Biomarkers of impaired placentation at 35-37 weeks' gestation in the prediction of adverse perinatal outcome

Anca Ciobanou,[1] Salma Jabak,[1] Hila De Castro,[1] Laurence Frei,[1] Ranjit Akolekar,[2,3] Kypros H. Nicolaides[1]

1. Fetal Medicine Research Institute, King's College Hospital, London, UK
2. Fetal Medicine Unit, Medway Maritime Hospital, Gillingham, UK
3. Institute of Medical Sciences, Canterbury Christ Church University, Chatham, UK

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Correspondence:
Professor KH Nicolaides, Fetal Medicine Research Institute,
King's College Hospital, 16-20 Windsor Walk, Denmark Hill, London SE5 8BB
Telephone: +442032998256; email: kypros@fetalmedicine.com

ABSTRACT

Objective: This screening study at 35-37 weeks' gestation investigates the potential value of uterine artery pulsatility index (UtA-PI) and serum levels of the angiogenic placental growth factor (PlGF) and antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFLT-1) in the prediction of adverse perinatal outcome in small for gestational age (SGA) and non-SGA neonates.

Methods: This was a prospective observational study in 19,209 singleton pregnancies attending for a routine hospital visit at 35^{+0} - 36^{+6} weeks' gestation. This visit included recording of maternal demographic characteristics and medical history, sonographic estimation of fetal weight (EFW), color Doppler ultrasound for measurement of the mean UtA-PI, and measurement of serum concentration of PlGF and sFLT. Multivariable logistic regression analysis was carried out to determine which of the factors from maternal or pregnancy characteristics and measurements of UtA-PI, PlGF and sFLT-1, provided a significant contribution in the prediction of each of four adverse outcome measures: first, stillbirth, second, cesarean section for presumed fetal compromise in labor, third, neonatal death or hypoxic ischemic encephalopathy grades 2 and 3, and fourth, admission to the neonatal unit (NNU) for ≥48 hours. Predicted probabilities from logistic regression analysis were used to construct receiver operating characteristic (ROC) curves to assess performance of screening for these adverse outcomes.

Results: First, 83% of stillbirths, 82% of cesarean sections for presumed fetal compromise in labor, 91% of cases of neonatal death or hypoxic ischemic encephalopathy and 86% of NNU admissions for ≥48 hours occurred in pregnancies with non-SGA babies. Second, UtA-PI >95^{th} percentile, sFLT-1 >95^{th} percentile and PLGF <5^{th} percentile were associated with increased risk of cesarean section for presumed fetal compromise in labor and NNU admission for ≥48 hours; the number of stillbirths and cases of neonatal death or hypoxic ischemic encephalopathy was too small to demonstrate significance in the observed differences from cases without these adverse outcomes. Third, multivariable regression analysis demonstrated that in prediction of cesarean section for presumed fetal compromise in labor there was no significant contribution from biomarkers; the prediction of NNU admission for ≥48 hours by maternal demographic characteristics and medical history was only marginally improved by the addition of UtA-PI, sFLT-1 or PlGF. Fourth, for each biomarker the detection rate of adverse outcomes was higher in SGA than in non-SGA neonates, but such increase was accompanied by an increase in false positive rate. Fifth, the relative risk of UtA-PI >95^{th}, sFLT-1 >95^{th} and PLGF <5^{th} percentiles for most adverse outcomes was <2.5 in both SGA and non-SGA neonates.

Conclusions: In pregnancies undergoing routine antenatal assessment at 35-37 weeks' gestation measurements of UtA-PI, sFLT-1 or PlGF provide poor prediction of adverse perinatal outcome in both SGA and non-SGA fetuses.

Key words: Third trimester screening, Small for gestational age, Uterine artery Doppler, Placental growth factor, Soluble fms-like tyrosine kinase-1, Cesarean section, Perinatal hypoxia, Stillbirth, Perinatal death, Hypoxic ischemic encephalopathy, Neonatal unit admission.

INTRODUCTION

Routine assessment of pregnancy at 35-37 weeks' gestation is useful in the prediction of subsequent development of pre-eclampsia (PE) and the birth of a small-for gestational-age (SGA) neonates.[1,2] Both conditions are associated with impaired placentation and/or placental dysfunction, reflected in increased pulsatility index (PI) in the uterine arteries (UtAs), reduced serum level of the angiogenic placental growth factor (PlGF) and increased level of the antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFLT-1).[1-6]

Small for gestational age fetuses / neonates are at increased risk of adverse perinatal outcome, including stillbirth, cesarean section for presumed fetal compromise and admission to the neonatal unit for ≥48 hours.[7-10] However, >80% of adverse perinatal events at term occur in babies with birthweight ≥10th percentile.[7-10] On the assumption that at least some of the adverse perinatal events in both SGA and non-SGA babies are a consequence of failure to reach a normal growth potential due to impaired placentation, it should be anticipated that high UtA-PI and sFLT-1 and low PlGF would be good predictors of adverse outcome.

The objective of this screening study at 35-37 weeks' gestation was to investigate the potential value of UtA-PI and serum levels of PlGF and sFLT-1 in the prediction of adverse perinatal outcome in SGA and non-SGA neonates.

METHODS

Study design and participants

This was a prospective study in women attending for a routine hospital visit at 35^{+0} - 36^{+6} weeks' gestation at King's College Hospital, London or Medway Maritime Hospital, Gillingham, UK between between March 2014 and September 2018. This visit included recording of maternal demographic characteristics and medical history, ultrasound examination for fetal anatomy and measurement of fetal head circumference, abdominal circumference and femur length for calculation of estimated fetal weight (EFW) (using the formula by Hadlock et al,[11] because a systematic review identified this as being the most accurate model[12]), transabdominal color Doppler ultrasound for measurement of the mean UtA-PI,[13] and measurement of serum concentration of PlGF and sFLT in pg/mL by an automated biochemical analyzer (Cobas e411 system, Roche Diagnostics, Penzberg, Germany, or BRAHMS KRYPTOR compact PLUS, Thermo Fisher Scientific, Hennigsdorf, Germany). Gestational age was determined by the measurement of fetal crown-rump length at 11-13 weeks or the fetal head circumference at 19-24 weeks.[14,15] The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

Inclusion / exclusion criteria

The inclusion criteria for this study were singleton pregnancies examined at 35^{+0} - 36^{+6} weeks' gestation and delivering a non-malformed live birth or stillbirth. We excluded pregnancies with aneuploidies and major fetal abnormalities. Some of the patients in this study were included in a previous publication (n=3,953).[16]

Patient characteristics

Patient characteristics recorded included maternal age, racial origin (White, Black, South Asian, East Asian and mixed), method of conception (spontaneous or assisted by use of ovulation induction drugs or *in vitro* fertilization), cigarette smoking during pregnancy, medical history of chronic hypertension or diabetes mellitus, obstetric history (nulliparous if

no previous pregnancies at ≥24 weeks and parous with or without previous history of delivery of SGA neonate with birthweight <10th percentile) and presence of PE, obstetric cholestasis or gestational diabetes mellitus in the current pregnancy. Maternal weight and height were measured and body mass index (BMI) was calculated.

Outcome measures

Data on pregnancy outcome were collected from the hospital delivery records. The following outcome measures were considered: first, stillbirth, second, cesarean section for presumed fetal compromise in labor, third, neonatal death or hypoxic ischemic encephalopathy grades 2 and 3, and fourth, admission to the neonatal unit (NNU) for ≥48 hours. Cesarean section for presumed fetal compromise in labor was carried out if there was evidence of a pathological electronic fetal heart rate pattern, abnormalities in ST waveform analysis of fetal electrocardiogram and / or abnormal fetal scalp blood pH.[17,18] Hypoxic-ischemic encephalopathy was diagnosed when there was disturbed neurologic function with evidence of perinatal hypoxia reflected in either a 5-minute Apgar score <5 or umbilical artery cord pH <7.0 or base deficit >12 mmol/L, supported by neuroimaging evidence of acute brain injury. The definition of SGA fetus or neonate was based on EFW and birthweight, respectively, <10th percentile for gestational age based on the Fetal Medicine Foundation fetal and neonatal population weight charts.[19]

Statistical analysis

Categorical data are presented as n (%) and continuous data as median and interquartile range (IQR). Mann-Whitney U-test and χ2-square test or Fisher's exact test, were used for comparing outcome groups for continuous and categorical data, respectively. Significance was assumed at 5%.

Univariable and multivariable logistic regression analysis was carried out to determine which of the factors from maternal or pregnancy characteristics and measurements of UtA-PI, PlGF and sFLT-1, provided a significant contribution in the prediction of each of the four outcome measures. Prior to the regression analysis, the continuous variables, such as age, weight and height were centred by subtracting the arithmetic mean from each value. Multiple categorical variables were dummy coded as binary variables to estimate the independent effect of each category. The measured, PlGF, sFLT-1 and UtA-PI were converted to multiples of the median (MoM) after adjustment for variables from maternal characteristics and medical history that affect these measurements.[1,20] The birth weight Z-score was derived from the Fetal Medicine Foundation fetal and neonatal population weight charts.[19] We estimated cut-offs for the 95th percentiles for UtA-PI and sFLT-1 and <5th percentile for PlGF and determined the prevalence of abnormal values in each of the outcome groups. The values of UtA-PI >95th percentile, sFLT-1 >95th percentile and PLGF <5th percentile were used as binary categorical variables in the multivariable regression analysis. Predicted probabilities from logistic regression analysis were used to construct receiver operating characteristic (ROC) curves to assess performance of screening for these adverse outcomes. We examined the detection rate (DR), false positive rate (FPR), relative risk and positive and negative likelihood ratios (LR) of UtA-PI >95th, sFLT-1 >95th and PLGF <5th percentiles for each adverse perinatal outcome in the sub-groups of SGA (birthweight <10th percentile) and non-SGA (birthweight ≥10th percentile) fetuses and neonates.

The statistical package SPSS 24.0 (IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY: IBM Corp; 2016) was used for data analyses.

RESULTS

Study population

The characteristics of the study population of 19,209 singleton pregnancies, including 19,174 with livebirths and 35 with stillbirths are summarized in Table 1.

The 19,174 pregnancies with livebirths, included 14,170 with vaginal delivery following spontaneous or induced labor, 72 with elective cesarean section for suspected fetal compromise due to abnormal Doppler findings or fetal-heart rate patterns in SGA fetuses and 2,201 with elective cesarean section for a variety of other indications (breech or transverse lie, placenta previa, previous cesarean section or traumatic birth, maternal medical disorder or maternal request) and 2,731 with cesarean section following spontaneous or induced labor; in the latter group, the indication for cesarean section was presumed fetal compromise in 1,007 cases.

In the livebirths there were 2 neonatal deaths and 30 cases with HIE grades 2 or 3 and 1,323 cases of NNU admission for ≥48 hours (including the 30 cases of HIE).

Biomarkers in pregnancies with adverse outcome

Stillbirth

In stillbirths, compared to livebirths, there was a higher median sFLT-1 MoM and a higher incidence of UtA-PI and sFLT-1 >95th and BW <10th percentiles (Table 1). Multivariable regression analysis demonstrated that the only significant predictors of stillbirth were maternal BMI and parous women with a previous delivery of SGA neonate, but not from UtA-PI >95th, sFLT-1 >95th, PLGF <5th or EFW <10th percentiles (R^2=0.018; p=0.010; Table S1).

Cesarean section for presumed fetal compromise in labor

In those delivering by cesarean section for presumed fetal compromise in labor, compared to those with vaginal delivery, there was first, higher median maternal weight and body mass index, and higher incidence of women of Black racial origin, conception by *in vitro* fertilization, nulliparity, chronic hypertension, diabetes mellitus, PE and gestational diabetes; second, higher median sFLT-1 MoM; and third, higher incidence of UtA-PI and sFLT-1 >95th, PLGF <5th and birthweight <10th percentiles (Table 1).

Multivariable regression analysis demonstrated that in prediction of cesarean section for presumed fetal compromise in labor there was a statistically significant contribution from maternal age, body mass index, smoking, Black and mixed racial origin, parity, chronic hypertension, diabetes mellitus and PE, but not from UtA-PI >95th, sFLT-1 >95th, PLGF <5th or EFW <10th percentiles (R^2=0.101; p<0.001; Table S1).

Neonatal death or hypoxic ischemic encephalopathy

In those with neonatal death or hypoxic ischemic encephalopathy, compared to those without these adverse events, there was higher incidence of Black racial origin, parous women without previous SGA and PE. There was no significant difference in median UtA-PI MoM, sFLT-1 MoM, PLGF MoM and there was no significant difference in the incidence of UtA-PI >95th, sFLT-1 >95th, PLGF <5th or EFW <10th percentiles (Table 1).

Multivariable regression analysis demonstrated that in prediction of neonatal death or hypoxic ischemic encephalopathy there was a statistically significant contribution from parity and PE, but not from UtA-PI >95th, sFLT-1 >95th, PLGF <5th or EFW <10th percentiles (R^2=0.022; p=0.006; Table S1).

Neonatal unit admission for ≥48 hours

In those with NNU admission for ≥48 hours, compared to those without this adverse event, there was first, higher median body mass index and lower maternal age, higher incidence of smokers, White and South Asian racial origin, conception with use of ovulation induction drugs, diabetes mellitus, gestational diabetes, obstetric cholestasis and PE and lower incidence of parous women with or without previous SGA, second, higher median sFLT-1 MoM and lower median PLGF MoM, and third, higher incidence of UtA-PI and sFLT-1 >95th, PLGF <5th, and birthweight <10th percentiles (Table 1).

Multivariable regression analysis demonstrated that in prediction of NNU admission for ≥48 hours there was a statistically significant contribution from maternal age, body mass index, Black and South Asian racial origin, parous women with or without previous SGA, obstetric cholestasis, PE, sFLT-1 >95th, PLGF <5th percentiles and estimated fetal weight <10th percentile (R^2=0.048; p<0.001; Table S1). There was a marginal improvement in prediction of admission to NNU ≥48 hours from addition of the biomarkers to the maternal factors (AUROC; 95% CI: 0.641; 0.625-0.657 vs. 0.634; 0.618-0.650, respectively; p=0.012)

Elective cesarean section for suspected fetal compromise

In the 72 cases of elective cesarean section for suspected fetal compromise, the UtA-PI was >95th percentile in 12 (16.7%), sFLT-1 was >95th percentile in 12 (16.7%), PLGF was <5th percentile in 17 (23.6%) and birthweight was <10th percentile in 58 (80.6%).

Performance of screening in pregnancies with SGA and non-SGA neonates

The predictive performance of UtA-PI >95th percentile, sFLT-1 >95th percentile and PLGF <5th percentile for adverse perinatal outcome SGA and non-SGA neonates Is shown in Tables 2-4.

Stillbirth

The incidence of stillbirth was 0.30% (6/2,034) in babies with birthweight <10th percentile and 0.17% (29/17,175) in those with birthweight ≥10th percentile (p=207). Consequently, 82.9% (29/35) of stillbirths occurred in non-SGA babies.

The UtA-PI was >95th percentile in 8.6% (3/35) pregnancies with stillbirth and in 5.0% (958/19,174) of those with livebirth. The PLGF was <5th percentile in 8.6% (3/35) pregnancies with stillbirth and in 5.0% (957/19,174) of those with livebirth. The sFLT-1 was >95th percentile in 11.4% (4/35) pregnancies with stillbirth and in 5.0% (956/19,174) of those with livebirth.

The relative risk for stillbirth in the group with UtA-PI >95th percentile was 1.71 in the SGA neonates and 1.63 in the non-SGA neonates (Table 2). The relative risk for stillbirth in the group with sFLT-1 >95th percentile was 1.66 in the SGA neonates and 2.56 in the non-SGA neonates (Table 3). The relative risk for stillbirth in the group with PLGF <5th percentile was 1.23 in the SGA neonates and 1.81 in the non-SGA neonates (Table 4).

Cesarean section for presumed fetal compromise

The incidence of cesarean section for presumed fetal compromise in labor in the livebirths was 10.9% (183/1,686) in babies with birthweight <10th percentile and 6.1% (824/13,491) in those with birthweight ≥10th percentile (p<0.001). Consequently, 81.8% (824/1,007) of cesarean sections for presumed fetal compromise occurred in non-SGA babies.

The UtA-PI was >95th percentile in 6.7% (67/1,007) pregnancies delivered by cesarean section for presumed fetal compromise and in 4.7% (664/14,170) of those with vaginal delivery. The PLGF was <5th percentile in 6.6% (66/1,007) pregnancies delivered by cesarean section for presumed fetal compromise and in 4.9% (697/14,170) of those with vaginal delivery. The sFLT-1 was >95th percentile in 7.7% (78/1,007) pregnancies delivered by cesarean section for presumed fetal compromise and in 4.7% (661/14,170) of those with vaginal delivery.

The relative risk for delivery by cesarean section for presumed fetal compromise in the group with UtA-PI >95th percentile was 1.33 in the SGA neonates and 1.29 in the non-SGA neonates (Table 2). The relative risk for delivery by cesarean section for presumed fetal compromise in the group with sFLT-1 >95th percentile was 1.33 in the SGA neonates and 1.61 in the non-SGA neonates (Table 3). The relative risk for delivery by cesarean section for presumed fetal compromise in the group with PLGF <5th percentile was 1.23 in the SGA neonates and 1.13 in the non-SGA neonates (Table 4).

Neonatal death or hypoxic ischemic encephalopathy

The incidence of neonatal death or hypoxic ischemic encephalopathy was 0.10% (3/2,034) in babies with birthweight <10th percentile and 0.17% (29/17,175) in those with birthweight ≥10th percentile (p=0.556). Consequently, 90.6% (29/32) of cases of neonatal death or hypoxic ischemic encephalopathy occurred in non-SGA babies.

The UtA-PI was >95th percentile in 9.4% (3/32) cases of neonatal death or hypoxic ischemic encephalopathy and in 5.0% (958/19,177) of those without this adverse outcome. The PLGF was <5th percentile in 3.1% (1/32) cases of neonatal death or hypoxic ischemic encephalopathy and in 5.0% (959/19,177) of those without this adverse outcome. The sFLT-1 was >95th percentile in 9.4% (3/32) cases of neonatal death or hypoxic ischemic encephalopathy and in 5.0% (957/19,177) of those without this adverse outcome.

The relative risk for neonatal death or hypoxic ischemic encephalopathy in the group with UtA-PI >95th percentile was 1.22 in the SGA neonates and 2.53 in the non-SGA neonates (Table 2). The relative risk for neonatal death or hypoxic ischemic encephalopathy in the group with sFLT-1 >95th percentile was 4.14 in the SGA neonates and 1.64 in the non-SGA neonates (Table 3). The relative risk for neonatal death or hypoxic ischemic encephalopathy in the group with PLGF <5th percentile was 2.04 in the SGA neonates and 0.87 in the non-SGA neonates (Table 4).

Neonatal unit admission for ≥48 hours

The incidence of NNU admission for ≥48 hours was 9.5% (192/2,028) in babies with birthweight <10th percentile and 6.6% (1,131/17,145) in those with birthweight ≥10th percentile (p<0.001). Consequently, 85.5% (1,131/1,323) of cases of NNU admission for ≥48 hours occurred in non-SGA babies.

The UtA-PI was >95th percentile in 7.0% (92/1,323) cases of NNU admission for ≥48 hours and in 4.9% (866/17,851) of those without this adverse outcome. The PLGF was <5th percentile in 8.3% (110/1,323) cases of NNU admission for ≥48 hours and in 4.7% (847/17,851) of those without this adverse outcome. The sFLT-1 was >95th percentile in 9.3% (123/1,323) cases of NNU admission for ≥48 hours and in 4.7% (833/17,851) of those without this adverse outcome.

The relative risk for NNU admission for ≥48 hours in the group with UtA-PI >95th percentile was 1.28 in the SGA neonates and 1.36 in the non-SGA neonates (Table 2). The relative

risk for NNU admission for ≥48 hours in the group with sFLT-1 >95th percentile was 2.32 in the SGA neonates and 1.72 in the non-SGA neonates (Table 3). The relative risk for NNU admission for ≥48 hours in the group with PLGF <5th percentile was 1.62 in the SGA neonates and 1.61 in the non-SGA neonates (Table 4).

DISCUSSION

Main findings of the study

The findings of this study of routine assessment of singleton pregnancies at 35-37 weeks' gestation demonstrate the following. First, 83% of stillbirths, 82% of cesarean sections for presumed fetal compromise in labor, 91% of cases of neonatal death or hypoxic ischemic encephalopathy and 86% of NNU admissions for ≥48 hours occurred in pregnancies with non-SGA babies. Second, UtA-PI >95th percentile, sFLT-1 >95th percentile and PLGF <5th percentile were associated with increased risk of of cesarean section for presumed fetal compromise in labor and NNU admission for ≥48 hours; the number of stillbirths and cases of neonatal death or hypoxic ischemic encephalopathy was too small to demonstrate significance in the observed differences from cases without these adverse outcomes. Third, multivariable regression analysis demonstrated that in prediction of cesarean section for presumed fetal compromise in labor there was no significant contribution from biomarkers; the prediction of NNU admission for ≥48 hours by maternal demographic characteristics and medical history was only marginally improved by the addition of UtA-PI, sFLT-1 or PLGF. Fourth, for each biomarker the DR of adverse outcomes was higher in SGA than in non-SGA babies, but such increase was accompanied by an increase in FPR. Fifth, the relative risk of UtA-PI >95th, sFLT-1 >95th and PLGF <5th percentiles for most adverse outcomes was <2.5 in both SGA and non-SGA neonates.

If it was to be assumed that the adverse outcomes we have investigated are the consequence of impaired placentation and that high UtA-PI and sFLT-1 and low PLGF are good markers of such impairment, it should be anticipated that these biomarkers would be good predictors of adverse outcome. However, the observed low performance of these biomarkers in the prediction of adverse perinatal outcomes suggests that first, they provide poor assessment of placentation or second, most cases of stillbirth at term are not associated with impaired placentation and the contribution of maternal and pregnancy characteristics as well as events in labor play a much greater role than impaired placentation in the development of fetal compromise in labor or adverse neonatal outcome.

Comparison with findings from previous studies

The results of this study are consistent with those of the only two previous screening studies in unselected populations that examined biomarkers of impaired placentation in 8,268 pregnancies at 30-34 weeks' gestation and 3,953 at 35-37 weeks, respectively, for prediction of adverse perinatal outcome; in both studies these markers were useful in the prediction of PE and birth of SGA neonates, but not of adverse events in labor or after birth.[16,21]

Our results are also consistent with those of a prospective study of 438 low-risk pregnancies in which serial measurements of serum PLGF were carried out from 36 weeks' gestation until delivery; the study found that low PLGF levels were associated with low birth weight and adverse intrapartum and neonatal outcome, but the predictive performance of low PLGF was poor with DR of 10-11% at FPR of 10%.[22] In contrast, a prospective study in 3,747 singleton pregnancies in nulliparous women reported that elevated sFLT1/PlGF ratio (>85th percentile) at 36 weeks' gestation in combination with EFW <10th percentile predicted 38% of adverse perinatal outcomes, at screen positive rate of 3%.[23] However, adverse outcome was defined as birth of a SGA neonate associated with PE or perinatal morbidity or mortality, and such definition would apply to a very small proportion of all cases of stillbirth, cesarean

section for presumed fetal compromise in labor, neonatal death or hypoxic ischemic encephalopathy or NNU admission for ≥48 hours. In a previous report of the same study population, the authors reported that SGA neonates contributed only 18% of neonatal morbidity and 15% of severe adverse perinatal outcome found in the total population,[24] which is consistent with our findings.

Implications for clinical practice

An integrated clinical assessment at 35-37 weeks' gestation, which includes fetal biometry and measurement of biomarkers, identifies a high proportion of pregnancies that subsequently develop PE and those delivering SGA neonates.[1-6] Contrary to the expectation that the same biomarkers would be useful in predicting adverse perinatal outcome, this did not prove to be the case.

Strengths and limitations of the study

The strengths of our study are first, examination of a large number of pregnancies attending for routine assessment of fetal growth and wellbeing at a prespecified gestational-age range at the end of the third trimester of pregnancy, second, measurement of UtA-PI by appropriately-trained doctors, third, measurement of sFLT-1 and PLGF by automated machines that provide reproducible results, fourth, expression of the values of the biomarkers as MoMs after adjustment for maternal factors and reagents used that affect the measurements, and fifth, use of a wide range of well accepted indicators for adverse perinatal outcome.

The main limitation of this and most previous studies investigating the biomarkers of impaired placentation in the prediction of adverse pregnancy outcome is that the results of the ultrasound scan were made available to the attending obstetricians who would have taken specific actions of further monitoring and planned delivery of the cases with suspected SGA and fetal compromise. In our study 72 such pregnancies had elective delivery by cesarean section; had this not been carried out it is possible that some of the cases would have resulted in stillbirth, cesarean section for fetal compromise in labor, birth asphyxia and NNU admission. Consequently, the performance of screening by UtA-PI, sFLT-1 and PLGF for adverse perinatal outcome in SGA fetuses would have been negatively biased. However, the number of these cases was very small and it is therefore unlikely that they would have had a major impact on the overall effect of adverse events in labor and after birth.

Conclusions

In pregnancies undergoing routine antenatal assessment at 35 - 37 weeks' gestation measurements of UtA-PI, sFLT-1 or PLGF, which has been previously found to be useful in prediction of PE and birth of SGA neonates, provide poor prediction of stillbirth, cesarean section for presumed fetal compromise in labor, NNU admission for ≥48 hours, neonatal death or hypoxic ischemic encephalopathy in both SGA and non-SGA fetuses.

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Table 1. Maternal and pregnancy characteristics in pregnancies with livebirths and stillbirths.

Maternal and pregnancy characteristic	Outcome		Method of delivery		NND or HIE 2/3		NNU admission	
	Livebirth (n=19,174)	Stillbirth (n=35)	Vaginal (n=14,170)	CS for FC (n=1,007)	No (n=19,177)	Yes (n=32)	No (n=17,851)	Yes (n=1,323)
Maternal age (years)	32.1 (38.0-35.7)	31.9 (28.7-34.9)	31.8 (27.6-35.3)	31.7 (27.7-35.3)	32.1 (28.0-35.7)	31.3 (27.7-34.7)	32.2 (28.1-35.7)	30.8 (26.7-34.8)**
Maternal weight (kg)	79.0 (71.0-89.8)	83.5 (73.5-90.3)	78.0 (70.0-88.7)	81.0 (72.0-92.0)**	79.0 (71.0-89.8)	82.7 (72.1-95.3)	79.0 (70.7-89.3)	82.0 (73.0-94.0)**
Maternal height (cm)	165 (161-169)	165 (161-170)	165 (161-170)	163 (159-168)**	165 (161-169)	165 (159-172)	165 (161-169)	164 (160-169)*
Maternal body mass (kg/m^2)	29.0 (26.1-32.8)	29.9 (27.6-34.0)	28.6 (25.9-32.2)	30.6 (27.0-34.0)**	29.0 (26.1-32.8)	29.6 (26.4-34.5)	29.0 (26.1-32.6)	30.3 (27.1-34.6**)
Cigarette smoker	1,319 (6.9)	5 (14.3)	1,019 (7.2)	82 (8.1)	1,323 (6.9)	1 (3.1)	1,208 (6.8)	111 (8.4)**
Racial origin								
White	15,022 (78.3)	23 (65.7)	11,180 (78.9)	714 (70.9)**	15,022 (78.3)	23 (71.9)	13,940 (78.1)	1,082 (81.8)**
Black	2,358 (12.3)	8 (22.9)	1,669 (11.8)	186 (18.5)**	2,359 (12.3)	7 (21.9)*	2,228(12.5)	130 (9.8)**
South Asian	826 (4.3)	1 (2.9)	604 (4.3)	49 (4.9)	825 (4.3)	2 (6.3)	754 (4.2)	72 (5.4)*
East Asian	400 (2.1)	1 (2.9)	304 (2.1)	17 (1.7)	401 (2.1)	0	387 (2.2)	13 (1.0)*
Mixed	568 (3.0)	2 (5.7)	413 (2.9)	41 (4.1)*	570 (3.0)	0	542 (3.0)	26 (2.0)*
Conception								
Natural	18,389 (95.9)	35 (100.0)	13,722 (96.8)	954 (94.7)	18,392 (95.9)	32 (100.0)	17,128 (95.9)	1,261 (95.3)
Ovulation induction drugs	110 (0.6)	0	78 (0.6)	6 (0.6)	110 (0.6)	0	98 (0.5)	12 (0.9)**
In vitro fertilization	675 (3.5)	0	370 (2.6)	47 (4.7)**	675 (3.5)	0	625 (3.5)	50 (3.8)
Obstetric history								
Nulliparous	8.997 (46.9)	13 (37.1)	6,439 (45.4)	739 (73.4)**	8,986 (46.9)	24 (75.0)	8,215 (46.0)	782 (59.1)
Parous, previous SGA	1,209 (6.3)	5 (14.3)	916 (6.5)	46 (4.6)*	1,214 (6.3)	0	1,154 (6.5)	55 (4.2)**
Parous, no previous SGA	8,968 (46.8)	17 (48.6)	6,815 (48.1)	222 (22.0)**	8,977 (46.8)	8 (25.0)*	8,482 (47.5)	486 (36.7)**
Medical disorders								
Chronic hypertension	209 (1.1)	0	107 (0.8)	25 (2.5)**	209 (1.1)	0	188 (1.1)	21 (1.6)
Diabetes mellitus	197 (1.0)	0	96 (0.7)	18 (1.8)**	197 (1.0)	0	175 (1.0)	22 (1.7)*
Pregnancy complications								
Preeclampsia	413 (2.2)	1 (2.9)	234 (1.7)	64 (6.4)**	411 (2.1)	3 (9.4)*	348 (1.9)	65 (4.9)**
Gestational diabetes	766 (4.0)	1 (2.9)	462 (3.3)	48 (4.8)*	767 (4.0)	0	697 (3.9)	69 (5.2)*
Obstetric cholestasis	151 (0.8)	1 (2.9)	108 (0.8)	10 (1.0)	151 (0.8)	1 (3.1)	121 (0.7)	30 (2.3)**
Biomarkers of impaired placentation								
Uterine artery PI (MoM)	0.96 (0.82-1.14)	1.10 (0.87-1.18)	0.96 (0.82-1.14)	0.94 (0.79-1.15)	0.96 (0.82-1.14)	0.97 (0.74-1.12)	0.96 (0.82-1.13)	0.96 (0.81-1.17)
Uterine artery PI >95th percentile	958 (5.0)	3 (8.6)*	664 (4.7)	67 (6.7)**	958 (5.0)	3 (9.4)	866 (4.9)	92 (7.0)**

Serum PLGF (MoM)	0.96 (0.53-1.73)	0.83 (0.39-1.22)	0.95 (0.53-1.72)	0.92 (0.49-1.73)	0.96 (0.53-1.73)	1.24 (0.58-2.18)	0.97 (0.54-1.73)	0.83 (0.45-1.61)**
Serum PLGF <5th percentile	957 (5.0)	3 (8.6)	697 (4.9)	66 (6.6)*	959 (5.0)	1 (3.1)	847 (4.7)	110 (8.3)**
Serum sFLT-1 (MoM)	0.96 (0.70-1.39)	1.37 (0.87-2.43)**	0.96 (0.70-1.38)	1.02 (0.72-1.50)*	0.96 (0.70-1.39)	1.04 (0.77-1.49)	0.96 (0.70-1.38)	1.04 (0.72-1.60)**
Serum sFLT-1 >95th percentile	956 (5.0)	4 (11.4)*	661 (4.7)	78 (7.7)**	957 (5.0)	3 (9.4)	833 (4.7)	123 (9.3)**
Estimated weight <10th percentile	1,488 (7.8)	4 (11.4)	1,165 (8.2)	97 (9.6)	1,491 (7.8)	1 (3.1)	1,367 (7.7)	121 (9.1)
GA at delivery in weeks	40.0 (39.1-40.9)	39.7 (38.7-41.3)	40.1 (39.3-40.9)	40.4 (39.4-41.3)*	40.0 (39.1-40.9)	40.1 (39.1-40.6)	40.0 (39.1-40.9)	39.9 (38.4-40.9)**
Birth weight (g)	3440 (3130-3755)	3250 (2920-3460)	3435 (3130-3740)	3400 (3050-3750)	3440 (3130-3755)	3596 (3282-3790)	3440 (3135-3750)	3410 (3030-3780)*
Birth weight <10th percentile	2,028 (10.6)	6 (17.1)**	1,503 (10.6)	183 (18.2)**	2,031 (10.6)	3 (9.4)	1,836 (10.3)	192 (14.5)**

Data are given as median (interquartile range) for continuous variables and n(%) for categorical variables.
CS = caesarean section; FC = presumed fetal compromise; NND = neonatal death; HIE = hypoxic ischemic encephalopathy; NNU = neonatal unit; SGA = small for gestational age with birthweight <10th percentile; PE = preeclampsia; PI = pulsatility index; MoM = multiple of the median

Significance value * $p<0.05$; ** $p<0.01$

Table S1. Multivariable logistic regression analysis in prediction of adverse perinatal outcomes from maternal and pregnancy characteristics and biomarkers of impaired placentation.

Maternal and pregnancy characteristic	Stillbirth		CS for fetal compromise		NND or HIE 2/3		NNU admission	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Maternal age - 30 (years)			1.04 (1.02-1.05)	<0.001			0.97 (0.96-0.98)	<0.001
Maternal BMI - 30 (kg/m^2)	1.08 (1.02-1.31)	0.007	1.07 (1.06-1.08)	<0.001			1.06 (1.05-1.07)	<0.001
Cigarette smoker			1.56 (1.22-2.00)	<0.001				
Racial origin								
White								
Black			1.85 (1.55-2.21)	<0.001			0.70 (0.58-0.85)	<0.001
South Asian							1.30 (1.01-1.68)	<0.001
East Asian								
Mixed			1.59 (1.13-2.23)	0.007				
Conception								
Natural								
Ovulation induction drugs								
In vitro fertilization								
Obstetric history								
Nulliparous								
Parous, previous SGA	2.64 (1.02-6.84)	0.045	0.36 (0.26-0.50)	<0.001			0.53 (0.40-0.70)	<0.001
Parous, no previous SGA			0.23 (0.20-0.27)	<0.001	0.40 (0.18-0.89)	0.024	0.61 (0.54-0.69)	<0.001
Medical disorders								
Chronic hypertension			2.13 (1.33-3.42)	0.002				
Diabetes mellitus			2.72 (1.60-4.61)	<0.001				
Pregnancy complications								
Preeclampsia			2.62 (1.94-3.53)	<0.001	4.05 (1.22-13.43)	0.022	1.60 (1.19-2.14)	0.002
Gestational diabetes								
Obstetric cholestasis							3.06 (2.02-4.64)	<0.001
Biomarkers of impaired placentation								

Uterine artery PI >95th percentile								
Serum PLGF <5th percentile							1.50 (1.20-1.89)	<0.001
Serum sFLT-1 >95th percentile							1.55 (1.24-1.93)	<0.001
Estimated weight <10th percentile							1.25 (1.02-1.52)	0.034

OR = odds ratio; CI = confidence interval; BMI = body mass index; NND = neonatal death; HIE = hypoxic ischemic encephalopathy; NNU = neonatal unit; SGA = small for gestational age with birthweight <10th percentile; PE = preeclampsia; PI = pulsatility index; MoM = multiple of the median.

Table 2. Predictive performance of uterine artery pulsatility index >95th percentile for adverse perinatal outcome in small and non-small for gestational age neonates.

Adverse outcome	**Uterine artery pulsatility index >95th percentile**	
	BW ≥10th percentile	**BW <10th percentile**
Stillbirth (n=35)		
Detection rate	6.9 (4.3-9.3)	16.7 (11.7-21.5)
False positive rate	4.4 (3.0-5.9)	10.5 (6.4-14.6)
Relative risk	1.63 (0.39-6.83)	1.71 (0.20-14.57)
Positive likelihood ratio	1.59 (0.42-6.05)	1.59 (0.27-9.59)
Negative likelihood ratio	0.97 (0.88-1.07)	0.93 (0.65-1.33)
Cesarean section for fetal compromise (n=1,007)		
Detection rate	5.3 (4.2-6.4)	12.6 (10.8-14.4)
False positive rate	4.1 (3.0-5.1)	9.4 (7.9-10.9)
Relative risk	1.29 (0.96-1.62)	1.33 (0.88-1.99)
Positive likelihood ratio	1.30 (0.96-1.75)	1.33 (0.88-2.01)
Negative likelihood ratio	0.99 (0.97-1.01)	0.97 (0.91-1.02)
Neonatal death or HIE (n=32)		
Detection rate	10.3 (9.5-11.6)	0
False positive rate	4.3 (4.0-4.6)	10.5 (10.1-10.9)
Relative risk	2.53 (0.77-8.35)	1.22 (0.06-23.47)
Positive likelihood ratio	2..38 (0.81-6.97)	0
Negative likelihood ratio	0.94 (0.83-1.06)	1.12 (1.10-1.13)
Neonatal unit admission for 48 hours (n=1,323)		
Detection rate	5.8 (5.5-6.3)	13.5 (12.0-15.1)
False positive rate	4.2 (3.9-4.5)	10.1 (8.7-11.5)
Relative risk	1.36 (1.07-1.73)	1.28 (0.86-1.93)
Positive likelihood ratio	1.37 (1.08-1.76)	1.28 (0.86-1.90)
Negative likelihood ratio	0.98 (0.97-1.00)	0.97 (0.91-1.03)

Values in brackets are 95% confidence intervals

Table 3. Predictive performance of serum soluble fms-like tyrosine kinase-1 >95th percentile for adverse perinatal outcome in small and non-small for gestational age neonates.

Adverse outcome	**Soluble fms-like tyrosine kinase-1 >95th percentile**	
	BW ≥10th percentile	**BW <10th percentile**
Stillbirth (n=35)		
Detection rate	10.3 (9.3-11.3)	16.7 (15.0-18.4)
False positive rate	4.3 (4.0-4.6)	10.7 (9.3-12.1)
Relative risk	2.56 (0.78-8.44)	1.66 (0.19-14.12)
Positive likelihood ratio	2.40 (0.82-7.03)	1.55 (0.26-9.32)
Negative likelihood ratio	0.94 (0.83-1.06)	0.93 (0.65-1.34)
Cesarean section for fetal compromise (n=1,007)		
Detection rate	6.7 (6.3-7.1)	12.6 (11.1-14.2)
False positive rate	4.1 (3.8-4.4)	9.4 (8.0-10.8)
Relative risk	1.61 (1.24-2.0)	1.33 (0.88-1.99)
Positive likelihood ratio	1.63 (1.25-2.13)	1.33 (0.88-2.01)
Negative likelihood ratio	0.97 (0.96-0.99)	0.97 (0.91-1.02)
Neonatal death or HIE (n=32)		
Detection rate	6.9 (6.4-7.3)	33.3 (30.4-37.1)
False positive rate	4.3 (4.0-4.6)	10.7 (9.3-12.1)
Relative risk	1.64 (0.39-6.90)	4.14 (0.38-15.51)
Positive likelihood ratio	1.60 (0.42-6.11)	3.11 (0.62-15.46)
Negative likelihood ratio	0.97 (0.88-1.07)	0.75 (0.34-1.66)
Neonatal unit admission for 48 hours (n=1,323)		
Detection rate	7.2 (6.8-7.6)	21.9 (19.2-23.4)
False positive rate	4.1 (3.8-4.4)	9.6 (8.2-10.9)
Relative risk	1.72 (1.39-2.12)	2.32 (1.71-3.17)
Positive likelihood ratio	1.75 (1.40-2.18)	2.28 (1.69-3.09)
Negative likelihood ratio	0.97 (0.95-0.98)	0.86 (0.80-0.93)

Values in brackets are 95% confidence intervals

Table 4. Predictive performance of serum placental growth factor <5th percentile for adverse perinatal outcome in small and non-small for gestational age neonates.

Adverse outcome	Placental growth factor <5th percentile	
	BW ≥10th percentile	BW <10th percentile
Stillbirth (n=35)		
Detection rate	6.9 (6.6-7.2)	16.7 (15.0-18.5)
False positive rate	3.9 (3.6-4.2)	14.0 (12.4-15.6)
Relative risk	1.81 (0.43-7.60)	1.23 (0.14-10.47)
Positive likelihood ratio	1.76 (0.46-6.71)	1.19 (0.20-7.15)
Negative likelihood ratio	0.97 (0.88-1.07)	0.97 (0.68-1.39)
Cesarean section for fetal compromise (n=1,007)		
Detection rate	4.5 (4.2-4.8)	15.8 (13.7-17.1)
False positive rate	4.0 (3.7-4.3)	13.0 (11.4-14.7)
Relative risk	1.13 (0.83-1.55)	1.23 (0.85-1.78)
Positive likelihood ratio	1.13 (0.82-1.57)	1.22 (0.85-1.75)
Negative likelihood ratio	0.99 (0.98-1.01)	0.97 (0.91-1.03)
Neonatal death or HIE (n=32)		
Detection rate	3.4 (2.9-3.8)	0
False positive rate	3.9 (3.6-4.2)	14.0 (12.4-15.6)
Relative risk	0.87 (0.12-6.41)	2.04 (0.08-49.94)
Positive likelihood ratio	0.88 (0.13-6.03)	0
Negative likelihood ratio	1.01 (0.94-1.08)	1.16 (1.14-1.18)
Neonatal unit admission for 48 hours (n=1,323)		
Detection rate	6.2 (5.9-6.5)	20.8 (18.9-22.7)
False positive rate	3.8 (3.5-4.1)	13.3 (11.7-14.9)
Relative risk	1.61 (1.28-2.03)	1.62 (1.17-2.24)
Positive likelihood ratio	1.64 (1.29-2.09)	1.57 (1.16-2.11)
Negative likelihood ratio	0.97 (0.96-0.99)	0.91 (0.85-0.98)

Values in brackets are 95% confidence intervals