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

A. CIOBANOU[1], S. JABAK[1], H. DE CASTRO[1], L. FREI[1], R. AKOLEKAR[2,3] and K. 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*

KEYWORDS: Cesarean section; hypoxic ischemic encephalopathy; neonatal unit admission; perinatal death; perinatal hypoxia; placental growth factor; small-for-gestational age; soluble fms-like tyrosine kinase-1; stillbirth; third-trimester screening; uterine artery Doppler

ABSTRACT

Objective *To investigate the potential value of uterine artery pulsatility index (UtA-PI) and serum levels of the angiogenic placental growth factor (PlGF) and the 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 at 35–37 weeks' gestation.*

Methods *This was a prospective observational study of 19 209 singleton pregnancies attending for a routine hospital visit at 35 + 0 to 36 + 6 weeks' gestation. This visit included recording of maternal demographic characteristics and medical history, sonographic estimation of fetal weight, color Doppler ultrasound for measurement of mean UtA-PI, and measurement of serum concentrations of PlGF and sFlt-1. 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 delivery for suspected fetal compromise in labor; third, neonatal death or hypoxic ischemic encephalopathy Grade 2 or 3; and, fourth, admission to the neonatal unit (NNU) for ≥ 48 h. Predicted probabilities from logistic regression analysis were used to construct receiver–operating characteristics curves to assess the 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 h occurred in pregnancies with a non-SGA neonate. Second, UtA-PI > 95th percentile, sFlt-1 > 95th percentile and PlGF < 5th percentile were associated with increased risk of Cesarean delivery for suspected fetal compromise in labor and NNU admission for ≥ 48 h; 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 logistic regression analysis demonstrated that, in the prediction of Cesarean delivery for suspected fetal compromise in labor, there was no significant contribution from biomarkers; the prediction of NNU admission for ≥ 48 h by maternal demographic characteristics and medical history was only marginally improved by the addition of sFlt-1 or PlGF. Fourth, for each biomarker, the detection rate of adverse outcome was higher in SGA than in non-SGA neonates, but this increase was accompanied by an increase in false-positive rate. 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.*

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. Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

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) neonate[1,2]. Both

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, Fetal Medicine Research Institute, King's College Hospital, 16–20 Windsor Walk, Denmark Hill, London SE5 8BB, UK (e-mail: kypros@fetalmedicine.com)

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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].

SGA fetuses/neonates are at increased risk of adverse perinatal outcome, including stillbirth, Cesarean delivery for suspected fetal compromise and admission to the neonatal unit (NNU) for ≥ 48 h[7–10]. However, > 80% of adverse perinatal events at term occur in neonates with birth weight ≥ 10th percentile[7–10]. On the assumption that at least some of the adverse perinatal events in both SGA and non-SGA neonates are a consequence of failure to reach 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 to 36 + 6 weeks' gestation at King's College Hospital, London or Medway Maritime Hospital, Gillingham, UK between March 2014 and September 2018. This visit included recording of maternal demographic characteristics and medical history, ultrasound examination for assessment of fetal anatomy and measurement of fetal head circumference, abdominal circumference and femur length for calculation of estimated fetal weight (EFW)[11,12], transabdominal color Doppler ultrasound for measurement of mean UtA-PI[13], and measurement of serum concentrations of PlGF and sFlt-1 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). EFW was calculated utilizing the Hadlock formula[11], because a systematic review identified this as being the most accurate model[12]. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks or fetal head circumference at 19–24 weeks[14,15]. The women gave written informed consent to participate in this study, which was approved by the NHS Research Ethics Committee.

Inclusion and exclusion criteria

The inclusion criteria for this study were singleton pregnancy examined at 35 + 0 to 36 + 6 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality. Some of the patients in this study were included in a previous publication ($n = 3953$)[16].

Patient characteristics

Patient characteristics recorded included maternal age and 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 pregnancy at ≥ 24 weeks or parous with or without history of delivery of a SGA neonate with birth weight < 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 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 delivery for suspected fetal compromise in labor; third, neonatal death or hypoxic ischemic encephalopathy Grade 2 or 3; and, fourth, admission to the NNU for ≥ 48 h. We included neonatal death and hypoxic ischemic encephalopathy in the same group because they are both severe adverse neonatal events but the numbers of each were too small for separate analysis. Cesarean delivery for suspected fetal compromise in labor was carried out if there was evidence of a pathological electronic fetal heart-rate pattern, abnormality on ST waveform analysis of fetal electrocardiography 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-min 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 and neonate was based on EFW and birth weight, 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 chi-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 analyses were 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 maternal age, weight and height, were centered 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 into 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 percentile for UtA-PI and sFlt-1 and the 5th percentile for PlGF and determined the prevalence of abnormal values in each of the outcome groups. The values of UtA-PI $>95^{th}$ percentile, sFlt-1 $>95^{th}$ percentile and PlGF $<5^{th}$ percentile were used as binary categorical variables in the multivariable regression analysis. Predicted probabilities from logistic regression analysis were used to construct receiver–operating characteristics (ROC) curves to assess performance of screening for these adverse outcomes. We examined the detection rate, false-positive rate (FPR), relative risk and positive and negative likelihood ratios of UtA-PI $>95^{th}$, sFlt-1 $>95^{th}$ and PlGF $<5^{th}$ percentiles for each adverse perinatal outcome in the subgroups of SGA (birth weight $<10^{th}$ percentile) and non-SGA (birth weight $\geq 10^{th}$ percentile) fetuses and neonates.

The statistical package SPSS for Windows version 24.0 (IBM Corp., Armonk, NY, USA) was used for data analyses.

RESULTS

Study population

The demographic and clinical characteristics of the study population of 19 209 singleton pregnancies, comprising 19 174 live births and 35 stillbirths, are summarized in Table 1.

The 19 174 pregnancies with live birth included 14 170 (73.9%) with vaginal delivery following spontaneous or induced labor and 5004 (26.1%) with Cesarean delivery. The latter included, first, 72 for suspected fetal compromise before the onset of labor due to abnormal Doppler findings or fetal heart-rate patterns in SGA fetuses, second, 2201 with elective Cesarean section for a variety of indications (breech or transverse lie, placenta previa, previous Cesarean section or traumatic birth, maternal medical disorder or maternal request), and third, 2731 Cesarean sections following spontaneous or induced labor (1007 for suspected fetal compromise and 1724 for other indications, mainly failure to progress).

In the live births, there were two neonatal deaths and 30 cases with hypoxic ischemic encephalopathy Grade 2 or 3 and 1323 cases of NNU admission for ≥ 48 h (including the 30 cases of hypoxic ischemic encephalopathy).

Biomarkers in pregnancies with adverse outcome

Stillbirth

In pregnancies complicated by stillbirth, compared to those with live birth, there was higher median sFlt-1 MoM and a higher incidence of UtA-PI and sFlt-1 $>95^{th}$ percentile and birth weight $<10^{th}$ percentile (Table 1). Multivariable logistic regression analysis demonstrated that the only significant predictors of stillbirth were maternal body mass index and being parous with a previous delivery of a SGA neonate, but not UtA-PI $>95^{th}$, sFlt-1 $>95^{th}$, PlGF $<5^{th}$ or EFW $<10^{th}$ percentile ($R^2=0.018$; $P=0.010$; Table S1).

Cesarean delivery for suspected fetal compromise in labor

In pregnancies delivering by Cesarean delivery for suspected fetal compromise in labor, compared to those with vaginal delivery, there was, first, higher median maternal weight and body mass index, and a 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 $>95^{th}$ percentile, PlGF $<5^{th}$ percentile and birth weight $<10^{th}$ percentile (Table 1).

Multivariable regression analysis demonstrated that, for the prediction of Cesarean delivery for suspected 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 $>95^{th}$, sFlt-1 $>95^{th}$, PlGF $<5^{th}$ or EFW $<10^{th}$ percentile ($R^2=0.101$; $P<0.001$; Table S1).

Neonatal death or hypoxic ischemic encephalopathy

In pregnancies with neonatal death or hypoxic ischemic encephalopathy, compared to those without these adverse events, there was higher incidence of black racial origin and PE, and lower incidence of parous women without previous SGA. There was no significant difference in median UtA-PI MoM, sFlt-1 MoM and PlGF MoM, and there was no significant difference in the incidence of UtA-PI $>95^{th}$, sFlt-1 $>95^{th}$, PlGF $<5^{th}$ or EFW $<10^{th}$ percentile (Table 1).

Multivariable regression analysis demonstrated that, for the prediction of neonatal death or hypoxic ischemic encephalopathy, there was a statistically significant contribution from parity and PE, but not from UtA-PI $>95^{th}$, sFlt-1 $>95^{th}$, PlGF $<5^{th}$ or EFW $<10^{th}$ percentile ($R^2=0.022$; $P=0.006$; Table S1).

Neonatal unit admission for ≥ 48 h

In pregnancies with NNU admission for ≥ 48 h, compared to those without this adverse event, there was, first, higher median body mass index and lower maternal age, a higher incidence of smokers, women of white or South Asian racial origin, conception with use of ovulation induction drugs, diabetes mellitus, gestational diabetes, obstetric cholestasis and PE, and a lower incidence of parous women with or without previous SGA, second, higher

median sFlt-1 MoM and lower median PlGF MoM, and, third, a higher incidence of UtA-PI and sFlt-1 > 95th percentile, PlGF < 5th percentile, and birth weight < 10th percentile (Table 1).

Multivariable regression analysis demonstrated that, for the prediction of NNU admission for ≥ 48 h, 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 percentile, PlGF < 5th percentile and EFW < 10th percentile ($R^2 = 0.048$; $P < 0.001$; Table S1). There was a marginal improvement

Table 1 Maternal and pregnancy characteristics in 19 209 pregnancies, according to outcome

	Outcome		*Method of delivery*		*NND or HIE Grade 2 or 3*		*NNU admission for ≥ 48 h*	
Characteristic	*Live birth (n = 19 174)*	*Stillbirth (n = 35)*	*Vaginal (n = 14 170)*	*CS for FC (n = 1007)*	*No (n = 19 177)*	*Yes (n = 32)*	*No (n = 17 851)*	*Yes (n = 1323)*
Maternal age (years)	32.1 (28.0–35.7)	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 BMI (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	1319 (6.9)	5 (14.3)	1019 (7.2)	82 (8.1)	1323 (6.9)	1 (3.1)	1208 (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)	1082 (81.8)†
Black	2358 (12.3)	8 (22.9)	1669 (11.8)	186 (18.5)†	2359 (12.3)	7 (21.9)*	2228 (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)	1261 (95.3)
Ovulation induction	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	8997 (46.9)	13 (37.1)	6439 (45.4)	739 (73.4)†	8986 (46.9)	24 (75.0)	8215 (46.0)	782 (59.1)
Parous								
Previous SGA	1209 (6.3)	5 (14.3)	916 (6.5)	46 (4.6)*	1214 (6.3)	0	1154 (6.5)	55 (4.2)†
No previous SGA	8968 (46.8)	17 (48.6)	6815 (48.1)	222 (22.0)†	8977 (46.8)	8 (25.0)*	8482 (47.5)	486 (36.7)†
Medical disorder								
CH	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 complication								
Pre-eclampsia	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								
UtA-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)
UtA-PI > 95th	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	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	956 (5.0)	4 (11.4)*	661 (4.7)	78 (7.7)†	957 (5.0)	3 (9.4)	833 (4.7)	123 (9.3)†
EFW < 10th	1488 (7.8)	4 (11.4)	1165 (8.2)	97 (9.6)	1491 (7.8)	1 (3.1)	1367 (7.7)	121 (9.1)
GA at delivery (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	2028 (10.6)	6 (17.1)†	1503 (10.6)	183 (18.2)†	2031 (10.6)	3 (9.4)	1836 (10.3)	192 (14.5)†

Data are given as median (interquartile range) or *n* (%). Compared between outcome subgroups: *$P < 0.05$; †$P < 0.01$. 5th, 5th percentile; 10th, 10th percentile; 95th, 95th percentile; BMI, body mass index; CH, chronic hypertension; CS, Cesarean section; EFW, estimated fetal weight; FC, presumed fetal compromise in labor; GA, gestational age; HIE, hypoxic ischemic encephalopathy; MoM, multiples of the median; NND, neonatal death; NNU, neonatal unit; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; SGA, small-for-gestational age with birth weight < 10th percentile; UtA-PI, uterine artery pulsatility index.

in the prediction of admission to NNU for ≥ 48 h from the addition of biomarkers to maternal factors (area under the ROC curve, 0.641 (95% CI, 0.625–0.657) *vs* 0.634 (95% CI, 0.618–0.650); $P = 0.012$).

Cesarean delivery for suspected fetal compromise before onset of labor

Of the 72 cases of Cesarean delivery for suspected fetal compromise on antenatal testing, 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 birth weight 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 in SGA and non-SGA neonates is shown in Table 2.

Stillbirth

The incidence of stillbirth was 0.30% (6/2034) in neonates with birth weight < 10th percentile and 0.17% (29/17 175) in those with birth weight ≥ 10th percentile ($P = 0.207$). Consequently, 82.9% (29/35) of stillbirths occurred in non-SGA neonates.

UtA-PI was > 95th percentile in 8.6% (3/35) of pregnancies with stillbirth and in 5.0% (958/19 174) of those with live birth. PlGF was < 5th percentile in 8.6% (3/35) of pregnancies with stillbirth and in 5.0% (957/19 174) of those with live birth. sFlt-1 was > 95th percentile in 11.4% (4/35) of pregnancies with stillbirth and in 5.0% (956/19 174) of those with live birth.

The relative risk for stillbirth in the group with UtA-PI > 95th percentile was 1.71 (95% CI, 0.20–14.57) in SGA neonates and 1.63 (95% CI, 0.39–6.83) in non-SGA neonates. The relative risk for stillbirth in the group with sFlt-1 > 95th percentile was 1.66 (95% CI, 0.19–14.12) in SGA neonates and 2.56 (95% CI, 0.78–8.44) in non-SGA neonates. The relative risk for stillbirth in the group with PlGF < 5th percentile was 1.23 (95% CI, 0.14–10.47) in SGA neonates and 1.81 (95% CI, 0.43–7.60) in non-SGA neonates (Table 2).

Cesarean delivery for suspected fetal compromise

The incidence of Cesarean delivery for suspected fetal compromise in labor in live births was 10.9% (183/1686) in neonates with birth weight < 10th percentile and 6.1% (824/13 491) in those with birth weight ≥ 10th percentile ($P < 0.001$). Consequently, 81.8% (824/1007) of Cesarean sections for presumed fetal compromise occurred in non-SGA neonates.

UtA-PI was > 95th percentile in 6.7% (67/1007) of pregnancies delivered by Cesarean delivery for suspected

Table 2 Predictive performance of uterine artery pulsatility index (UtA-PI) > 95th percentile, serum soluble fms-like tyrosine kinase-1 (sFlt-1) > 95th percentile and serum placental growth factor (PlGF) < 5th percentile for adverse perinatal outcome in small-for-gestational-age (SGA) and non-SGA neonates

	UtA-PI > 95th percentile		*sFlt-1 > 95th percentile*		*PlGF < 5th percentile*	
Adverse outcome	*Non-SGA*	*SGA*	*Non-SGA*	*SGA*	*Non-SGA*	*SGA*
Stillbirth ($n = 35$)						
Detection rate (%)	6.9 (4.3–9.3)	16.7 (11.7–21.5)	10.3 (9.3–11.3)	16.7 (15.0–18.4)	6.9 (6.6–7.2)	16.7 (15.0–18.5)
False-positive rate (%)	4.4 (3.0–5.9)	10.5 (6.4–14.6)	4.3 (4.0–4.6)	10.7 (9.3–12.1)	3.9 (3.6–4.2)	14.0 (12.4–15.6)
Relative risk	1.63 (0.39–6.83)	1.71 (0.20–14.57)	2.56 (0.78–8.44)	1.66 (0.19–14.12)	1.81 (0.43–7.60)	1.23 (0.14–10.47)
LR+	1.59 (0.42–6.05)	1.59 (0.27–9.59)	2.40 (0.82–7.03)	1.55 (0.26–9.32)	1.76 (0.46–6.71)	1.19 (0.20–7.15)
LR−	0.97 (0.88–1.07)	0.93 (0.65–1.33)	0.94 (0.83–1.06)	0.93 (0.65–1.34)	0.97 (0.88–1.07)	0.97 (0.68–1.39)
CS for fetal compromise ($n = 1007$)						
Detection rate (%)	5.3 (4.2–6.4)	12.6 (10.8–14.4)	6.7 (6.3–7.1)	12.6 (11.1–14.2)	4.5 (4.2–4.8)	15.8 (13.7–17.1)
False-positive rate (%)	4.1 (3.0–5.1)	9.4 (7.9–10.9)	4.1 (3.8–4.4)	9.4 (8.0–10.8)	4.0 (3.7–4.3)	13.0 (11.4–14.7)
Relative risk	1.29 (0.96–1.62)	1.33 (0.88–1.99)	1.61 (1.24–2.0)	1.33 (0.88–1.99)	1.13 (0.83–1.55)	1.23 (0.85–1.78)
LR+	1.30 (0.96–1.75)	1.33 (0.88–2.01)	1.63 (1.25–2.13)	1.33 (0.88–2.01)	1.13 (0.82–1.57)	1.22 (0.85–1.75)
LR−	0.99 (0.97–1.01)	0.97 (0.91–1.02)	0.97 (0.96–0.99)	0.97 (0.91–1.02)	0.99 (0.98–1.01)	0.97 (0.91–1.03)
Neonatal death or HIE ($n = 32$)						
Detection rate (%)	10.3 (9.5–11.6)	0	6.9 (6.4–7.3)	33.3 (30.4–37.1)	3.4 (2.9–3.8)	0
False-positive rate (%)	4.3 (4.0–4.6)	10.5 (10.1–10.9)	4.3 (4.0–4.6)	10.7 (9.3–12.1)	3.9 (3.6–4.2)	14.0 (12.4–15.6)
Relative risk	2.53 (0.77–8.35)	1.22 (0.06–23.47)	1.64 (0.39–6.90)	4.14 (0.38–15.51)	0.87 (0.12–6.41)	2.04 (0.08–49.94)
LR+	2.38 (0.81–6.97)	0	1.60 (0.42–6.11)	3.11 (0.62–15.46)	0.88 (0.13–6.03)	0
LR−	0.94 (0.83–1.06)	1.12 (1.10–1.13)	0.97 (0.88–1.07)	0.75 (0.34–1.66)	1.01 (0.94–1.08)	1.16 (1.14–1.18)
NNU admission for ≥ 48 h ($n = 1323$)						
Detection rate (%)	5.8 (5.5–6.3)	13.5 (12.0–15.1)	7.2 (6.8–7.6)	21.9 (19.2–23.4)	6.2 (5.9–6.5)	20.8 (18.9–22.7)
False-positive rate (%)	4.2 (3.9–4.5)	10.1 (8.7–11.5)	4.1 (3.8–4.4)	9.6 (8.2–10.9)	3.8 (3.5–4.1)	13.3 (11.7–14.9)
Relative risk	1.36 (1.07–1.73)	1.28 (0.86–1.93)	1.72 (1.39–2.12)	2.32 (1.71–3.17)	1.61 (1.28–2.03)	1.62 (1.17–2.24)
LR+	1.37 (1.08–1.76)	1.28 (0.86–1.90)	1.75 (1.40–2.18)	2.28 (1.69–3.09)	1.64 (1.29–2.09)	1.57 (1.16–2.11)
LR−	0.98 (0.97–1.00)	0.97 (0.91–1.03)	0.97 (0.95–0.98)	0.86 (0.80–0.93)	0.97 (0.96–0.99)	0.91 (0.85–0.98)

Values in parentheses are 95% CI. CS, Cesarean section; HIE, hypoxic ischemic encephalopathy; LR+/−, positive/negative likelihood ratio; NNU, neonatal unit.

fetal compromise and in 4.7% (664/14 170) of those with vaginal delivery. PlGF was $< 5^{th}$ percentile in 6.6% (66/1007) of pregnancies delivered by Cesarean delivery for suspected fetal compromise and in 4.9% (697/14 170) of those with vaginal delivery. sFlt-1 was $> 95^{th}$ percentile in 7.7% (78/1007) of pregnancies delivered by Cesarean delivery for suspected fetal compromise and in 4.7% (661/14 170) of those with vaginal delivery.

The relative risk for delivery by Cesarean delivery for suspected fetal compromise in the group with UtA-PI $> 95^{th}$ percentile was 1.33 (95% CI, 0.88–1.99) in SGA neonates and 1.29 (95% CI, 0.96–1.62) in non-SGA neonates. The relative risk for delivery by Cesarean delivery for suspected fetal compromise in the group with sFlt-1 $> 95^{th}$ percentile was 1.33 (95% CI, 0.88–1.99) in SGA neonates and 1.61 (95% CI, 1.24–2.0) in non-SGA neonates. The relative risk for delivery by Cesarean delivery for suspected fetal compromise in the group with PlGF $< 5^{th}$ percentile was 1.23 (95% CI, 0.85–1.78) in SGA neonates and 1.13 (95% CI, 0.83–1.55) in non-SGA neonates (Table 2).

Neonatal death or hypoxic ischemic encephalopathy

The incidence of neonatal death or hypoxic ischemic encephalopathy was 0.10% (3/2034) in neonates with birth weight $< 10^{th}$ percentile and 0.17% (29/17 175) in those with birth weight $\geq 10^{th}$ percentile ($P = 0.556$). Consequently, 90.6% (29/32) of cases of neonatal death or hypoxic ischemic encephalopathy occurred in non-SGA neonates.

UtA-PI was $> 95^{th}$ percentile in 9.4% (3/32) of cases with neonatal death or hypoxic ischemic encephalopathy and in 5.0% (958/19 177) of those without this adverse outcome. PlGF was $< 5^{th}$ percentile in 3.1% (1/32) of cases with neonatal death or hypoxic ischemic encephalopathy and in 5.0% (959/19 177) of those without this adverse outcome. sFlt-1 was $> 95^{th}$ percentile in 9.4% (3/32) of cases with 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 $> 95^{th}$ percentile was 1.22 (95% CI, 0.06–23.47) in SGA neonates and 2.53 (95% CI, 0.77–8.35) in non-SGA neonates. The relative risk for neonatal death or hypoxic ischemic encephalopathy in the group with sFlt-1 $> 95^{th}$ percentile was 4.14 (95% CI, 0.38–15.51) in SGA neonates and 1.64 (95% CI, 0.39–6.90) in non-SGA neonates. The relative risk for neonatal death or hypoxic ischemic encephalopathy in the group with PlGF $< 5^{th}$ percentile was 2.04 (95% CI, 0.08–49.94) in SGA neonates and 0.87 (95% CI, 0.12–6.41) in non-SGA neonates (Table 2).

Neonatal unit admission for ≥ 48 h

The incidence of NNU admission for ≥ 48 hours was 9.5% (192/2028) in neonates with birth weight $< 10^{th}$ percentile and 6.6% (1131/17 145) in those with birth weight $\geq 10^{th}$ percentile ($P < 0.001$). Consequently, 85.5% (1131/1323) of cases of NNU admission for ≥ 48 h occurred in non-SGA neonates.

UtA-PI was $> 95^{th}$ percentile in 7.0% (92/1323) of cases with NNU admission for ≥ 48 h and in 4.9% (866/17 851) of those without this adverse outcome. PlGF was $< 5^{th}$ percentile in 8.3% (110/1323) of cases with NNU admission for ≥ 48 h and in 4.7% (847/17 851) of those without this adverse outcome. sFlt-1 was $> 95^{th}$ percentile in 9.3% (123/1323) of cases with NNU admission for ≥ 48 h and in 4.7% (833/17 851) of those without this adverse outcome.

The relative risk for NNU admission for ≥ 48 h in the group with UtA-PI $> 95^{th}$ percentile was 1.28 (95% CI, 0.86–1.93) in SGA neonates and 1.36 (95% CI, 1.07–1.73) in non-SGA neonates. The relative risk for NNU admission for ≥ 48 h in the group with sFlt-1 $> 95^{th}$ percentile was 2.32 (95% CI, 1.71–3.17) in SGA neonates and 1.72 (95% CI, 1.39–2.12) in non-SGA neonates. The relative risk for NNU admission for ≥ 48 h in the group with PlGF $< 5^{th}$ percentile was 1.62 (95% CI, 1.17–2.24) in SGA neonates and 1.61 (95% CI, 1.28–2.03) in non-SGA neonates (Table 2).

DISCUSSION

Main findings

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 h occurred in pregnancies with a non-SGA neonate. Second, UtA-PI $> 95^{th}$ percentile, sFlt-1 $> 95^{th}$ percentile and PlGF $< 5^{th}$ percentile were associated with increased risk of Cesarean delivery for suspected fetal compromise in labor and NNU admission for ≥ 48 h; 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 the prediction of Cesarean delivery for suspected fetal compromise in labor, there was no significant contribution from biomarkers; the prediction of NNU admission for ≥ 48 h by maternal demographic characteristics and medical history was only marginally improved by the addition of 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 FPR. Fifth, the relative risk of UtA-PI $> 95^{th}$, sFlt-1 $> 95^{th}$ and PlGF $< 5^{th}$ percentile 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 does 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 8268 pregnancies at 30–34 weeks' gestation and 3953 at 35–37 weeks for prediction of adverse perinatal outcome; in both studies, these markers were useful in the prediction of PE and delivery of a SGA neonate, but not of adverse events in labor or after delivery[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 level was associated with low birth weight and adverse intrapartum and neonatal outcomes, but the predictive performance of low PlGF was poor, with detection rate of 10–11% at FPR of 10%[22]. In contrast, a prospective study in 3747 singleton pregnancies of nulliparous women reported that elevated sFlt-1/PlGF ratio (> 85th percentile) at 36 weeks' gestation in combination with EFW < 10th percentile predicted 38% of adverse perinatal outcomes, at a 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 delivery for suspected fetal compromise in labor, neonatal death or hypoxic ischemic encephalopathy, or NNU admission for ≥ 48 h. 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.

Two studies examined the possible value of UtA-PI in the prediction of adverse perinatal outcome. The first study examined 30 780 pregnancies undergoing routine screening at 30–34 weeks' gestation and reported that UtA-PI > 95th percentile may be useful in the prediction of adverse perinatal outcome in SGA fetuses, but not in non-SGA fetuses[25]. The second study examined 946 low-risk pregnancies at 37 weeks' gestation and reported that UtA-PI did not improve the prediction of adverse perinatal outcome provided by EFW alone[26].

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 a SGA neonate[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

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 study and most previous ones investigating 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 in cases with suspected SGA and fetal compromise. In our study, 72 such pregnancies had prelabor 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 or NNU admission. Consequently, the performance of screening by UtA-PI, sFlt-1 and PlGF for adverse perinatal outcome in SGA fetuses could 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 provide poor prediction of stillbirth, Cesarean delivery for suspected fetal compromise in labor, NNU admission for ≥ 48 h and neonatal death or hypoxic ischemic encephalopathy in both SGA and non-SGA fetuses.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

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