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Serum placental growth factor and soluble fms-like tyrosine kinase 1 at mid-gestation in healthy women: association with small for gestational age neonates

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Running title: Placental growth factor and SGA

Abstract

Aims: This study was performed to determine the associations between serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) levels at mid-gestation with the risk of small for gestational age (SGA) neonates born at gestational week (GW) ≥ 36 in healthy women.

Methods: PlGF and sFlt-1 concentrations were determined at GW 24 – 27 in 183 women with births at GW ≥ 36, but without gestational diabetes mellitus and hypertension.

Results: Thirteen (7.1%) SGA neonates were born. Median (range) GW at blood sampling was similar between women with and without SGA (25 [24 – 25] and 24 [24 – 27], respectively, $P = 0.671$). Pre-pregnancy body mass index (BMI) and PlGF levels were significantly lower in women with than without SGA, while sFlt-1 levels and sFlt-1:PlGF ratio (sFlt-1/PlGF) did not differ significantly between the two groups. PlGF and sFlt-1/PlGF, but not BMI or sFlt-1, showed significant correlations with birthweight z-score; the correlation was positive for PlGF and negative for sFlt-1/PlGF. Women with PlGF level < 10^{th} percentile and those with sFlt-1/PlGF level > 90^{th} percentile showed significantly increased risk of SGA compared to those with respective counterpart characteristics; relative risk was 3.8 (95% CI, 1.3 – 11.3; 21% [4/19] vs. 5.5% [9/164]) for PlGF and 7.9 (3.0 – 20.8, 33.3% [6/18] vs. 4.2% [7/165]) for sFlt-1/PlGF.

Conclusions: Maternal PlGF and sFlt-1/PlGF determined during GW 24 – 27 were associated with the risk of SGA born at GW ≥ 36, even in women with uncomplicated pregnancies.

Key words: biomarker, fetal growth restriction, placental growth factor, small for gestational age

Introduction

Small for gestational age (SGA) neonates are born as a result of intrauterine fetal growth restriction (FGR). Even SGA neonates born at term are at increased risk of morbidity and mortality.[1,2] FGR is caused not only by intrinsic problems in the fetus, such as infection and chromosomal aberrations, but also by placental dysfunction, i.e., failure of the placenta to meet the increasing demands of the fetus as pregnancy progresses.[3] Optimal management improves the outcome of SGA infants born to women complicated with placental dysfunction due to hypertensive disorders of pregnancy (HDP) or glucose intolerance, including diabetes mellitus (DM) and gestational DM (GDM). However, some SGA infants are born to mothers without these complications.

Failure of trophoblast invasion leads to altered placental production and systemic release of antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1), and proangiogenic factors, such as placental growth factor (PlGF).[4] Both sFlt-1 and PlGF have been implicated in the pathogenesis of preeclampsia[5, 6] and were suggested to be possible biomarkers for prediction of SGA.[7 – 17] In all except one previous report,[17] low PlGF levels determined during 1st trimester,[7,12] 2nd trimester,[8 – 10,13,15] and 3rd trimester[11,14 – 16] were associated with the birth of SGA infants among women with or without later development of preeclampsia. In addition, sFlt-1:PlGF ratio (sFlt-1/PlGF) determined at different stages of pregnancy are associated with substantially different risks of SGA.[8,12] Thus, most studies acknowledge that PlGF can be used as a biomarker of SGA.

Fetal growth can be affected by complications, such as HDP, DM, GDM, and anti-phospholipid antibody syndrome. In addition, SGA is more likely in women with preterm than term deliveries.[18] To our knowledge, there have been no studies regarding whether the 2nd trimester PlGF and sFlt-1/PlGF can be used to predict the risk of SGA at term among women with neither HDP, DM, GDM, nor anti-phospholipid antibody syndrome. This study was performed to resolve this issue.

Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki and with the approval of the Institutional Review Board of Hokkaido University Hospital.

Participants and assay of PlGF

A total of 250 women with singleton pregnancies in the 2nd trimester of pregnancy participated in this study. However, 67 women with various conditions in the current pregnancy were excluded: 35 with DM/GDM, 23 with diagnoses of preeclampsia/gestational hypertension, five with chronic hypertension, two with fetal malformation, and two with unknown pregnancy outcome. The remaining 183 women that fulfilled all of the following criteria were included in the study: (1) normotensive singleton pregnancy and neither DM, thyroid dysfunction, nor anti-phospholipid

antibody syndrome at the time of blood sampling; (2) no development of GDM, thyroid dysfunction, anti-phospholipid antibody syndrome, gestational hypertension, or preeclampsia in the current pregnancy; and (3) giving birth to a normally formed infant without proven chromosomal aberrations at gestational week (GW) ≥ 36. SGA was diagnosed in neonates with birthweight less than the 10th percentile corrected by maternal parity (nulliparous or multiparous), GW at birth, and gender for Japanese infants.[19] Determination of GW and diagnoses of DM, GDM, and HDP were based on Japanese guidelines for obstetric practice.[20] Both birthweight and placental weight were transformed to z-score using data specific for Japanese infants.[19,21]

Serum samples were prepared according to the standard operating procedure and stored at –20°C until measurement of sFlt-1 and PlGF using commercial ELISA kits (R&D Systems, Minneapolis, MN). Serum samples were diluted in the range of 1:10 – 1:100 for sFlt-1 assay and 1:1 – 1:10 for PlGF assay.

Statistical analyses

Data are presented as the median (range). Statistical analyses were performed using the JMP10© statistical software package (SAS, Cary, NC). The Mann–Whitney U test was used to compare median values between two groups. The Kruskal–Wallis test was used for comparison of medians of three groups. The Spearman's rank-order correlation was used to test associations between two variables. Receiver operating characteristic (ROC) curves were constructed for the biomarkers to assess their ability to differentiate women with later SGA infants. In all analyses, $P < 0.05$ was taken to indicate statistical significance. However, a significant finding regarding a linear correlation between two variables was defined as that meeting both $P < 0.05$ and correlation coefficient (r) > 0.20 or < –0.20.

Results

Thirteen of the 183 neonates (7.1%) were diagnosed as SGA (Table 1). Pre-pregnancy body mass index (BMI) was significantly lower in women with than without SGA (P = 0.043). Neither GW at blood sampling for determination of serum PlGF and sFlt-1 nor GW at delivery differed significantly between the two groups. However, z-scores of placental weight and placental weight were significantly lower in women with than without SGA infants.

The PlGF concentration differed significantly according to GW (Kruskal–Wallis test), while sFlt-1 and sFlt-1/PlGF did not differ significantly according to GW at determination (Fig. 1A). PlGF level was significantly lower in women with than without SGA infants (Fig. 1B, left), although GW at blood sampling for PlGF did not differ between the two groups (Table 1). Neither sFlt-1 level nor sFlt-1:PlGF differed significantly between the two groups (Fig. 1B, middle two panels).

The associations of birthweight z-score with PlGF, sFlt-1, sFlt-1/PlGF, and BMI were analyzed (Fig. 2). The PlGF level was significantly positively correlated with birthweight z-score, while sFlt-1/PlGF was significantly negatively correlated with

birthweight z-score. Neither sFlt-1 nor BMI was significantly correlated with birthweight z-score ($P > 0.5$ for sFlt-1 and $P < 0.5$, but $r = 0.168$ for BMI). These results suggested that certain cut-off values of serum PlGF and sFlt-1/PlGF levels can distinguish between women that will and those that will not later deliver SGA infants. Neither PlGF, sFlt-1, sFlt-1/PlGF, nor BMI was significantly correlated with placental weight z-score ($P < 0.5$, but $r = 0.190$ for PlGF, $P > 0.5$ for sFlt-1, $P < 0.5$, but $r = 0.159$ for sFlt-1/PlGF, $P < 0.5$, but $r = 0.172$ for BMI).

Ability of PlGF and sFlt-1/PlGF to differentiate between women with higher and lower risks of SGA infants

First, we used the 10th or 90th percentile as the cut-off. As PlGF, but not sFlt-1 and sFlt-1/PlGF levels, appeared to increase with advancing gestation (Fig. 1), a different cut-off of 10th percentile specific for each GW was applied for PlGF, but not for sFlt-1 and sFlt-1/PlGF (Fig. 3). Of the 13 SGA infants, four were born to 19 women with PlGF < 10th and nine were born to 164 women with PlGF ≥ 10th percentile (Fig. 3, left). Thus, the relative risks (RR) of SGA were 3.8 (95% CI, 1.3 – 11.3; 21% [4/19] vs. 5.5% [9/164]) for women with PlGF < 10th percentile compared to women with PlGF ≥ 10th percentile. Similarly, the RR of SGA was 1.7 (95% CI, 0.40 – 6.9; 11% [2/18] vs. 6.7% [11/165]) for women with sFlt-1 > 90th percentile, 7.9 (95% CI, 3.0 – 20.8; 33% [6/18] vs. 4.2% [7/165]) for women with sFlt-1/PlGF > 90th percentile, and 1.8 (95% CI, 0.43 – 7.4; 12% [2/17] vs. 6.7% [11/166]) for women with BMI < 10th percentile compared to women with counterpart characteristics. Thus, women with PlGF < 10th percentile and those with sFlt-1/PlGF > 90th percentile at mid-gestation (GW 24 – 27) had a significantly increased risk of SGA.

Second, we used ROC to determine appropriate cut-off values. The area under the curve (AUC) of the ROC curve was greater for PlGF than for sFlt-1/PlGF (0.760 vs. 0.613, respectively) (Fig. 4). The cut-off suggested by the ROC curve gave PlGF (500 pg/mL) a sensitivity of 100% (13/13), specificity of 54% (92/170), positive predictive value of 14% (13/91), and negative predictive value of 100% (92/92). The corresponding values for sFlt-1/PlGF (4.1) were 46% (6/13), 93% (158/170), 33% (6/18), and 96% (158/165), respectively.

Discussion

Although the study population was small, the results of this study indicated that PlGF and sFlt-1/PlGF ratio determined at mid-gestation (GW 24 – 27) were more efficient than pre-pregnancy BMI for prediction of SGA risk among healthy women that later gave birth at GW ≥ 36.

In this study, pre-pregnancy BMI was significantly lower in women with than without SGA neonates, consistent with previous reports that higher pre-pregnancy BMI is associated with greater birthweight.[22 – 24] Unexpectedly, however, the risk of SGA among women with term or near-term delivery in this study did not differ significantly between women with pre-pregnancy BMI < 10th percentile and those with pre-pregnancy BMI ≥ 10th percentile. Women with PlGF < 10th percentile and/or

sFlt-1/PlGF ratio level > 90th percentile had significantly higher risks of giving birth to SGA infants compared to those with respective counterpart characteristics. These observations suggested that PlGF and sFlt-1/PlGF ratio were more closely associated with birthweight of term or near term infants. This was also confirmed by correlation analyses, which indicated that PlGF and sFlt-1/PlGF, but not BMI, were significantly correlated with birthweight z-score (Fig. 2).

This study suggested that even in healthy women not complicated with HDP, DM, or GDM, PlGF was associated with the risk of giving birth to SGA infants at term or near-term. The antiangiogenic factor, sFlt-1, and proangiogenic factor, PlGF, have been implicated in the pathogenesis of preeclampsia,[5] and sFlt-1/PlGF has been accepted as a biomarker of preeclampsia.[6] Women with preeclampsia are likely to suffer from FGR and give birth to preterm SGA neonates.[25] In addition, women carrying fetuses with FGR are at increased risk of spontaneous preterm delivery.[18] In most previous reports,[7 – 12, 14 – 16] it was unclear whether PlGF and sFlt-1/PlGF at mid-gestation predicted SGA neonates not associated with DM, GDM, and HDP women with term or near-term deliveries. Some studies did not exclude women with DM, GDM, and preeclampsia from the study population,[3,7] some studies dealt with PlGF level at the 3rd trimester only,[14,16] and others included considerable numbers of women with preterm births in their study population.[7 – 12,15] In only one study by Lesmes *et al.*,[13] the value of PlGF at mid-gestation in the prediction of SGA at term was investigated in which the PlGF (transformed to multiples of median [MoM]) determined at GW 19 – 24 was significantly lower in women with than without SGA infants born at term among women not complicated with preeclampsia.[13]

In this study, PlGF and sFlt-1 levels were determined at GW 24 – 27. Risk of SGA was 7.9-fold higher in women with sFlt-1/PlGF ≥ 90th percentile than < 90th percentile. However, as serum levels of sFlt-1 and PlGF do not change in parallel during pregnancy,[8] the clinical significance of sFlt-1/PlGF ratio differs greatly markedly to GW at blood sampling. A higher sFlt-1/PlGF ratio in the 1st trimester is associated with lower risk of SGA, while higher sFlt-1/PlGF ratio in the 2nd trimester is associated with higher risk of SGA,[8,12] consistent with the present results. The sFlt-1/PlGF ratio determined at GW 18 – 25 was suggested to be less predictive of SGA.[8,13,17]

To date, no effective treatments to facilitate fetal growth regarding weight have been reported. However, as gestational weight gain (GWG) as well as pre-pregnancy BMI are positively associated with birthweight,[22 – 24] interventions such as counseling on GWG in women with lower BMI at mid-gestation with uncomplicated pregnancies can be considered if women at high risk of giving birth to SGA infants could be detected efficiently during the 2nd trimester. This study suggested that PlGF and or sFlt-1/PlGF determined at GW24 – 27 are candidate biomarkers for this purpose.

The major limitation of this study was the small size of the study population. Therefore, the possibility that selection bias distorted the results to some extent could not be excluded. The PlGF concentration increases with advancing GW in women that do not give birth to SGA neonates.[8] The number of study subjects differed according to GW at enrollment: 91, 76, 14, and two women underwent determination of PlGF and sFlt-1 at

GW 24, 25, 26, and 27, respectively. Therefore, cut-off values of PlGF and sFlt-1/PlGF levels suggested by ROC curve analyses in this study did not represent those for the general population.

In this study performed in 183 healthy Japanese women, including 13 that gave birth to SGA neonates, the level of maternal serum PlGF and sFlt-1/PlGF ratio determined at GW 24 – 27 were significantly positively and negatively correlated with birthweight z-score of neonates born at GW ≥ 36, respectively, and could differentiate between women with lower and higher risks of having SGA neonates. Women with PlGF < 10^{th} percentile (specific for each GW) had RR of SGA = 3.8 (1.3 – 11.3) compared to those with PlGF ≥ 10^{th} percentile, and women with sFlt-1/PlGF ratio > 90^{th} percentile had RR of SGA = 7.9 (3.0 – 20.8) compared to those with sFlt-1/PlGF ratio ≤ 90^{th} percentile.

Acknowledgments

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Disclosure

The authors declare no conflicts of interest.

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Figure legends

Figure 1 Serum PlGF, sFlt-1, and sFlt-1/PlGF according to gestational week (A) and in women with and without SGA (B)

The numbers of women tested are indicated in parentheses. Box-and-whisker plots indicate outliers, 25^{th}, 75^{th}, and median. A: Median values at GW 24, 25, and 26 – 27 were as follows: PlGF, 454, 579, and 672 pg/mL, respectively; sFlt-1, 741, 765, and 848 pg/mL, respectively; and sFlt-1/PlGF, 1.76, 1.40, and 1.40, respectively. B: Median values of women with vs. without SGA infants were 365 vs. 535 pg/mL, respectively,

for PlGF, 696 vs. 766 pg/mL, respectively, for sFlt-1, 1.82 vs. 1.56, respectively, for sFlt-1/PlGF, and 18.9 vs. 20.5 kg/m^2 (Table 1), respectively, for pre-pregnancy BMI.

Figure 2 Correlations of birthweight z-score with PlGF, sFlt-1, sFlt-1/PlGF, and pre-pregnancy BMI

The birthweight z-score was significantly positively and negatively correlated with PlGF and sFlt-1/PlGF, respectively. Between birthweight z-score and BMI, $P < 0.5$, but the correlation was considered not significant based on the correlation coefficient (r) value of 0.168.

Figure 3 Possibility of PlGF, sFlt-1, sFlt-1/PlGF, and pre-pregnancy BMI at mid-gestation for differentiation of women with higher and lower risks of SGA

The horizontal lines with numerals in the figure indicate the cut-off values of 10th or 90th percentile. Of the 13 SGA infants, 4 (31%), 2 (15%), 6 (46%), and 2 (15%) were born to women with PlGF < 10th percentile, sFlt-1 > 90th percentile, sFlt-1/PlGF > 90th percentile, and BMI < 10th percentile, respectively.

Figure 4 Receiver operating characteristic curves for PlGF and sFlt-1/PlGF determined at GW 24 – 27 for differentiation of women with higher and lower risks of SGA

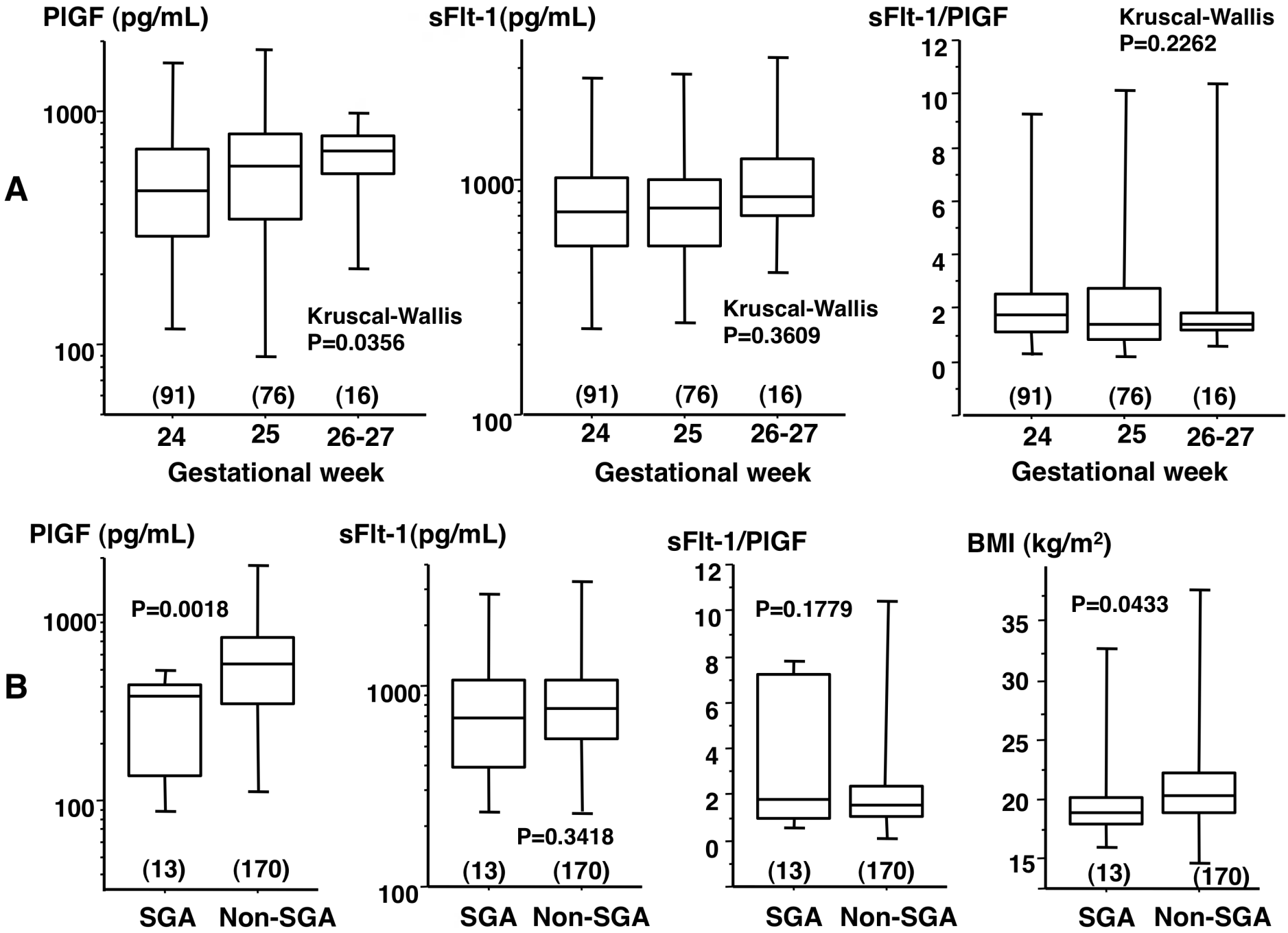
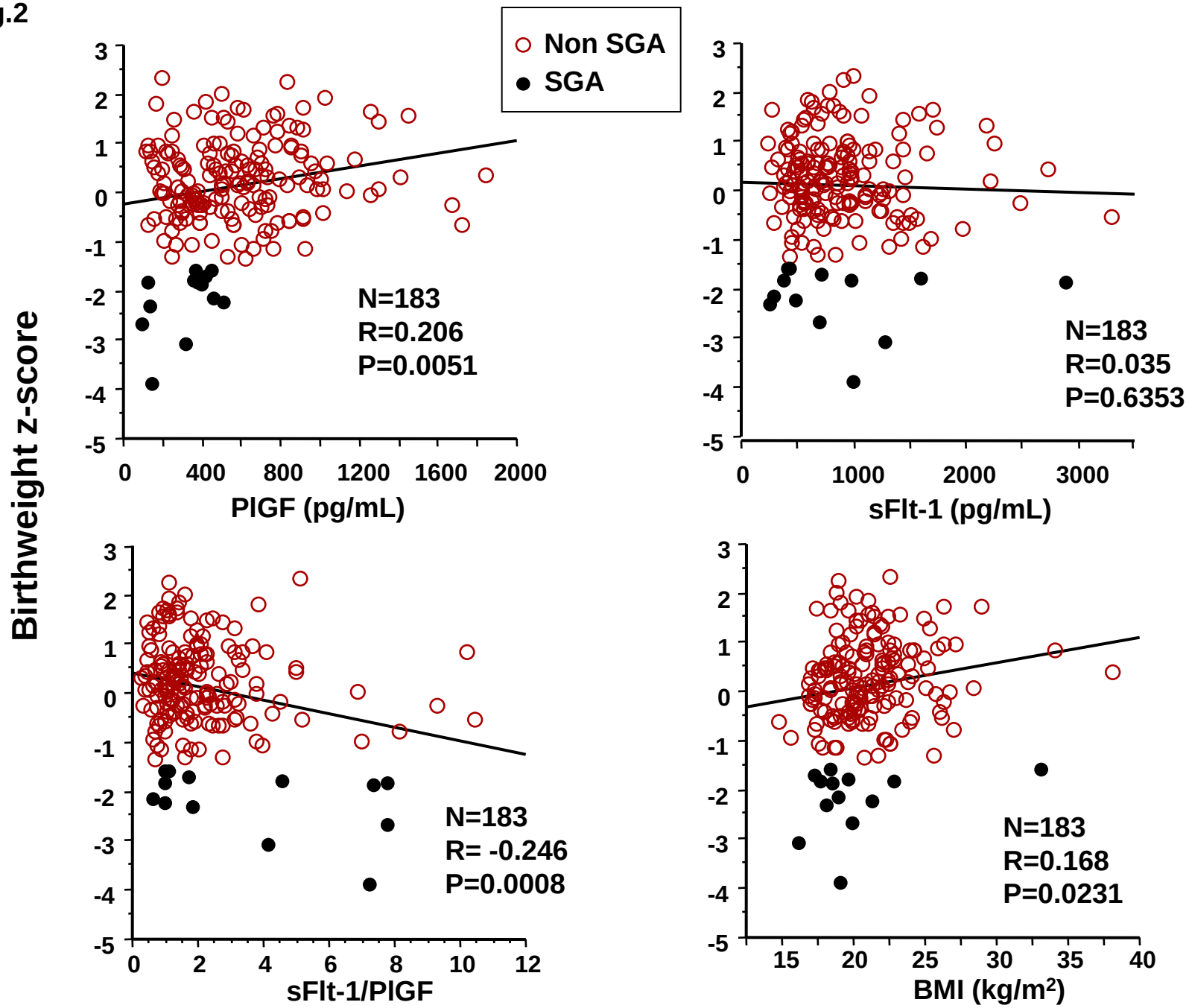

A
PlGF (pg/mL)
1000
100
Kruscal-Wallis
P=0.0356
(91)
(76)
(16)
24
25
26-27
Gestational week
sFlt-1(pg/mL)
1000
100
Kruscal-Wallis
P=0.3609
(91)
(76)
(16)
24
25
26-27
Gestational week
sFlt-1/PlGF
Kruscal-Wallis
P=0.2262
12
10
8
6
4
2
0
(91)
(76)
(16)
24
25
26-27
Gestational week
B
PlGF (pg/mL)
1000
100
P=0.0018
(13)
(170)
SGA
Non-SGA
sFlt-1(pg/mL)
1000
100
P=0.3418
(13)
(170)
SGA
Non-SGA
sFlt-1/PlGF
12
10
8
6
4
2
0
P=0.1779
(13)
(170)
SGA
Non-SGA
BMI (kg/m2)
35
30
25
20
15
P=0.0433
(13)
(170)
SGA
Non-SGA

Fig.2


Non SGA
SGA
Birthweight z-score
N=183
R=0.206
P=0.0051
PlGF (pg/mL)
N=183
R=0.035
P=0.6353
sFlt-1 (pg/mL)
N=183
R= -0.246
P=0.0008
sFlt-1/PlGF
N=183
R=0.168
P=0.0231
BMI (kg/m2)

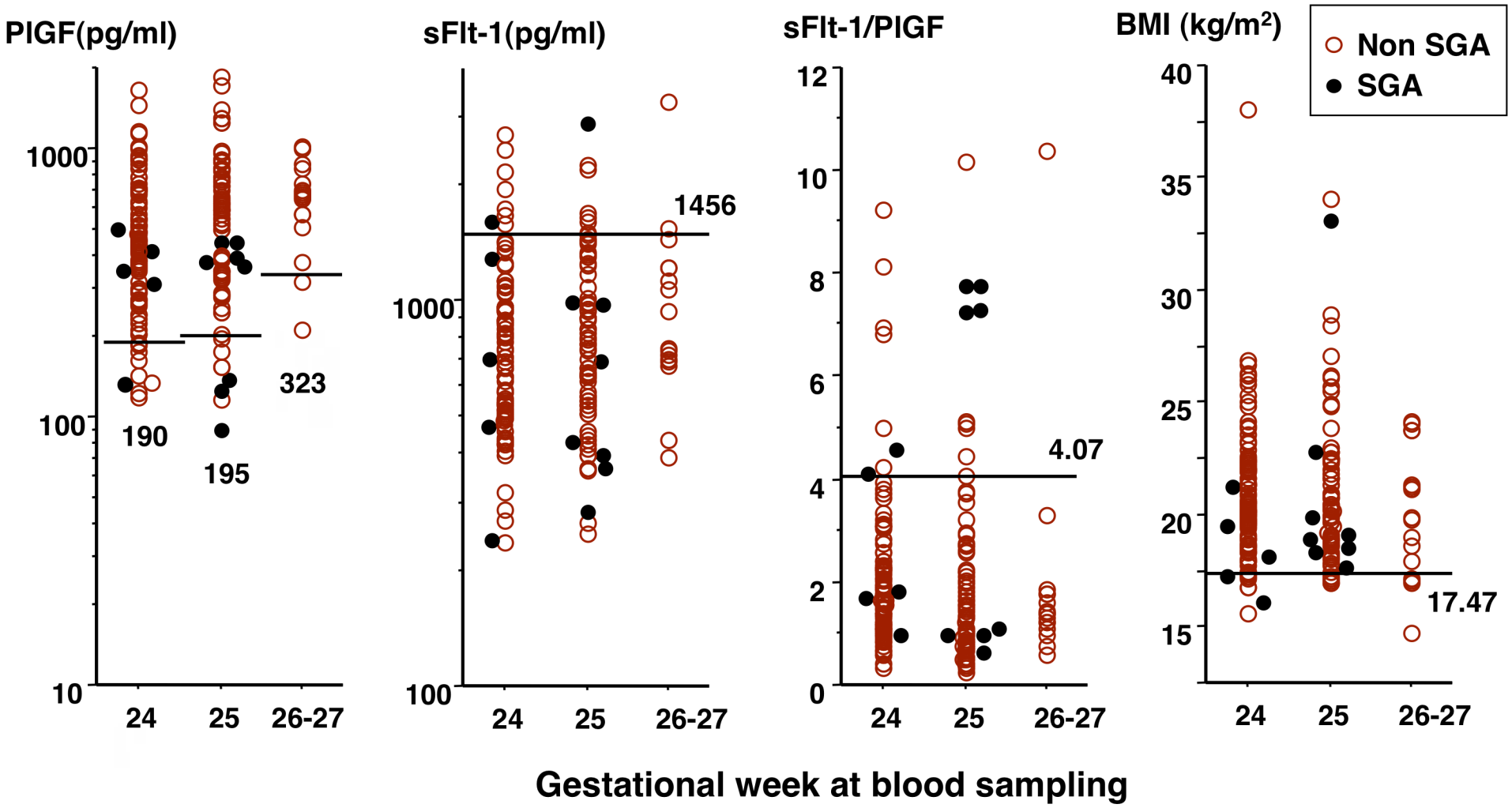
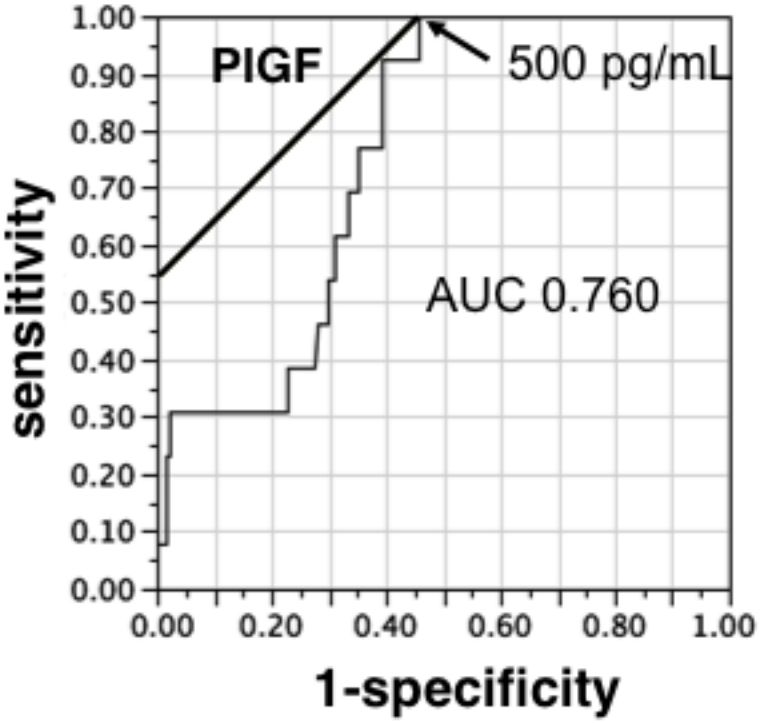

PlGF(pg/ml)
1000
100
10
190
195
323
24
25
26-27
sFlt-1(pg/ml)
1000
100
1456
24
25
26-27
sFlt-1/PlGF
12
10
8
6
4
2
0
4.07
24
25
26-27
BMI (kg/m²)
40
35
30
25
20
15
17.47
24
25
26-27
Non SGA
SGA
Gestational week at blood sampling

Fig.4


PlGF
500 pg/mL
AUC 0.760
sensitivity
1-specificity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.20
0.40
0.60
0.80
1.00

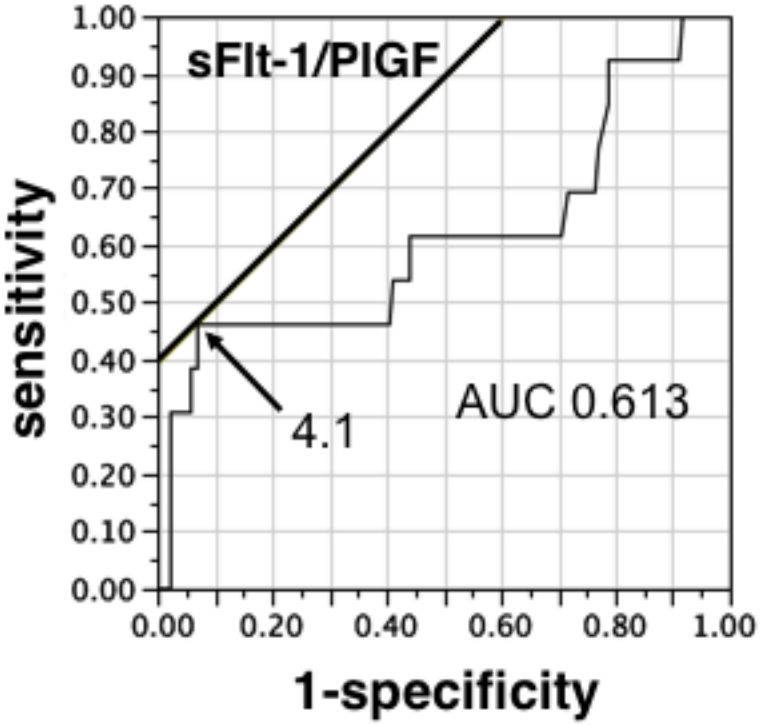

sFlt-1/PlGF
4.1
AUC 0.613
sensitivity
1-specificity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.20
0.40
0.60
0.80
1.00

Table 1. Demographic characteristics of 183 women

	Small for gestational age (SGA) neonates	
	Yes (n=13)	No (n=170)
Maternal age (years)	31 (25 – 39)	34 (20 - 46)
≥ 35	2 (15%)	74 (44%)
≥ 40	0 (0.0%)	18 (11%)
Nulliparous	7 (53.8%)	94 (55.3%)
Height (m)	1.58 (1.48 – 1.68)	1.59 (1.38 – 1.74)
Pre-pregnancy weight (kg)	47.0 (40.1 – 77.5)	52.0 (37.6 – 100)
Pre-pregnancy BMI (kg/m^2)	18.9 (16.1 – 33.1)	20.5 (14.7 – 38.1)*
≥ 25	1 (7.7%)	18 (10.6%)
GW at blood sampling	25 (24 – 25)	24 (24 – 27)
24	5 (38%)	86 (51%)
25	8 (62%)	68 (40%)
26	0 (0.0%)	14 (8.2%)
27	0 (0.0%)	2 (1.2%)
GW at delivery	38 (37 – 41)	38 (36 – 41)
36	0 (0%)	6 (3.5%)
37 - 38	8 (62%)	89 (52%)
39 - 40	3 (23%)	64 (38%)
41	2 (15%)	11 (6.5%)
Abnormal cord insertion†	0 (0.0%)	13 (7.6%)
Placental weight (kg)	0.42 (0.27 – 0.66)	0.57 (0.39 – 0.90) *
Placental weight z-score	–1.367 (–3.186 – 0.936)	0.137 (–2.0 – 2.669) *
Infant birthweight (kg)	2.22 (1.48 – 2.86)	2.97 (2.48 – 4.02)*
Birthweight z-score	–1.848 (–3.885 – –1.547)	0.22 (–1.31 – 2.346) *

Data are presented as the median (range). *, $P < 0.05$ vs. SGA group;†, including velamentous cord insertion and marginal insertion of the umbilical cord; BMI, body mass Index; GW, gestational week;