sFlt-1 to PlGF ratio in different stages of early-onset fetal growth restriction and small for gestational age

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ABSTRACT

Introduction

Increased soluble fms-like tyrosine kinase to placental growth factor ratio (sFlt-1/PlGF) has been demonstrated in early-onset fetal growth restriction (FGR) and small for gestational age (SGA). sFlt-1/PlGF cutoffs have been described to assess preeclampsia (PE) severity; however, sFlt-1/PlGF values present in early-onset SGA and different FGR severity stages remain unknown. Thus, the objective of this study was to describe and compare the sFlt-1/PlGF values and pregnancy outcomes among early-onset SGA/FGR stages.

Material and methods

This is a prospective case-control study conducted at Vall d'Hebron University Hospital. Singleton pregnancies with estimated fetal weight (EFW) below the 10th centile and a control group of uncomplicated pregnancies between 20+0 and 31+6 weeks of gestation were enrolled. Study women were classified at diagnosis into different stages, according to EFW centile and Doppler ultrasound. sFlt-1/PlGF serum concentrations were measured at diagnosis and together with pregnancy outcomes, compared among FGR severity stages, SGA and controls. Finally, correlations between sFlt-1/PlGF values and time to delivery, gestational age at delivery, days of neonatal admission and birthweight z-scores were investigated.

Results

Among the 207 women enrolled, 32 (15.4%) were uncomplicated, 49 (23.7%) SGA, and 126 (60.9%) FGR (92 being stage I, 17 stage II and 17 stage III). SGA and controls had similar median sFlt-1/PlGF values (25.7 vs 27.1, P>0.05) and pregnancy outcomes. However, all FGR stages had significantly poorer outcomes and greater concentrations of sFlt-1/PlGF than those of SGA and controls. Furthermore, median values differed significantly among all FGR severity stages (9.76 for stage I; 284.3 for stage II and 625.02 for stage III, P<0.05) increasing with FGR severity as well as the frequency of adverse pregnancy outcomes. Additionally, a significant correlation was found between greater sFlt-1/PlGF ratio values and GA at delivery, time from diagnosis to delivery, birthweight z-scores and time in neonatal intensive care unit (r= -0.637, r= -0.576, r= -0.161 and r=0.311, respectively).

Conclusions

sFlt-1/PlGF values at diagnosis permit early-onset FGR/SGA severity classification with good correlation with Doppler ultrasound findings and the occurrence of adverse outcomes. Thus, sFlt-1/PlGF could aid in early-onset FGR/SGA severity classification and clinical management when Doppler assessment is not feasible.

Keywords: adverse pregnancy outcomes, angiogenic factors, FGR, PlGF, sFlt-1, SGA

Abbreviations: FGR, fetal growth restriction; SGA, small for gestational age; sFlt-1, fms-like tyrosine kinase-1; PlGF, placental growth factor; GA, gestational age; PE, preeclampsia; EFW, estimated fetal weight; CTG, cardiotocography; UtA, uterine artery; PI, pulsatility index; UA, umbilical artery; MCA, middle cerebral artery; DV, ductus venosus; CPR, cerebroplacental ratio; IQR, interquartile range; N-ICU, neonatal intensive care unit.

Key message: sFlt-1/PlGF ratio at the time of FGR diagnosis allows FGR/SGA classification with good correlation with Doppler severity. Angiogenic factors could be useful in clinical practice to classify FGR severity when Doppler assessment is not feasible.

INTRODUCTION

Small-for-gestational-age (SGA) fetuses and fetal growth restriction (FGR) affect around 10% of pregnancies.[1] SGA are considered constitutionally small fetuses and are commonly defined as estimated fetal weight (EFW) between the 3rd and the 10th centiles with no abnormalities in fetal and/or uterine Doppler assessment, whereas FGR is described as EFW below the 3rd centile or EFW below the 10thcentile with fetoplacental Doppler abnormalities.[2] Additionally, FGR and SGA fetuses can also be divided in two groups: early- and late-onset, depending on the gestational age (GA) at which diagnosis is made (before or after 32 weeks of gestation).[3,4] SGA and FGR, and specially early-onset FGR, are at increased risk of adverse pregnancy outcomes and are known as major causes of morbidity and mortality.[5] Fetoplacental Doppler assessment has been widely studied and has proved to predict adverse pregnancy outcomes;[6] thereby, different severity stages have been described and are frequently used to tailor follow-up.[2,4] However, accurate Doppler measurements require intensive training and may be influenced by mild-to-moderate intra- and inter-observer variability.[7–9] Additionally, approaches to surveillance and timing for delivery can vary considerably between countries.[10] On the other hand, maternal serum concentrations of angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), can be precisely measured in automated assay platforms.[11] These placental biomarkers have been consistently related to poorer outcomes in women with placental dysfunction such as preeclampsia (PE) and/or FGR. For this reason, different cutoff values (<38, 38-84 and ≥85) have been established in PE for their implementation in clinical practice since they have proved to be associated to disease severity and prognosis.[12–15] Increased sFlt-1/PlGF ratio in early-onset FGR has also recently shown to be related to more severe forms of the disease and also to shorter time to delivery and other obstetric complications, such as placental abruption and fetal demise.[14,16,17]Although adverse pregnancy outcomes in early-onset FGR due to placental ischemia can be studied and predicted either by fetoplacental Doppler or sFlt-1/PlGF ratio, no previous studies have analyzed if an association exists between the degree of fetal circulation abnormalities and the maternal serum concentrations of angiogenic factors. Thus, the objective of this study was to describe and compare sFlt-1/PlGF ratio values and pregnancy outcomes among the different severity stages of early-onset SGA/FGR.

MATERIALS AND METHODS

Study design and participants

This was a prospective case-control study conducted at Vall d'Hebron University Hospital, Barcelona (Spain), between July 2017 and July 2019. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was followed for writing this report. The study population were women referred for fetal growth assessment and EFW below the 10th centile between 20+0 and 31+6 weeks of gestation. Exclusion criteria were: stillbirth, known fetal chromosomal abnormalities and/or congenital defects and need for immediate delivery (ductus venosus "a"-wave reversal, non-reassuring cardiotocography (CTG) or placental abruption). After all study women were recruited, a group of consecutive pregnancies with EFW>10th centile and not complicated with PE at diagnosis served as the control group, in whom the following baseline characteristics were matched to the study women: maternal age, prepregnancy BMI, smoking status, obstetric history, maternal history, ethnicity, mode of conception and GA at enrolment. Matching was verified after each control participant was recruited. Since controls were only included as a means of setting in context the sFlt-/PlGF values in FGR and SGA groups, the minimum number of cases were enrolled.

GA was determined by the measurement of fetal crown-rump length at 11+0-13+6 weeks of gestation.[18] After EFW below the 10th centile was confirmed by customized birthweight standards for a Spanish population,[19,20] fetoplacental circulation was assessed by Doppler ultrasound consisting of the measurement of mean uterine artery (UtA) pulsatility index (PI), umbilical artery (UA) PI, middle cerebral artery (MCA) PI and *ductus venosus* (DV) PI and diastolic flow ("a" wave present, absent or reversed). Above 28 weeks of gestation, fetal wellbeing was also assessed by conventional CTG. Additionally, maternal serum concentrations of PlGF and sFlt-1 in pg/ml were analyzed at the time of diagnosis by means of the fully automated Elecsys assays for sFlt-1 and PlGF on an electrochemiluminescenseimmunoassay platform (cobas e analyzers; Roche Diagnostics, Penzberg, Germany). sFlt-1/PlGF ratio values were calculated and classified in three categories (<38, 38-84 and ≥85) in order to simplify its analysis. These cutoffs were selected since they are commonly used in PE.[15] Additionally,

optimal cutoffs to distinguish between FGR stages were assessed and their sensitivity, specificity, positive predictive value and negative predictive value were calculated.

SGA was defined as EFW between the 3rd and 10th centiles with normal Doppler assessment. FGR was defined as EFW below the 3rd centile or between the 3rd and 10th centiles with fetomaternal Doppler abnormality.[2,21] FGR was classified into four stages according to the Doppler findings: stage I, UA PI>95th centile, cerebroplacental ratio (CPR)<5th centile, MCA PI<5th centile and/or UtA PI >95th centile; stage II, UA absent end-diastolic flow; stage III, reversed end-diastolic flow and/or DV PI>95th centile; and stage IV, DV reversed a-wave.[1]

All cases were assessed and followed up until delivery at a specific Placental Insufficiency Unit by the same group of experienced obstetricians, accredited by the Fetal Medicine Foundation for Doppler assessment.

Outcome measures

Adverse pregnancy outcomes were compared between groups. Adverse perinatal outcomes were defined as any adverse fetal outcome (stillbirth and Cesarean for non-reassuring CTG) or any adverse neonatal outcome (neonatal death, respiratory distress syndrome, bronchopulmonary dysplasia, neonatal sepsis, retinopathy of prematurity stage III-IV, necrotizing enterocolitis, intraventricular hemorrhage grade III-IV, periventricular leukomalacia, 5-min Apgar score <5 or umbilical artery cord pH <7). Adverse maternal outcome was defined as any of the following: placental abruption, PE, eclampsia or admission to the obstetric intensive care unit for ≥48h. PE was defined as new onset of high blood pressure (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) or worsening of previous high blood pressure added to new-onset proteinuria (≥ 300mg per 24-hour urine collection or protein/creatinine ≥ 0.3 or dipstick reading of 1+) or worsening of previous proteinuria or to at least one of the following signs and symptoms: cerebral or visual symptoms, raised liver enzymes, low platelet count, renal insufficiency or pulmonary edema. Women with PE and any of these signs and symptoms were classified as PE with severity features.[22] Birthweight z-scores were calculated according to local birthweight charts.[23]

Indications for elective delivery

All cases were assessed by the same group of specialist who were not blinded to angiogenic factor levels since they are routinely measured in clinical practice at the time of early-onset FGR/SGA diagnosis to rule out PE.[13] Nevertheless, the mode and timing of delivery was made in all the cases according to GA, Doppler and CTG findings and maternal signs and symptoms following the current hospital protocols and irrespectively of the results of the sFlt-1/PlGF ratio.[1] Elective delivery was recommended at >37 weeks in stage I, >34 weeks in stage II, >30 weeks in stage III and >26 weeks in stage IV.[2] CTG indications for elective delivery were: fetal heart rate sinusoidal tracing or absent fetal heart rate variability accompanied by recurrent late decelerations, recurrent variable decelerations or bradycardia. Immediate delivery was also indicated in severe PE at 34+0 weeks or later, whereas in non-severe forms of PE expectant management was recommended until 37+0 weeks.[24]

Statistical analysis

Categorial data are presented as frequency and percentage and continuous data as median and interquartile range (IQR). Comparisons between groups were estimated by chi-squared or Fisher's tests, as appropriate. Continuous variables were described as median and IQR and Mann-Whitney U test was used to assess differences between groups. Correlations between quantitative variables were assessed by Pearson's correlation coefficient. Receiver–operating characteristic (ROC) curves were constructed and the resulting areas under the curve (AUC) were used to assess the optimal sFlt-1/PlGF cutoff to distinguish between FGR severity stages. The statistical software R Commander, R package version 2.3–1 was used for all data analyses. Statistical significance level was set at $P<0.05$.All P values are two-tailed and Bonferroni correction method was used in multiple comparisons.

Ethical approval

Approval for this study (PR(AMI)349/2016) was provided by the Ethics Committee CEIC Vall d'Hebron Research Institute, Barcelona, Spain. All women provided their written informed consent.

RESULTS

Study population/baseline characteristics

A total of 207 women were included in the study: 32 women in the control group, 49 singleton pregnancies diagnosed with SGA, 92 women diagnosed with FGR stage I, 17 with FGR stage II, and 17 pregnancies with FGR stage III. A recruitment flowchart can be seen in Figure 1. Demographic and clinical characteristics of the studied population are summarized in Table 1 with no significant differences observed among them.

sFlt-1/PlGF ratio values in different groups

Controls and SGA showed similar sFlt-1/PlGF values with no statistically significant differences between them (4.79 vs. 4.14, respectively). Nevertheless, all forms of FGR had significantly greater values of sflt-1/PlGF compared to both SGA and controls. In the cases with FGR, those with more severe forms showed higher sFlt-1/PlGF values, these differences being statistically significant among all FGR stages (stage I, 9.76; stage II, 284.30; stage III, 625.02).

The sFlt-1/PlGF cutoff categories were then compared among participants. Few cases showed ratio values between 38 and 84; thus, no statistically significant differences were found between groups. By contrast, 93.8% of women in the control group and 98.0% of SGA showed a sFlt-1/PlGF ratio <38, these differences not being statistically significant. The majority (72.9%) of the stage I FGR showed also a sFlt-1/PlGF<38; however, the frequency was significantly lower than that of SGA. Only one case had a sFlt-1/PlGF ratio <38 in stages II and III with a ratio ≥85 being the most frequent category present in these cases. No cases with a ratio ≥85 were present neither in the control group nor in SGA; being only found in FGR (stage I, 21.7%; stage II, 88.2%; stage III, 100%). The proportions of women with a ratio ≥85 differed significantly among all groups except between stage II and III FGR. More details on ratio values and categories can be seen in Table 1 and Figure 2.

Optimal cutoffs and their respective AUC to distinguish between FGR severity stages where obtained from the ROC curves. Between stage I and stage II, the optimal sFlt-1/PlGF was 97.46 (AUC: 0.852, 95% CI: 0.772 - 0.932) and between stage II and III, the optimal sFlt-1/PlGF was 523.77 (AUC: 0.852, 95% CI: 0.772 - 0.932). The Sn, Sp, PPV and NPV of these cutoffs can be seen in Table 2.

Pregnancy outcomes

Birthweight in the control group was significantly greater than that of all the other groups; however, no differences were observed between controls and SGA regarding GA at delivery, days from diagnosis to delivery, adverse maternal outcome or composite adverse perinatal outcome. By contrast, lower GA at delivery, shorter time from diagnosis to delivery and poorer composite perinatal outcomes were found in all FGR stages compared to the control group and SGA. Additionally, stages II and III showed similar pregnancy outcomes as being overall significantly worse than those of the other groups. More details on pregnancy outcomes can be seen in Table 3.

Correlations between the sFlt-1/PlGF values and quantitative pregnancy outcomes were analyzed (Figure 3). A significant correlation was found with GA at delivery (r= -0.637 (-0.713 to -0.546), *P*<0.001), time from diagnosis to delivery (r= -0.576 (-0.662 to -0.475), *P*<0.001), birthweight z-score (r= -0.161 (-0.297 to -0.020), *P*=0.026) and days in the neonatal intensive care unit (N-ICU) (r=0.311 (0.095 to 0.499), *P*=0.006).

DISCUSSION

This study provides evidence that early-onset FGR severity stages assessed by Doppler can be identified by the angiogenic profile at the time of diagnosis since they have significantly different sFlt-1/PlGF values, being greater in the more severe forms. We provide sFlt-1/PlGF cutoffs, which distinguish the different FGR severity stages with good sensitivity, specificity, positive predictive value and negative predictive value. We also show that more than 93% of SGA and uncomplicated pregnancies have sFlt-1/PlGF values <38, while in FGR stage II-III it is ≥85 in more than 88% of the cases. Furthermore, a significant correlation was found between higher sFlt-1/PlGF values and a lower GA at delivery, a shorter time from diagnosis to delivery, a lower birthweight z-scores and a longer stay in N-ICU. No differences were observed between controls and SGA regarding sFlt-1/PlGF or pregnancy outcomes.

Overall, SGA are more frequent than FGR; however, in this study 72.0% of the cases were FGR while only 28.0% were SGA. This may be explained by the fact that FGR are more likely to be identified earlier by ultrasound due to a lower fundal height or the presence of maternal risk factors for FGR.

Increased values of sFlt-1/PlGF are a consequence of placental insufficiency; which can be detected several weeks before the onset of clinical disease.[11] Greater values of sFlt-1/PlGF are not only present in PE, they have also been found in FGR (with and without preeclampsia), preterm labor, stillbirth and placental abruption, among others.[12,14,17,25–27] Previous studies have shown that 63.3 % of FGR cases with severe Doppler abnormalities (Stage II and III) are highly likely to later become preeclamptic.[28] Since high sFlt-1/PlGF values and abnormal fetal Doppler are both a consequence of the underlying placental disfunction, it was awaited that the most severe stages of FGR had more severe Doppler abnormalities as well as higher sFlt-1/PlGF, which may explain why they are at increased risk of preeclampsia.

Several studies have investigated the role of angiogenic factors in the prognosis of pregnancies complicated by FGR. A previous study has analyzed the capacity of a single compared to multiple determinations of angiogenic factors to anticipate the need of elective delivery or the occurrence of adverse pregnancy outcomes in early-onset FGR. It showed that 73% and 75% of cases complicated by early-onset FGR without PE exceeded the cutoff point of 38 and 85 respectively, four weeks before delivery; nevertheless, multiple measurements showed no improvement in their predictive capacity.[29] A larger study showed that sFlt-1/PlGF<85 at diagnosis identifies pregnancies with early-onset FGR stage I at a lower risk of need to deliver.[14] Moreover, a secondary analysis of the STRIDER trial proved that the sFlt-1/PlGF ratio and EFW were independent predictors of poor pregnancy outcomes.[12]Additionally, Doppler ultrasound has shown to predict short-term adverse pregnancy outcomes and the need for elective delivery.[2,4,6] However, no previous studies have analyzed if an association exists between the Doppler FGR stages and sFlt-1/PlGF ratio values or the correlation between angiogenic factors and specific pregnancy outcomes such as: birthweight z-scores, time to delivery, days at N-ICU and GA at delivery.

Doppler is often used in clinical practice for fetal surveillance and for planning elective delivery; however, it requires longitudinal follow-up and intensive training; thus, even in skilled examiners, it has mild-to-moderate inter- and intra-observer variability. By contrast, angiogenic factors are objective markers with low variability[30] and good correlation with adverse pregnancy outcomes and severity of the disease. In addition, we have shown that the cutoffs of 97.47 and 523.77 are highly accurate to identify the current FGR severity, shedding some light on the relationship between angiogenic

factors and placental insufficiency that underlies in early-onset FGR and SGA. Thus, angiogenic factors could aid in FGR classification when Doppler assessment or skilled examiners are not available. For these reasons, we believe that they will probably be incorporated into FGR management guidelines in the future; however, further research is still needed to better understand how sFlt-1/PlGF could be implemented in clinical practice.

The strengths of this study are the prospective design in a relatively large cohort of early-onset FGR and being the first study to demonstrate a correlation between sFlt-1/PlGF and FGR severity stages. Thus, angiogenic factors could aid in fetal surveillance since they can distinguish different profiles of FGR that correlate well with the severity of the disease. Therefore, the same cutoff would not be appropriate for all FGR stages. Our results could be considered for designing future studies focused on the incorporation of sFlt-1/PlGF in the management of each early-onset FGR stage.

Among the limitations, we acknowledge that a greater amount of participants, especially of the two more severe forms of FGR, might have allowed to identify some differences that could not be detected due to insufficient sample size. Furthermore, the angiogenic values were not blinded to practitioners since they are routinely measured in FGR to rule out PE and this could have affected clinical decisions although management was intended to be based on current protocols. Another important aspect is that our results could only be valid with the FGR classification used in our study and our findings might not be valid for other FGR severity classifications.[1] And finally, pregnancies with congenital defects, chromosomal anomalies or genetic syndromes were excluded from this study; thus, our results might not be applicable to these conditions.

CONCLUSION

In conclusion, sFlt-1/PlGF ratio values at the time of diagnosis permit early-onset FGR severity classification with good correlation with Doppler ultrasound findings. Thus, angiogenic factors could be useful in clinical management and aid severity classification in settings where Doppler assessment is not feasible. Since FGR stages have shown to have different angiogenic profiles, further research is needed to establish appropriate cutoffs for each FGR stage to permit their most likely incorporation to clinical management of early-onset FGR.

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Tweetable abstract

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Table 1: Baseline characteristics

	Uncomplicated (n=32)	SGA (n=49)	Stage I (n=92)	Stage II (n=17)	Stage III (n=17)
Maternal age (years)	33.0 (32.0-36.3)	33.0 (30.0-35.0)	31.5 (27.0-36.0)	37.0 (31.0-39.0)	37.0 (32.0-39.0)
Prepregnancy BMI (kg/m2)	25.6 (23.3-29.8)	22.7 (20.5-25.2)	24.7 (22.1-28.6)	25.3 (22.4-26.9)	25.0 (22.6-26.3)
Smoking	5 (15.6%)	5 (10.2%)	15 (16.3%)	3 (17.6%)	5 (29.4%)
Obstetric history					
FGR previous pregnancy	2 (6.2%)	9 (18.4%)	8 (8.7%)	2 (11.8%)	2 (11.8%)
SGA previous pregnancy	0	1 (2.0%)	3 (3.3%)	0	0
First pregnancy	12 (37.5%)	19 (38.8%)	55 (59.8%)	14 (82.4%)	11 (64.7%)
Previous preeclampsia	4 (12.5%)	4 (8.2%)	2 (2.2%)	0	1 (5.9%)
Maternal history					
Chronic hypertension	3 (9.4%)	1 (2.0%)	0	2 (11.8%)	1 (5.9%)
Pre-pregnancy diabetes	4 (12.5%)	2 (4.1%)	1 (1.1%)	0	0
Chronic kidney disease	0	1 (2.0%)	0	0	1 (5.9%)
SLE	0	0	1 (1.1%)	0	0
Antiphospholipid syndrome	2 (6.2%)	0	4 (4.3%)	1 (5.9%)	0
Ethnicity					
White	26 (81.3%)	41 (83.7%)	79 (85.9%)	13 (76.5%)	14 (82.4%)
Black	2 (6.2%)	4 (8.2%)	3 (3.3%)	2 (11.8%)	1 (5.9%)
Asian	2 (6.2%)	1 (2.0%)	1 (1.1%)	0	0
Southeast Asia	2 (6.2%)	3 (6.1%)	9 (9.8%)	2 (11.8%)	1 (5.9%)
Other	0	0	0	0	1 (5.9%)
Mode of conception					
Spontaneous	29 (90.6%)	48 (98.0%)	87 (94.6%)	13 (76.5%)	16 (94.1%)
IVF	3 (9.4%)	1 (2.0%)	4 (4.3%)	4 (23.5%)	1 (5.9%)
Insemination	0	0	1 (1.1%)	0	0
Gestational age (weeks) at diagnosis	25.7 (23.2-27.5)	27.1 (24.2-29.7)	25.8 (23.6-29.1)	26.0 (25.0-29.1)	25.0 (24.0-26.4)
Preeclampsia at diagnosis	0[e]	0[e]	7 (7.6%)	3 (17.6%)	5 (29.4%)[ab]
Ratio sFlt-1 to PlGF	4.79 (3.71-7.34)[cde]	4.14 (2.12-7.42)[cde]	9.76 (3.42-45.71)[abde]	284.30 (119.11-550.40)[abce]	625.02 (508.83-989.99)[abcd]
Ratio sFlt-1 to PlGF categories					
<38	30 (93.8%)[de]	48 (98.0%)[cde]	67 (72.9%)[bde]	1 (5.9%)[abc]	0[abc]
38-84	2 (6.2%)	1 (2.0%)	5 (5.4%)	1 (5.9%)	0
≥85	0[bcde]	0[acde]	20 (21.7%)[abde]	15 (88.2%)[abc]	17 (100.0%)[abc]
Mean uterine artery Doppler >95th centile	4 (12.5%)[cde]	0[cde]	64 (69.6%)[abc]	13 (76.5%)[ab]	17 (100.0%)[abc]

Continuous data are given as median and interquartile range. Categorical data as frequency and percentage.

BMI, body mass index; FGR, fetal growth restriction, IVF, in vitro fertilization; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine-kinase-1; SGA, small for gestational age; SLE, systemic lupus erythematosus.

a, $P<0.05$ compared to controls; b, $P<0.05$ compared to SGA; c, $P<0.05$ compared to FGR I; d, $P<0.05$ compared to FGR II; e, $P<0.05$ compared to FGR III.

Table 2. Sensitivity, specificity, positive predictive value, negative predictive value of different sFlt-1/PlGF cutoffs to identify SGA and FGR stages

	sFlt-1/PlGF	Sn (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
FGR Stage I	<97.46	78.3% (68.4-86.2)	97.1% (84.7-99.9)	98.6% (92.6-100)	62.3% (47.9-75.2)
FGR Stage II	97.47-523.77	70.6% (44.0-89.7)	85.3% (77.3-91.4)	42.9% (24.5-62.8)	94.9% (88.5-98.3)
FGR Stage III	≥523.78	70.6% (44.0-89.7)	87.2% (79.4-92.8)	46.2% (26.6-66.6)	95.0% (77.5-90.7)

CI, confidence interval; FGR, fetal growth restriction; NPV, negative predictive value; PlGF, placental growth factor; PPV, positive predictive value; sFlt-1, soluble fms-like tyrosine kinase-1; SGA, small for gestational age; Sn, sensitivity; Sp, specificity.

Table 3: Pregnancy outcomes

	Uncomplicated (n=32)	SGA (n=49)	Stage I (n=92)	Stage II (n=17)	Stage III (n=17)
Neonatal weight (grams)	3200 (2957.5-3397.5)[bcde]	2590 (2220-2937.5)[acde]	2050 (1450-2492.5)[abde]	960 (787.5-1018.8)[abce]	500 (400-730)[abcd]
Birthweight z-score	-0.08 (-0.41 to 0.63)bcde	-1.35 (-1.72 to -0.74)acde	-1.80 (-2.37 to -1.20)abde	-2.75 (-.31 to -1.70)abc	-2.94 (-4.10 to -2.32)abc
GA at delivery (weeks)	38.9 (37.5-40.0)[cde]	38.0 (37.1-39.4)[cde]	37.0 (34.0-37.8)[abde]	30.0 (28.7-31.4)[abc]	27.1 (25.1-28.6)[abc]
Delivery <37w	3 (9.4%)[cde]	5 (10.2%)[cde]	40 (43.5%)[abcd]	17 (100.0%)[abc]	17 (100.0%)[abc]
Delivery <34w	0[cde]	1 (2.0%)[cde]	22 (23.9%)[abcd]	15 (88.2%)[abc]	17 (100.0%)[abc]
Delivery <30w	0[de]	0[de]	10 (10.9%)[de]	8 (47.1%)[abc]	14 (82.4%)[abc]
Days from diagnosis to delivery	91.0 (74.0-108.5)[cde]	75.0 (65.0-97.0)[cde]	54.0 (35.8-83.0)[abde]	17.0 (6.0-25.0)[abc]	7.0 (3.0-16.0)[abc]
Adverse maternal outcomes					
Placental abruption	0	1 (2.0%)	4 (4.3%)	0	0
Preeclampsia	3 (9.4%)[de]	1 (2.0%)[cde]	24 (26.09%)[be]	10 (58.8%)[a]	13 (76.5%)[abc]
Without severity features	2 (6.2%)	1 (2.0%)	5 (5.4%)	1 (5.9%)	2 (11.8%)
With severity features	1 (3.1%)	0	19 (20.7%)	9 (52.9%)	11 (64.7%)
HELLP syndrome	1 (3.1%)	0	2 (2.2%)	4 (23.5%)	3 (17.6%)
Eclampsia	0	0	1 (1.1%)	0	0
OBICU admission ≥48h	1 (3.1%)[de]	1 (2.0%)[de]	19 (20.7%)[e]	10 (58.8%)[ab]	14 (82.4%)[abc]
Days in OBICU	4.0 (4.0-4.0)	2.0 (2.0-2.0)	6.0 (3.5-7.5)	5.5 (4.0-11.3)	4.0 (4.0-8.0)
Any adverse maternal outcome	3 (9.4%)[de]	3 (6.1%)[de]	22 (23.9%)[de]	11 (64.7%)[abc]	14 (82.4%)[abc]
Adverse Fetal outcomes					
Stilbirth	0	0	0[e]	1 (5.9%)	3 (17.6%)[e]
Cesarean for non-rassuring CTG	1 (3.1%)	3 (6.1%)	21 (22.8%)	2 (11.8%)	3 (17.6%)
TOP	0	0	2 (2.2%)	1 (5.9%)	3 (17.6%)
Adverse neonatal outcomes					
N-ICU admission ≥48h	3 (9.4%)[cde]	9 (18.4%)[cde]	41 (44.6%)[abd]	14 (82.4%)[abc]	12 (70.6%)[abc]
Days in N-ICU	11.0 (9.5-12.5)	17.0 (8.0-46.0)	22.0 (11.0-42.0)	32.5 (13.5-42.5)	63.0 (32.3-103.8)
Neonatal death	0	0	2 (2.2%)	1 (5.9%)	1 (5.9%)
RDS	2 (6.2%)[de]	3 (6.1%)[de]	20 (21.7%)[de]	10 (58.8%)[abc]	10 (58.8%)[abc]
BPD	0[e]	0[e]	8 (8.7%)	2 (11.8%)	5 (29.4%)[ab]
Sepsis	0[e]	1 (2.0%)[e]	4 (4.3%)[e]	2 (11.8%)	6 (35.3%)[abc]
Retinopathy (stage III-IV)	0	0	0	0	1 (5.9%)
NEC	0	1 (2.0%)	2 (2.2%)	1 (5.9%)	1 (5.9%)
Intraventricular hemorrhage grade III or IV	0	1 (2.0%)	0	1 (5.9%)	0
Periventricular leukomalacia	0	0	1 (1.1%)	0	0
Apgar 5 min <7	1 (3.1%)[e]	2 (4.1%)[e]	11 (12.0%)[e]	2 (11.8%)	10 (58.8%)[abc]
Artery pH≤7.0	0	0	3 (3.3%)	0	1 (5.9%)
Composite adverse perinatal outcomes (any fetal or neonatal adverse outcome)	3 (9.4%)[cde]	10 (20.4%)[cde]	56 (60.9%)[abde]	17 (100.0%)[abc]	17 (100.0%)[abc]
Stage FGR at delivery	[bcde]	[acde]	[abde]	[abc]	[abc]
SGA	2 (6.2%)[b]	42 (85.7%)[acde]	0[b]	0[b]	0[b]
Stage I	1 (3.1%)[c]	7 (14.3%)[c]	82 (89.1%)[abde]	0[c]	0[c]
Stage II	0[d]	0[d]	6 (6.5%)[d]	8 (47.1%)[abc]	1 (5.9%)
Stage III	0	0[e]	0[e]	1 (5.9%)	4 (23.5%)[bc]
Stage IV	0[de]	0[de]	4 (4.3%)[de]	8 (47.1%)[abc]	12 (70.6%)[abc]
Deliveryindication					
Spontaneous	25[bcde]	8 (16.3%)[a]	20 (21.7%)[a]	0[a]	0[a]
Preeclampsia	3 (9.4%)	0[cd]	14 (15.2%)[b]	6 (35.3%)[b]	3 (17.6%)
Fetal Doppler	0[de]	1 (2.0%)[de]	9 (9.8%)[de]	7 (41.2%)[abc]	7 (41.2%)[abc]
CTG	1 (3.1%)	1 (2.0%)	6 (6.5%)	2 (11.8%)	3 (17.6%)
Abruption	0	1 (2.0%)	3 (3.3%)	0	0
Stillbirth	0	0	1 (1.1%)	2 (11.8%)	4 (23.5%)
EFW	1 (3.1%)[bc]	30 (61.2%)[acde]	32 (34.8%)[abde]	0[bc]	0[bc]
Other	2 (6.2%)	8 (16.3%)	7 (7.6%)	0	0

Continuous data are given as median and interquartile range. Categorical data as frequency and percentage.

BPD, bronchopulmonary dysplasia; CTG, cardiotocography; EFW, estimated fetal weight; NEC, necrotizing enterocolitis; N-ICU, neonatal intensive care unit; OBICU, obstetric intensive care unit;RDS, respiratory distress syndrome.

a, $P<0.05$ compared to controls; b, $P<0.05$ compared to SGA; c, $P<0.05$ compared to FGR I; d, $P<0.05$ compared to FGR II; e, $P<0.05$ compared to FGR III.

Figure 1. Recruitment flowchart

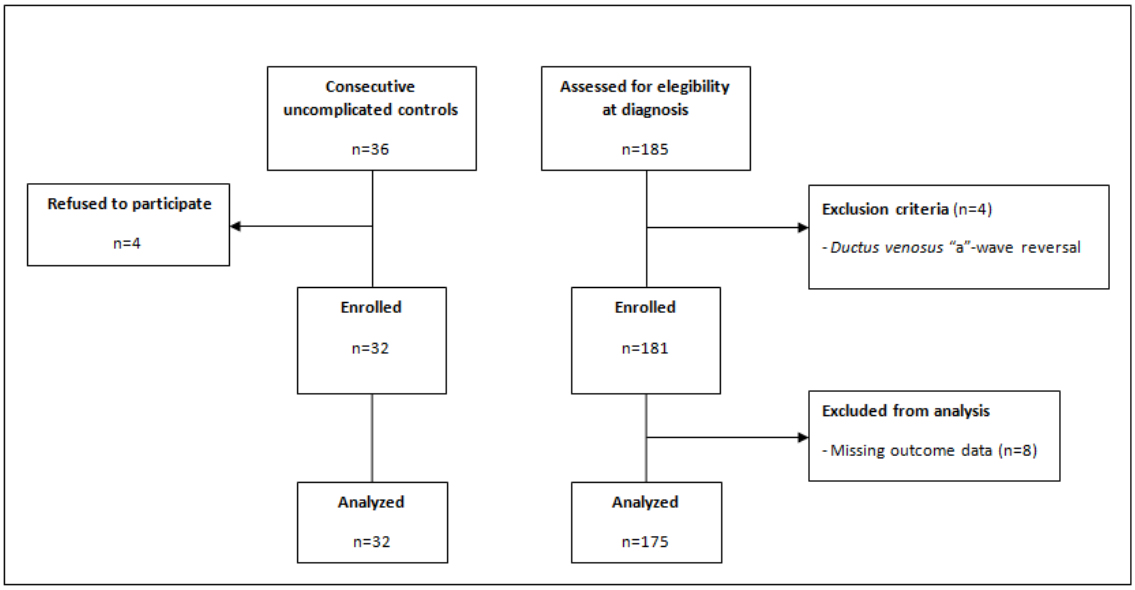
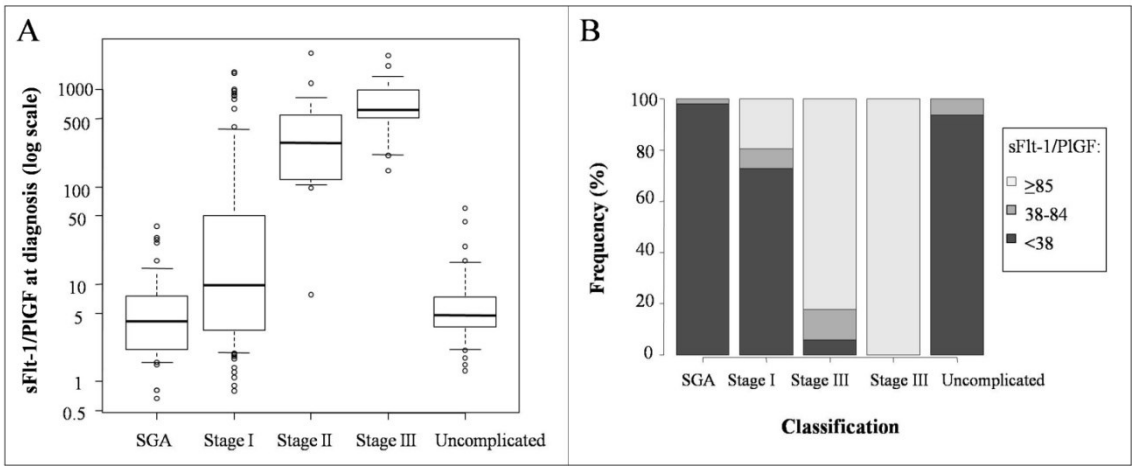


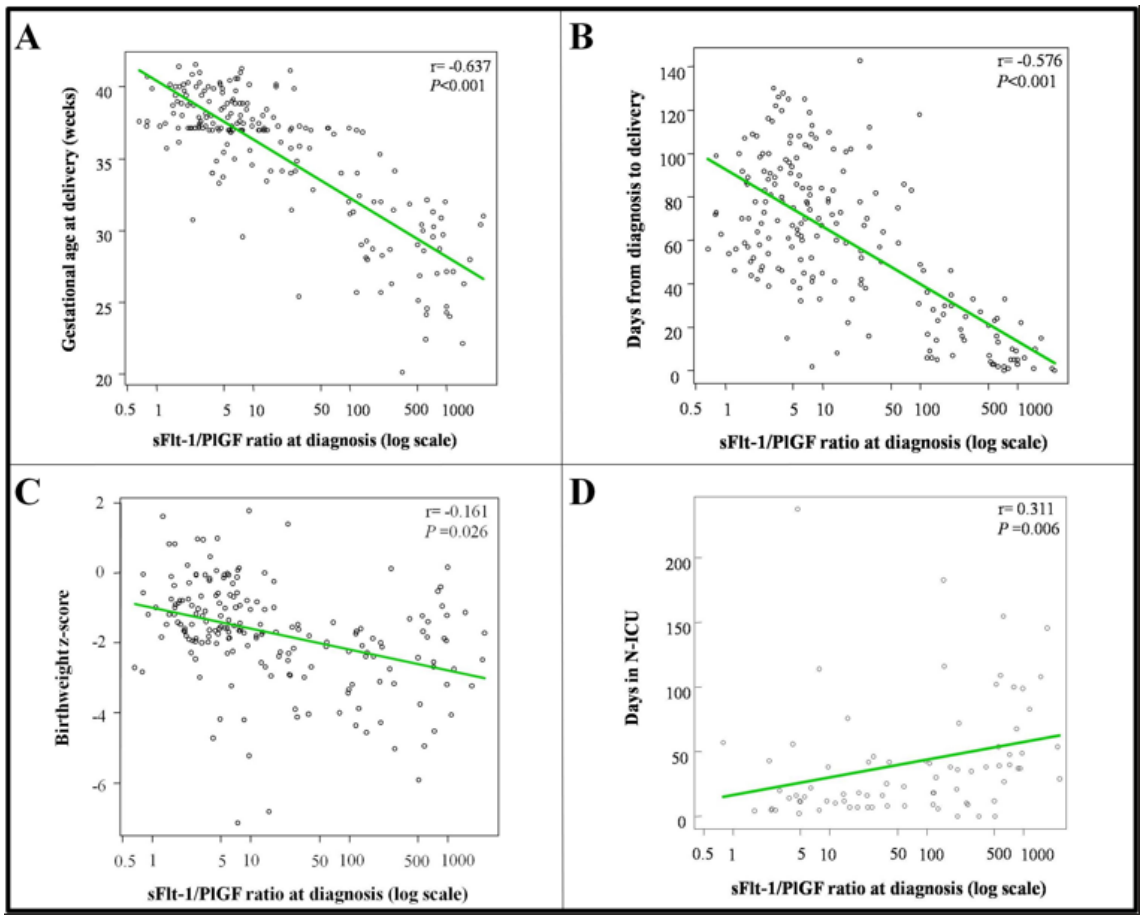
Figure 2: sFlt-1/PlGF values (A) and frequency of sFlt-1/PlGF cutoffs (B) in SGA, FGR stage I, II, III and reference women.



Note: The bottom and top edges of each box represent the first and third quartiles, respectively, the band within the box represents the median value and the whiskers represent values that are 1.5 times the interquartile range.

SGA, small for gestational age.

Figure 3: Correlation of sFlt-1/PlGF to adverse pregnancy outcomes.



Note: Line and scatter plots displaying the correlation of the sFlt-1/PlGF ratio to gestational age at delivery (A), days from diagnosis to delivery (B), birthweight z-scores (C) and days in N-ICU (D). *Dots* indicate individual sFlt-1/PlGF ratios; *line* indicates regression line.

N-ICU, neonatal intensive care unit.