24 and 30–34 weeks, respectively, included ultrasound examination for the assessment of fetal anatomy, growth and well-being. In all three visits, samples of serum were stored at -80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the NHS Research Ethics Committee.

The entry criteria for this study were singleton pregnancies with serum samples stored at 11–13, 20–24 and 30–34 weeks' gestation. The study population was comprised of 50 pregnancies that subsequently developed PE delivering after 37 weeks (late PE) and 99 cases that delivered SGA neonates after 37 weeks (late SGA). The controls were comprised of 298 cases matched with the cases for storage time; they did not develop any pregnancy complications and resulted in the live birth of phenotypically normal neonates. Four cases of controls had undetectable levels of PIGF-1 and PIGF-2 at either 20–24 or 30–34 weeks and were excluded from this study.

Maternal History

Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian or Mixed), cigarette smoking during pregnancy (yes or no), the method of conception (spontaneous, use of ovulation drugs and in vitro fertilization) and parity (parous or nulliparous if no delivery beyond 23 weeks). The maternal weight and height were measured and recorded.

Outcome Measures

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Data on pregnancy outcomes were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine whether the condition was chronic hypertension, PE or non-proteinuric gestational hypertension. The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [10]. The definition of SGA was a birth weight below the 5th percentile of a reference range derived from our population [11]. The selected cases were ones with PE or SGA delivering after 37 weeks' gestation.

Sample Analysis

Single measurements were performed for PIGF and the specific isoform PIGF-2 on the fully automated KRYPTOR compact PLUS system (BRAHMS PIGF KRYPTOR and BRAHMS PIGF-2 KRYPTOR; Thermo Fisher Scientific). Both assays were homogeneous sandwich immunoassays based on TRACE technology [12]. The total duration of the assays was 29 min and the sample volume was 70 μl . The PIGF assay was calibrated with recombinant human PIGF and standardized against the Quantikine PIGF ELISA (R&D Systems Europe Ltd., Abingdon, UK). According to the manufacturer's instructions for use, the BRAHMS PIGF KRYPTOR assay covered a measuring range of 3.6–7,000 pg/ml. The limit of detection was 3.6 pg/ml and the limit of quantitation (functional sensitivity) was <6.9 pg/ml. The intra-assay and inter-assay imprecisions at a PIGF concentration of 35 pg/ml were 4.6 and 7.3%, respectively; at a concentration of 103 pg/ml they were 2.1 and 3.1% and at a concentration of 430 pg/ml they were 0.9 and 2.3%. The cross-reactivity of the PIGF assay, which was determined in accordance with CLSI EP7-A2 (Clinical Laboratory Standards Institute guidelines), was 13% for PIGF-2 and 4% for PIGF-3 and in this study this assay is referred to as PIGF-1.

The specific PIGF-2 immunoassay was set up using a polyclonal anti-human PIGF antibody (R&D Systems Europe) and a monoclonal anti-human PIGF-2 antibody generated by immunization of female BALB/c mice with human PIGF-2 recombinant protein

Table 1. Characteristics of the study population

Characteristics	Normal (n = 294)	PE (n = 50)	SGA (n = 99)
11–13 weeks' gestation			
Maternal weight, kg	64.8 (59.0–73.6)	69.5 (63.1–81.4) ^a	61.6 (54.0–73.0)
Gestation at screening, weeks	12.6 (12.2–13.0)	12.6 (12.2–13.0)	12.6 (57.5–73.9)
20–24 weeks' gestation			
Maternal weight, kg	69.0 (62.9–77.1)	72.0 (21.9–22.1)	64.4 (57.5–73.9) ^a
Gestation at screening, weeks	22.0 (21.9–22.1)	22.0 (21.8–22.1)	22.0 (21.3–22.1)
30–34 weeks' gestation			
Maternal weight, kg	74.0 (67.8–83.9)	79.9 (72.5–89.9) ^a	69.0 (61.6–78.7) ^a
Gestation at screening, weeks	32.0 (32.0–32.3)	32.0 (32.0–32.3)	32.0 (32.0–32.3)
Maternal age, years	32.8 (29.0–35.4)	34.2 (28.7–36.5)	31.1 (25.6–35.1)
Maternal height, cm	165 (160–168)	164 (159–169)	162 (157–166) ^a
Racial origin			
Caucasian	181 (61.6)	31 (62.0)	54 (54.5)
Afro-Caribbean	70 (23.8)	14 (28.0)	33 (33.3)
South Asian	19 (6.5)	2 (4.0)	6 (6.1)
East Asian	14 (4.8)	2 (4.0)	2 (2.0)
Mixed	10 (3.4)	1 (2.0)	4 (4.0)
Nulliparous	159 (54.1)	33 (66.0)	68 (68.7) ^a
Cigarette smoker	8 (2.7)	3 (6.0)	18 (18.2) ^a
Conception			
Spontaneous	277 (94.2)	47 (94.0)	94 (94.9)
Ovulation drugs	5 (1.7)	0	0
In vitro fertilization	12 (4.1)	3 (6.0)	5 (5.1)
Gestation at delivery, weeks	40.3 (39.4–40.9)	39.8 (38.7–41.0)	39.7 (38.7–40.7) ^a
Birth weight, g	3,277 (3,094–3,523)	3,239 (2,975–3,612)	2,600 (2,384–2,758) ^a

Values are presented as medians (IQR) or numbers (%). Comparisons between outcome groups were made using a Mann-Whitney U test with post hoc Bonferroni correction and a χ^2 test or Fisher's exact test for categorical variables. ^a Adjusted significance level $p < 0.025$.

(R&D Systems Europe). This assay was calibrated with recombinant human PIGF-2. BRAHMS PIGF-2 KRYPTOR had an assay range of 15–10,000 pg/ml. The performance characteristics of the PIGF-2 assay were determined for the control samples in 26 runs with 2 replicates. The calibration curve of the first run was used as a reference curve during a 7-day period. The limit of detection was 15 pg/ml and the limit of quantitation (functional sensitivity) was 42 pg/ml. The intra-assay and inter-assay imprecisions at a PIGF-2 concentration of 100 pg/ml were 8.8 and 9.4%, respectively; at a concentration of 505 pg/ml they were 2.3 and 2.9% and at a concentration of 1,018 pg/ml they were 1.3 and 2.4%.

Statistical Analysis

Comparisons of pregnancy characteristics between outcome groups were made using a Mann-Whitney U test or a χ^2 test or Fisher's exact test for categorical variables, with post hoc Bonferroni correction (adjusted significance level $p < 0.025$).

The distributions of PIGF-1 and PIGF-2 at 11–13 weeks were converted to multiples of the median (MoM) in cases and controls, corrected for maternal characteristics as previously described [7]. The distributions of PIGF-1 and PIGF-2 at 20–24 and 30–34 weeks were expressed as MoM of the values at 11–13 weeks in cases and controls. Wilcoxon's signed-rank test was used to compare PIGF-1

and PIGF-2 within each outcome group. Cuzick's trend test was used to compare PIGF-1 and PIGF-2 across trimesters within each outcome group. The Mann-Whitney U test was used to determine the significance of differences in the median values in each outcome group versus the controls. Regression analysis was used to determine the significance of the association between the \log_{10} MoM values of PIGF-1 and PIGF-2 and between the \log_{10} MoM values of PIGF-1 and PIGF-2 with gestational age at delivery in the PE and SGA groups. The performance of the screening was determined by receiver operating characteristic (ROC) curves.

The statistical software package SPSS 20.0 (IBM SPSS Statistics for Windows, version 20.0; IBM Corp., Armonk, N.Y., USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

The maternal characteristics of each of the outcome groups are summarized in table 1. The median measured concentration of serum PIGF-2 was 3.6, 2.4 and 2.7 times

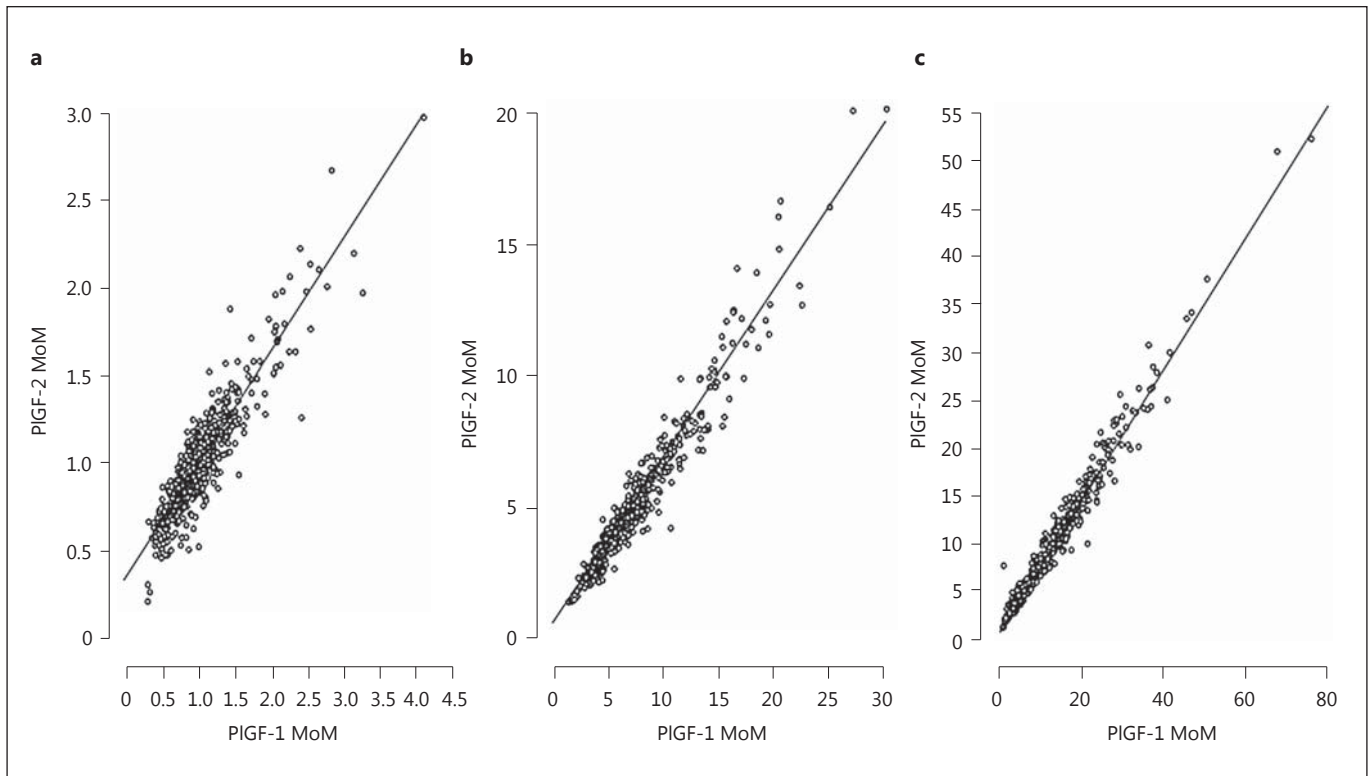


Fig. 1. Relationship between the MoM values of PIGF-1 and PIGF-2 in all pregnancies at 11–13 weeks (a), 20–24 weeks (b) and 30–33 weeks (c).

Table 2. Levels of PIGF-1 and PIGF-2

	PIGF-1, pg/ml	PIGF-2, pg/ml	p value
Level at 11–13 weeks	38.8 (28.7–52.8)	140.2 (112.7–173.1)	<0.0001*
Level at 20–24 weeks	257.0 (184.6–378.7)	627.1 (486.3–887.4)	<0.0001*
Level at 30–34 weeks	449.0 (241.1–703.7)	1,219.0 (721.9–1,837.6)	<0.0001*
Comparison across trimesters (p value)	<0.0001**	<0.0001**	

Values are presented as medians (IQR). Comparisons between isoforms in each trimester were made using Wilcoxon's signed-rank test. * $p < 0.05$. Cuzick's Trend test was used to compare PIGF-1 and PIGF-2 across trimesters within each outcome group. ** $p < 0.05$.

higher than that of PIGF-1 at 11–13, 20–24 and 30–34 weeks' gestation, respectively (table 2). There was a significant direct correlation between the \log_{10} MoM values of the two PIGF isoforms at 11–13 weeks, 20–24 weeks and 30–34 weeks in the controls ($r = 0.891$, $p < 0.0001$; $r = 0.967$, $p < 0.0001$, and $r = 0.983$, $p < 0.0001$), in the PE group ($r = 0.877$, $p < 0.0001$; $r = 0.977$, $p < 0.0001$, and $r = 0.978$, $p < 0.0001$) and in the SGA group ($r = 0.887$, $p < 0.0001$; $r = 0.973$, $p < 0.0001$, and $r = 0.982$, $p < 0.0001$) (fig. 1).

Normal Pregnancy Outcome

In the normal controls, there was a significant increase in both PIGF-1 MoM and PIGF-2 MoM across the first, second and third trimesters ($p < 0.0001$; table 3; fig. 2). The median MoM of PIGF-1 and PIGF-2 were significantly different at 20–24 and 30–34 weeks but not at 11–13 weeks.

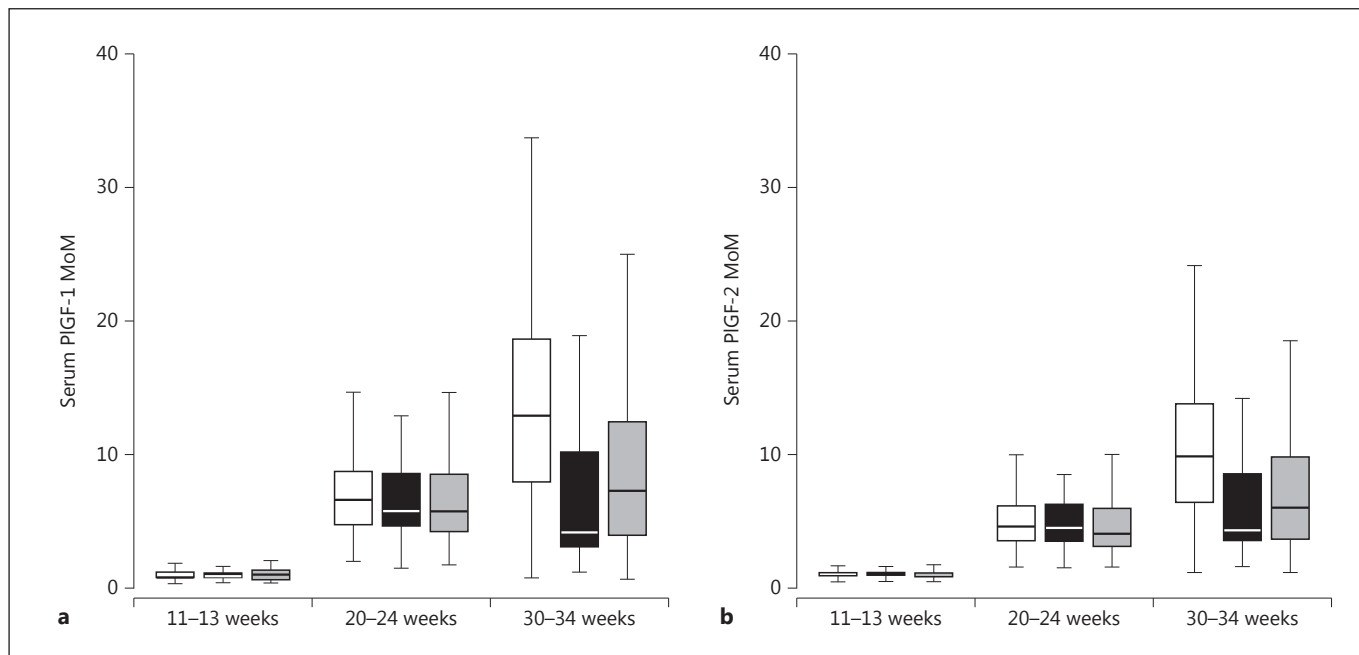


Fig. 2. Box plot of the median serum concentrations of PIGF-1 MoM (a) and PIGF-2 MoM (b) at 11–13, 20–24 and 30–34 weeks in the normal controls (white bars), the PE group (black bars) and the SGA group (grey bars).

Table 3. Comparison of the median of MoM values of PIGF-1 and PIGF-2 of each adverse outcome group with the controls

Outcome group	PIGF-1 median MoM (IQR)	PIGF-2 median MoM (IQR)
Control (n = 294)		
11–13 weeks	0.968 (0.756–1.201)	0.969 (0.839–1.179)
20–24 weeks	6.594 (4.700–8.777)	4.555 (3.544–6.147) ^b
30–34 weeks	12.820 (7.897–18.653)	9.878 (6.327–13.772) ^b
Comparison across trimesters (p value)	<0.0001 ^c	<0.0001 ^c
Late PE (n = 50)		
11–13 weeks	0.870 (0.730–1.171)	0.948 (0.828–1.227) ^b
20–24 weeks	5.802 (4.591–8.651)	4.514 (3.355–6.276) ^b
30–34 weeks	4.166 (3.018–10.251) ^a	4.303 (3.535–8.579) ^a
Comparison across trimesters (p value)	<0.0001 ^c	<0.0001 ^c
Late SGA (n = 99)		
11–13 weeks	0.966 (0.678–1.233)	0.965 (0.792–1.185)
20–24 weeks	5.752 (4.198–8.740)	4.010 (3.099–6.176) ^b
30–34 weeks	7.190 (3.741–12.453) ^a	6.047 (3.527–9.788) ^{a, b}
Comparison across trimesters (p value)	<0.0001 ^c	<0.0001 ^c

Comparisons between late PE or SGA and controls were made using the Mann-Whitney U test with post hoc Bonferroni correction. Comparisons of PIGF-1 and PIGF-2 within each outcome group were made using Wilcoxon's signed-rank test. Comparisons of PIGF-1 and PIGF-2 across trimesters within each outcome group were made using Cuzick's trend test. ^a Adjusted significance level $p < 0.025$; significant differences in PIGF-1 and PIGF-2 between PE or SGA and controls were observed only at 30–34 weeks. ^b Significance level $p < 0.05$; significant differences between PIGF-1 and PIGF-2 were observed at 20–24 and 30–34 weeks for the controls, at 11–13 and 20–24 weeks for the PE group, and at 20–24 and 30–34 weeks for the SGA group. ^c Significance level $p < 0.05$; significant trends across trimesters were observed for both isoforms in all three outcome groups.

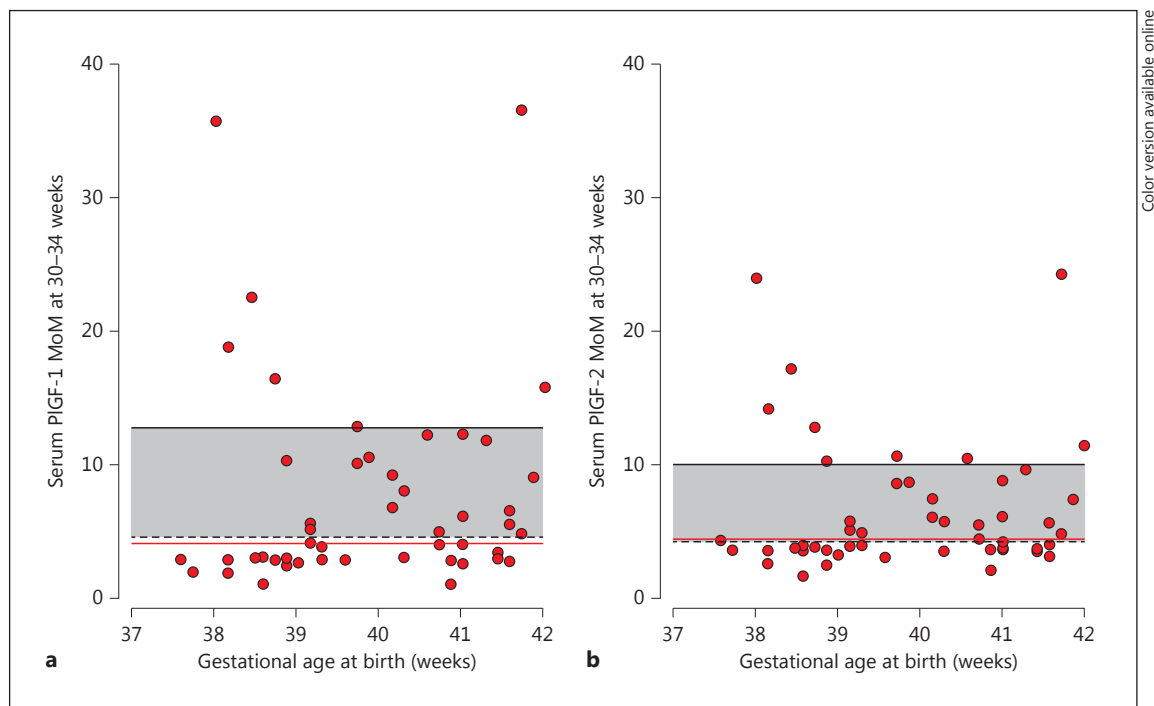


Fig. 3. Serum PIGF-1 (a) and PIGF-2 (b) MoM values in pregnancies that developed PE, plotted on the reference range for gestation in normal pregnancies (median and 10th percentile).

Pre-Eclampsia

In the PE group, there was a significant increase in both PIGF-1 MoM and PIGF-2 MoM across the first, second and third trimesters ($p < 0.0001$; table 3; fig. 2). In the PE group, compared to the normal controls, the median MoM of PIGF-1 and PIGF-2 were not significantly different at 11–13 weeks ($p = 0.159$ and $p = 0.709$) or at 20–24 weeks ($p = 0.260$ and $p = 0.579$), but at 30–34 weeks they were both lower ($p < 0.0001$). At 30–34 weeks, there was no significant association with gestational age at delivery for either PIGF-1 \log_{10} MoM ($r = 0.147$, $p = 0.309$; fig. 3) or PIGF-2 \log_{10} MoM ($r = 0.072$, $p = 0.621$; fig. 3).

In the prediction of PE, multivariate logistic regression analysis demonstrated that there were significant independent contributions from PIGF-1 \log_{10} MoM and PIGF-2 \log_{10} MoM at 30–34 weeks ($R^2 = 0.237$, $p < 0.0001$). However, the area under the ROC curve (AUROC) of the combination of PIGF-1 and PIGF-2 was not significantly different from that of PIGF-1 ($p = 0.912$) or PIGF-2 ($p = 0.585$). The AUROC and the detection rate of PE for a 10% false-positive rate in screening by PIGF-1, PIGF-2 and their combination at 30–34 weeks are given in table 4.

Small for Gestational Age

In the SGA group, there was a significant increase in both PIGF-1 MoM and PIGF-2 MoM across the first, second and third trimesters ($p < 0.0001$; table 3; fig. 2). In the SGA group, compared to the normal controls, the median MoM of PIGF-1 and PIGF-2 were not significantly different at 11–13 weeks ($p = 0.642$ and $p = 0.601$) or at 20–24 weeks ($p = 0.071$ and $p = 0.069$), but at 30–34 weeks both PIGF-1 and PIGF-2 were lower ($p < 0.0001$). At 30–34 weeks, there was no significant association with gestational age at delivery for either PIGF-1 \log_{10} MoM ($r = 0.139$, $p = 0.171$; fig. 3) or PIGF-2 \log_{10} MoM ($r = 0.193$, $p = 0.056$; fig. 4).

In the prediction of SGA, multivariate logistic regression analysis demonstrated that there was an independent significant contribution from PIGF-1 \log_{10} MoM but not from PIGF-2 \log_{10} MoM. The AUROC and the detection rate of SGA for a 10% false-positive rate in screening by PIGF-1 and PIGF-2 are given in table 4. The AUROC was not significantly different between PIGF-1 and PIGF-2 ($p = 0.769$).

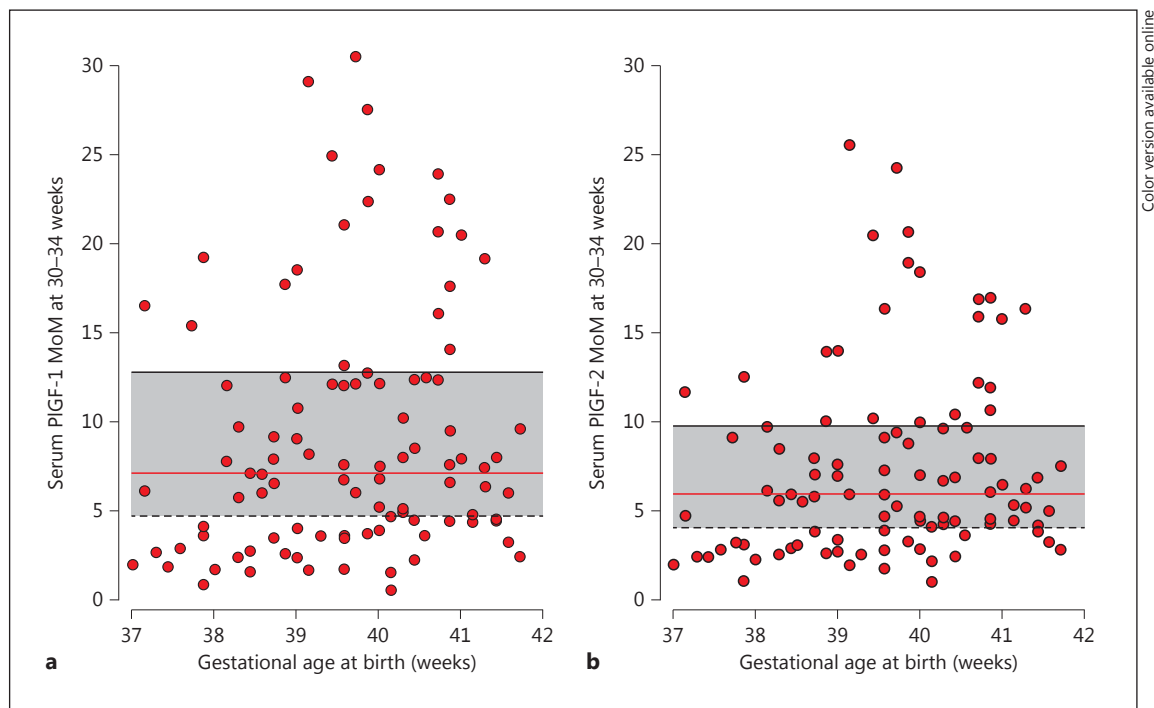


Fig. 4. Serum PIGF-1 (a) and PIGF-2 (b) MoM values in pregnancies that delivered SGA neonates, plotted on the reference range for gestation in normal pregnancies (median and 10th percentile).

Table 4. Performance of PIGF-1 and PIGF-2 in the detection of late PE and late SGA

Screening test	AUROC (95% CI)	DR for a 10% FPR (95% CI)
PE		
PIGF-1	0.777 (0.730–0.820)	52.0 (37.4–66.3)
PIGF-2	0.760 (0.711–0.804)	48.0 (33.7–62.6)
PIGF-1 and PIGF-2	0.775 (0.727–0.818)	56.0 (41.3–70.0)
SGA		
PIGF-1	0.690 (0.642–0.735)	33.3 (24.2–43.5)
PIGF-2	0.692 (0.644–0.737)	29.3 (20.6–39.3)

DR = Detection rate; FPR = false-positive rate.

Discussion

The findings of this study demonstrate that the serum levels of PIGF-1 and PIGF-2 are highly correlated in both normal and pathological pregnancies in all 3 trimesters of pregnancy. This high correlation presumably reflects the

common origin and control mechanisms of the production of these isoforms. The measured concentrations of PIGF were adjusted for maternal characteristics and the values were expressed as MoM [7]. Compared to the levels of PIGF-1 and PIGF-2 at 11–13 weeks' gestation, in normal pregnancies there was a 5-fold increase at 20–24 weeks and a 10-fold increase at 30–34 weeks. This is compatible with the results of previous longitudinal studies in normal pregnancies which have reported that serum PIGF increases with gestation to a peak at around 30 weeks and decreases thereafter until delivery, presumably because of deterioration in placental growth and function towards term [2, 13].

In pregnancies that developed late PE or delivered SGA neonates after 37 weeks' gestation, the levels of serum PIGF-1 and PIGF-2 at 11–13 and 20–24 weeks were not significantly different from those of the normal controls, but at 30–34 weeks the levels in the late PE group were about 60% lower and in the late SGA group they were about 40% lower than in the normal controls. Previous studies have reported that in PE and SGA reduced levels of PIGF are apparent from the first trimester of pregnancy [4, 5, 7]. The underlying cause for this decrease

has been attributed to impaired placentation leading to early and persisting placental hypoxia [6, 14]. However, the early decrease in serum PIGF in pregnancies with PE or SGA has been inversely related to the gestational age at delivery, with major deviations from normal in those with severe disease requiring delivery before 34 weeks but only mild or no deviations in affected cases delivering after 37 weeks [4, 5, 7]. The finding that in the late PE and late SGA groups the levels of PIGF were reduced at 30–34 weeks but not at 11–13 and 20–24 weeks' gestation is compatible with the results of a case-control study which reported that in pregnancies developing late PE the serum PIGF was significantly reduced at 30–33 weeks' gestation but not at 11–13 weeks [15].

We previously reported that in pathological pregnancies at 11–13 weeks' gestation, the deviation from normal was greater for PIGF-1 than for PIGF-2 and concluded that the performance of screening by serum PIGF is likely to be inversely related to the percentage of cross-reactivity of the assay to PIGF-2 [7]. In contrast, in this study the performance of screening for late PE and late SGA by PIGF-2 at 30–34 weeks was similar to that of PIGF-1; consequently, the performance of serum PIGF in screening for these pregnancy complications in the third trimester may be unrelated to the cross-reactivity of the assay to PIGF-2. Supportive evidence is provided by the results of a screening study for PE in 4,914 singleton pregnancies at 30–33 weeks, in which the cross-reactivity of the PIGF assay to PIGF-2 was about 28%; the serum PIGF was below

the 10th percentile in 93, 63 and 35% of pregnancies developing PE and requiring delivery at <37, 37–40 and >40 weeks' gestation, respectively [16]. These results concur with our findings that measurement of the serum PIGF, by an assay with 13% cross-reactivity to PIGF-2, could detect about 50% of pregnancies with late PE with a false-positive rate of 10%.

In accordance with our new model of pregnancy care, we proposed a 2-stage strategy for the identification of pregnancies at risk of PE and SGA [17, 18]. The first stage, at 11–13 weeks' gestation, should be primarily aimed at the effective prediction and prevention of severe early-onset disease [19, 20]. The second stage, at 30–34 weeks, should be aimed at the effective prediction of intermediate and late disease because close monitoring of such pregnancies to define the optimal time for delivery could improve the perinatal outcome. An important component of screening, both at 11–13 and at 30–34 weeks, is measurement of the serum PIGF [4, 5, 15, 16]. Although in the first trimester a high cross-reactivity of the PIGF assay to PIGF-2 may be detrimental to the performance of the screening [7], our findings suggest that this may not be the case in screening during the third trimester.

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