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Biophysical and biochemical markers at 35–37 weeks' gestation in the prediction of adverse perinatal outcome

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ABSTRACT

Objective *To investigate the potential value of biophysical and biochemical markers at 35–37 weeks' gestation in the prediction of adverse perinatal outcome.*

Methods *This was a screening study in 3953 singleton pregnancies at 35–37 weeks' gestation. Estimated fetal weight (EFW), uterine artery pulsatility index (UtA-PI), umbilical artery (UA)-PI, fetal middle cerebral artery (MCA)-PI, mean arterial pressure (MAP), serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured. The detection rate (DR) and false-positive rate (FPR) of screening by each biomarker were estimated for pre-eclampsia (PE), delivery of small-for-gestational-age (SGA) neonates, Cesarean section for fetal distress before or during labor, umbilical arterial cord blood pH ≤ 7.0 or umbilical venous cord blood pH ≤ 7.1, 5-min Apgar score < 7 and admission to the neonatal unit (NNU).*

Results *Multivariable regression analysis demonstrated that significant prediction of PE was provided by PlGF, sFlt-1 and MAP, with a DR of 73% at a 10% FPR. Prediction of SGA was provided by EFW, PlGF and UtA-PI, with a DR of 63% at a 10% FPR. Prediction of Cesarean section for fetal distress before labor was provided by EFW and UA-PI with DR of 100% at 10% FPR. Prediction of fetal distress in labor was provided by EFW and sFlt-1, with a DR of 15% at a 10% FPR. There were no significant differences between those with a normal outcome and those with low cord blood pH, low Apgar score or NNU admission for any of the biomarkers assessed.*

Conclusion *At 35–37 weeks' gestation biomarkers of impaired placentation and fetal hypoxemia provide good prediction of PE, SGA and fetal distress before labor, but poor or no prediction of adverse events in labor or after birth.*

INTRODUCTION

Impaired placentation and/or placental dysfunction, reflected in an increased pulsatility index (PI) in the uterine arteries (UtAs), reduced serum placental growth factor (PlGF) and increased soluble fms-like tyrosine kinase-1 (sFlt-1), are associated with the subsequent development of pre-eclampsia (PE) and the birth of a small-for-gestational-age (SGA) neonate[1–3]. In the 1980s, analysis of fetal blood obtained by cordocentesis demonstrated that some SGA fetuses are hypoxemic and acidemic and have a metabolic profile compatible with intrauterine starvation[4–6]. In SGA fetuses, hypoxemia was found to be associated with increased impedance to flow in the UtA and umbilical arteries (UAs) and reduced impedance in the fetal middle cerebral artery (MCA)[7–9]. It was recognized that the majority of fetuses that are growth restricted due to uteroplacental insufficiency may not actually be SGA[10] and it was speculated that if the biomarkers of fetal hypoxia are as useful in identifying affected fetuses that are not small as they are for identifying SGA fetuses, the biomarkers may replace measurement of fetal size for the antenatal prediction of fetal asphyxia[7].

A large screening study at 30–34 weeks' gestation reported that biomarkers of impaired placentation/placental dysfunction and fetal hypoxemia are useful in the prediction of PE and the birth of SGA neonates[11]. However, the biomarkers provide poor or no prediction of adverse events in labor or after birth, including Cesarean section for fetal distress, low cord blood pH, low Apgar score or neonatal unit (NNU) admission for cases other than those with PE and/or SGA[11]. Possible explanations

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for poor performance of screening at 30–34 weeks in prediction of adverse events in labor or after birth are, first, the premise that non-SGA starved fetuses are as hypoxic as SGA starved fetuses is not true[12], second, the outcome measures are primarily the consequence of problems which start in labor and therefore cannot be predicted using markers of chronic fetoplacental hypoxia, and third, assessment at 30–34 weeks is too remote from events in labor and that assessment at 35–37 weeks may improve the screening performance for labor-related adverse outcomes.

The objective of this screening study was to investigate the potential value of UtA-PI, UA-PI, MCA-PI, mean arterial pressure (MAP) and serum levels of PlGF and sFlt-1, at 35–37 weeks' gestation, in the prediction of adverse perinatal outcomes, including development of PE, birth of an SGA neonate, Cesarean section for fetal distress before or during labor, umbilical arterial cord blood pH ≤ 7.0 or umbilical venous cord blood pH ≤ 7.1, 5-min Apgar score < 7 and NNU admission.

METHODS

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit in the third trimester of pregnancy at King's College Hospital, London, and Medway Maritime Hospital, Gillingham, Kent, between February 2014 and December 2014. This visit, which is attended at 35 + 0 to 37 + 6 weeks' gestation, included the recording of maternal characteristics and medical history, estimation of fetal size from ultrasound measurement of fetal head circumference, abdominal circumference and femur length[13], transabdominal Doppler assessment of the UtA-PI, UA-PI and fetal MCA-PI[14–16], measurement of MAP[17] and serum concentrations of PlGF and sFlt-1 (Cobas e411 system, Roche Diagnostics, Penzberg, 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[13,18].

Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the ethics committee of each participating hospital. The pregnancies included in the study were those with data available on all eight biomarkers and resulted in the live birth or stillbirth of a phenotypically normal baby at ≥ 24 weeks' gestation.

Patient characteristics

Patient characteristics that were recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous/use of ovulation drugs/*in-vitro* fertilization), cigarette smoking during pregnancy, medical history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS) and parity (parous/nulliparous if no previous pregnancies ≥ 24 weeks). Maternal weight and height were also measured.

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The outcome measures of the study were PE, SGA, Cesarean section for fetal distress before or during labor, umbilical arterial cord blood pH ≤ 7.0, umbilical venous cord blood pH ≤ 7.1, 5-min Apgar score < 7 and admission to NNU. The newborn was considered to be SGA if the birth weight was < 10th percentile after correcting for gestational age at delivery[19]. The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy[20].

Statistical analysis

Comparison between the outcome groups was performed by chi-square test or Fisher's exact test for categorical variables and Mann–Whitney *U*-test for continuous variables. Categorical data are presented as *n* (%) and continuous data as median (interquartile range (IQR)).

The measured values of UtA-PI, UA-PI, MCA-PI, MAP, PlGF and sFlt-1 were expressed as multiples of the median (MoM) after adjustment for variables from maternal characteristics and medical history that affect these measurements[21–25]. Univariable and multivariable logistic regression analyses were used to determine if $\log_{10}$MoM of each biomarker had a significant contribution in predicting each adverse outcome. The detection rate (DR) and false-positive rate (FPR) of screening were estimated for each adverse outcome. The performance of screening was determined by receiver–operating characteristics (ROC) curve analysis.

The statistical software package SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp, Armonk, NY, USA) was used for the data analyses.

RESULTS

Study population

During the study period, 3953 women with a singleton pregnancy were examined and included in the analyses. The characteristics of the study population and the various subgroups according to adverse perinatal outcome are given in Table 1.

Biomarkers in pregnancies with PE or birth of SGA neonate

For pregnancies resulting in PE or the birth of a SGA neonate, the median MoM values of each biomarker and the proportion of pregnancies with values above or below a cut-off percentile were compared to those of unaffected pregnancies with normal outcome (Table 2). Compared to the unaffected group, pregnancies that developed PE had significantly higher UtA-PI, MAP and serum sFlt-1 and lower serum PlGF (Table 2). Multivariable logistic regression analysis demonstrated that significant

Table 1 Maternal and pregnancy characteristics of study population of 3953 women with singleton pregnancy and those subgroups with adverse perinatal outcome of pre-eclampsia (PE), small-for-gestational-age (SGA) neonate, fetal distress before or during labor leading to Cesarean section, low umbilical arterial or venous cord blood pH, 5-min Apgar score < 7 or admission to neonatal unit (NNU)

Characteristic	*All pregnancies (n = 3953)*	*PE (n = 65)*	*SGA (n = 379)*	*Prelabor fetal distress (n = 7)*	*Intrapartum fetal distress (n = 275)*	*Low cord blood pH (n = 52)*	*Low Apgar score (n = 20)*	*NNU admission (n = 232)*
Maternal age (years)	37.7 (26.9–35.3)	33.1 (27.8–35.8)	30.0 (25.0–34.3)	25.9 (22.0–38.8)	32.0 (27.0–35.4)	31.6 (27.5–35.3)	29.8 (27.5–34.5)	31.4 (26.3–35.1)
Maternal weight (kg)	79.0 (70.9–89.6)	85.0 (77.0–98.9)	73.1 (65.1–82.5)	71.0 (64.3–82.3)	81.6 (73.5–91.5)	78.0 (71.6–87.8)	76.0 (69.2–86.5)	82.2 (72.0–95.0)
Maternal height (m)	1.64 (1.60–1.69)	1.65 (1.61–1.72)	1.62 (1.58–1.66)	1.59 (1.55–1.63)	1.62 (1.58–1.66)	1.62 (1.59–1.67)	1.62 (1.57–1.65)	1.64 (1.59–1.69)
Cigarette smoker	365 (9.2)	3 (4.6)	70 (18.5)†	2 (28.6)	27 (9.8)	5 (9.6)	3 (15.0)	18 (7.8)
Racial origin								
Caucasian	2921 (73.9)	47 (72.3)	237 (62.5)	4 (57.1)	174 (63.3)	40 (76.9)	17 (85.0)	184 (79.3)
Afro-Caribbean	679 (17.2)	11 (16.9)	93 (24.5)†	3 (42.9)	73 (26.5)†	7 (13.5)	3 (15.0)	39 (16.8)
South Asian	146 (3.7)	3 (4.6)	29 (7.7)	0 (0.0)	13 (4.7)	4 (7.7)	0 (0.0)	4 (1.7)
East Asian	87 (2.2)	1 (1.5)	6 (1.6)	0 (0.0)	6 (2.2)	0 (0.0)	0 (0.0)	2 (0.9)
Mixed	120 (3.0)	3 (4.6)	14 (3.7)	0 (0.0)	9 (3.3)	1 (1.9)	0 (0.0)	3 (1.3)
Conception								
Spontaneous	3839 (97.1)	59 (90.8)	368 (97.1)	7 (100.0)	263 (95.6)	52 (100.0)	19 (95.0)	225 (97.0)
Assisted	114 (2.9)	6 (9.2)	11 (2.9)	0 (0.0)	12 (4.4)	0 (0.)	1 (5.0)	7 (3.0)
Medical disorder								
Chronic hypertension	55 (1.4)	5 (7.7)*	6 (1.6)	0 (0.0)	11 (4.0)†	1 (1.9)	1 (5.0)	9 (3.9)*
SLE/APS	11 (0.3)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Diabetes mellitus	47 (1.2)	2 (3.1)	1 (0.3)	0 (0.0)	2 (0.7)	0 (0.0)	0 (0.0)	3 (1.3)
Obstetric history								
Parous	1987 (50.3)	17 (26.2)	146 (38.5)	5 (71.4)	67 (24.4)	16 (30.8)	9 (45.0)	97 (41.8)
Nulliparous	1966 (49.7)	48 (73.8)†	233 (61.5)†	2 (28.6)	208 (75.6)†	36 (69.2)	11 (55.0)	135 (58.2)
Pregnancy complication								
Gestational diabetes	115 (2.9)	3 (4.6)	9 (2.4)	0 (0.0)	13 (4.7)	2 (3.8)	0 (0.0)	11 (4.7)
SROM	201 (5.1)	1 (1.5)	25 (6.6)	0 (0.0)	44 (16.0)†	4 (7.7)	1 (5.0)	22 (9.5)*
Onset of labor and mode of delivery								
Spontaneous labor								
Vaginal delivery	2461 (62.3)	3 (4.6)	232 (61.2)	0 (0.0)	0 (0.0)	22 (42.3)	10 (50.0)	116 (50.0)
Cesarean section	303 (7.7)	5 (7.7)	28 (7.4)	0 (0.0)	164 (59.6)	12 (23.1)	2 (10.0)	31 (13.4)*
Induced labor								
Vaginal delivery	556 (14.1)	30 (46.2)†	70 (18.5)	0 (0.0)	0 (0.0)	7 (13.5)	0 (0.0)	38 (16.4)
Cesarean section	197 (5.0)	14 (21.5)†	20 (5.3)	0 (0.0)	111 (40.4)†	6 (11.5)	5 (25.0)	21 (9.1)*
Elective Cesarean section	436 (11.0)	13 (20.0)	29 (7.7)	7 (100.0)	0 (0.0)	5 (9.6)	3 (15.0)	26 (11.2)
Assessment								
GA at assessment (weeks)	36.1 (36.0–36.4)	36.1 (35.9–36.4)	36.1 (36.0–36.6)	36.3 (35.4–36.4)	36.1 (36.0–36.4)	36.3 (36.0–36.4)	36.1 (36.0–36.6)	36.1 (35.9–36.4)
EFW percentile	49.7 (25.7–73.3)	47.8 (26.4–79.4)	12.2 (4.2–24.8)	0.6 (0.3–2.0)	55.5 (29.3–76.2)	50.2 (26.5–73.7)	59.1 (30.8–67.5)	55.5 (28.1–78.6)
Outcome								
GA at delivery (weeks)	40.0 (39.1–40.9)	39.1 (37.4–40.4)	39.9 (39.0–40.9)	37.0 (35.6–37.3)	40.6 (39.6–41.3)	40.4 (39.4–41.0)	40.1 (38.9–40.9)	39.7 (38.5–40.9)
Birth-weight percentile	48.7 (24.4–74.8)	42.7 (19.8–70.8)	5.9 (3.2–8.1)	3.5 (1.8–7.5)	42.1 (17.7–73.3)	39.1 (17.9–63.0)	38.0 (18.5–69.4)	54.9 (21.0–83.0)

Data are given as median (interquartile range) for continuous variables and *n* (%) for categorical variables. SGA defined as birth weight < 10th percentile. Significant difference from cohort without adverse outcome adjusted for multiple comparisons with *post-hoc* Bonferroni correction: *$P < 0.01$; †$P < 0.001$. APS, antiphospholipid syndrome; EFW, estimated fetal weight; GA, gestational age; SLE, systemic lupus erythematosus; SROM, spontaneous rupture of membranes.

Table 2 Biochemical and biophysical markers in pregnancies resulting in pre-eclampsia (PE), birth of small-for-gestational-age (SGA) neonate and those unaffected by these adverse outcomes

Biomarker	*Unaffected* (n = *3509*)	*PE* (n = *65*)	*SGA* (n = *379*)
Biochemical marker			
PlGF (MoM)	1.02 (0.59–1.79)	0.34 (0.24–0.48)†	0.66 (0.37–1.15)†
PlGF < 5th percentile	132 (3.8)	18 (27.7)†	48 (12.7)†
sFlt-1 (MoM)	0.97 (0.73–1.35)	2.08 (1.45–3.24)†	0.94 (0.71–1.37)
sFlt-1 > 95th percentile	141 (4.0)	29 (44.6)†	28 (7.4)*
Biophysical marker			
UtA-PI (MoM)	0.97 (0.83–1.15)	1.15 (0.89–1.40)†	1.06 (0.86–1.29)†
UtA-PI > 95th percentile	150 (4.3)	11 (16.9)†	37 (9.8)†
UA-PI (MoM)	1.00 (0.90–1.13)	0.99 (0.89–1.10)	1.07 (0.97–1.18)†
UA-PI > 95th percentile	147 (4.2)	3 (4.6)	48 (12.7)†
MCA-PI (MoM)	1.01 (0.90–1.13)	0.96 (0.87–1.11)	0.99 (0.89–1.11)
MCA-PI < 5th percentile	169 (4.8)	7 (10.8)	22 (5.8)
MAP (MoM)	1.00 (0.95–1.05)	1.09 (1.03–1.17)†	1.02 (0.96–1.08)†
MAP > 95th percentile	159 (4.5)	19 (29.2)†	20 (5.3)
EFW percentile	53.5 (30.8–75.4)	47.8 (26.3–79.4)	12.2 (4.2–24.5)†
EFW < 5th percentile	51 (1.5)	4 (6.2)*	104 (27.4)†

Data are given as median (interquartile range) or *n* (%). SGA defined as birth weight < 10th percentile. Significant difference from cohort without adverse outcome adjusted for multiple comparisons with *post-hoc* Bonferroni correction: *$P < 0.01$; †$P < 0.0001$. EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

contributions to prediction of PE were provided by MAP, sFlt-1 and PlGF, (area under ROC curve (AUC), 0.913 (95% CI, 0.874–0.952)). The DR of combined screening by all significant contributors was 73.3%, at a FPR of 10% (Figure 1a).

Compared to the unaffected group, pregnancies that delivered a SGA neonate had significantly higher UtA-PI, UA-PI and MAP and lower EFW and serum PlGF (Table 2). Multivariable logistic regression analysis demonstrated that significant contributions to prediction of SGA were provided by EFW, PlGF and UtA-PI (AUC, 0.883 (95% CI, 0.867–0.899)). The DR of combined screening by all significant contributors was 62.8%, at a FPR of 10% (Figure 1a).

Biomarkers in pregnancies with stillbirth

In the study population there was one stillbirth. The interval between assessment and delivery was 6 + 0 weeks. There were no risk factors noted in the maternal demographic characteristics as the pregnancy was in a nulliparous woman following a spontaneous conception, who was a non-smoker with a body mass index within normal range, no medical illness of note and no obstetric complications in the current pregnancy. The UtA-PI, MAP and serum sFlt-1 were < 95th percentile and serum PlGF was > 5th percentile. The estimated birth-weight *Z*-score was −2.4.

Biomarkers in pregnancies delivered by Cesarean section for fetal distress

In the 3952 pregnancies with live births, there were 3016 with vaginal delivery following spontaneous or induced labor, 436 with elective Cesarean section for a variety of indications and 500 with Cesarean section following spontaneous or induced labor; in the latter group the indication for Cesarean section was fetal distress in 275 cases. In the elective Cesarean-section group ($n = 436$) there were a variety of indications, including breech or transverse lie ($n = 83$), placenta previa ($n = 22$), previous Cesarean section, traumatic birth or maternal request ($n = 291$), maternal medical disorder ($n = 33$) and SGA fetuses with fetal compromise diagnosed by abnormal fetal heart rate patterns or fetal Doppler indices ($n = 7$).

For pregnancies resulting in delivery by Cesarean section for fetal distress, before labor ($n = 7$) or during labor ($n = 275$), the median MoM values of each biomarker and the proportion of pregnancies with values above or below a cut-off percentile were compared to those of pregnancies that delivered vaginally (Table 3).

Compared to those that delivered vaginally, the group that underwent Cesarean section for fetal distress before labor showed significant alteration in all biomarkers (Table 3). In the study population for assessment of fetal distress during labor, the median interval from assessment to delivery was 4.0 (IQR, 3.0–4.7) weeks. Compared to those that delivered vaginally, the group with Cesarean section for fetal distress in labor showed higher EFW and serum sFlt-1, but no significant differences for the median MoM of the other biomarkers were seen. Multivariable logistic regression analysis demonstrated that significant contribution to prediction of Cesarean section for fetal distress was provided by EFW and sFlt-1 (AUC, 0.566 (95% CI, 0.531–0.601)). The DR was 15.3%, at a 10% FPR (Figure 1b).

Biomarkers in pregnancies with adverse outcome after delivery

The median MoM values of biochemical and biophysical markers, and the proportion of pregnancies with values

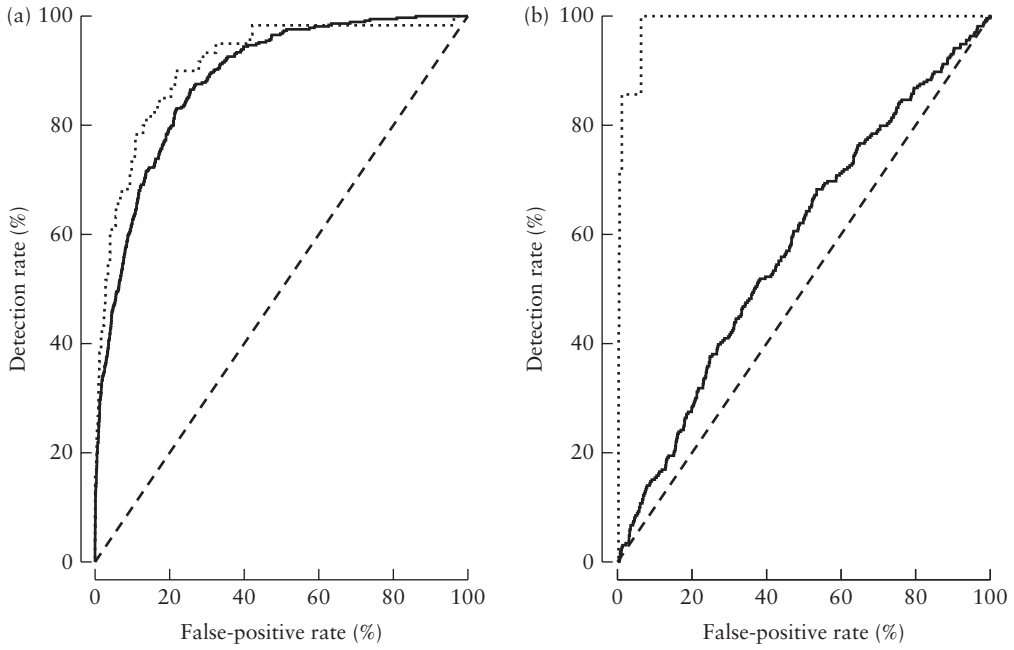

Figure 1 Receiver–operating characteristics curves for prediction of: (a) pre-eclampsia (·····) and small-for-gestational-age neonate (——); and (b) fetal distress before (·····) and during (——) labor resulting in delivery by Cesarean section, using a combination of significant contributors from biophysical and biochemical markers at 35–37 weeks' gestation.

Table 3 Biochemical and biophysical markers in pregnancies delivered by Cesarean section (CS) for fetal distress occurring during or before labor and those that delivered vaginally

		CS for fetal distress	
Biomarker	*Vaginal delivery (n = 3017)*	*During labor (n = 275)*	*Before labor (n = 7)*
Biochemical marker			
PlGF (MoM)	0.98 (0.55–1.68)	0.90 (0.49–1.76)	0.34 (0.10–1.36)†
PlGF < 5th percentile	141 (4.7)	25 (9.1)†	4 (57.1)‡
sFlt-1 (MoM)	0.96 (0.73–1.34)	1.08 (0.77–1.44)†	1.91 (1.44–2.87)*
sFlt-1 > 95th percentile	135 (4.5)	17 (6.2)	4 (57.1)‡
Biophysical marker			
UtA-PI (MoM)	0.98 (0.84–1.14)	1.01 (0.85–1.20)	1.65 (1.13–1.85)†
UtA-PI > 95th percentile	141 (4.7)	11 (4.0)	4 (57.1)‡
UA-PI (MoM)	1.01 (0.90–1.12)	1.02 (0.91–1.12)	1.30 (1.28–1.49)‡
UA-PI > 95th percentile	148 (4.9)	12 (4.4)	6 (85.7)‡
MCA-PI (MoM)	1.01 (0.90–1.12)	1.00 (0.89–1.14)	0.78 (0.77–0.95)†
MCA-PI < 5th percentile	140 (4.6)	12 (4.4)	4 (57.1)‡
MAP (MoM)	1.00 (0.95–1.06)	1.01 (0.96–1.07)	1.08 (0.98–1.18)
MAP > 95th percentile	139 (4.6)	16 (5.8)	3 (42.9)†
EFW percentile	47.9 (24.4–70.7)	55.5 (29.3–76.2)†	0.64 (0.27–2.02)‡
EFW < 5th percentile	127 (4.2)	8 (2.9)	7 (100.0)‡

Data are given as median (interquartile range) or *n* (%). Significant difference from cohort without adverse outcome adjusted for multiple comparisons with *post-hoc* Bonferroni correction: *$P < 0.025$; †$P < 0.01$; ‡$P < 0.0001$. EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

above or below a cut-off percentile, in pregnancies with and those without low cord blood pH, low 5-min Apgar score and NNU admission, are shown in Table 4. No significant differences in the median MoM values of any of the biomarkers were found in the group with low cord blood pH compared to those with normal pH, in those with low 5-min Apgar score compared to those with normal score, and in those with NNU admission compared to those without admission.

DISCUSSION

Main findings of the study

The findings of this study demonstrate that, in pregnancies that develop PE and those that result in delivery of a SGA neonate, there is biophysical and biochemical evidence of impaired placentation or placental dysfunction reflected in increased UtA-PI, MAP and serum sFlt-1 and reduced serum PlGF. Screening by a combination of biomarkers at

Table 4 Biochemical and biophysical markers in pregnancies with and those without low cord blood pH, 5-min Apgar score < 7 or admission to neonatal unit (NNU)

	Umbilical cord blood pH		*5-min Apgar score*		*Admission to NNU*	
Biomarker	*Unaffected (n = 1336)*	*Low pH (n = 52)*	*Unaffected (n = 3586)*	*Score < 7 (n = 20)*	*Unaffected (n = 3721)*	*Admitted (n = 232)*
Biochemical marker						
PlGF (MoM)	0.95 (0.53–1.68)	0.70 (0.43–1.52)	0.96 (0.55–1.67)	1.15 (0.67–2.25)	0.98 (0.55–1.68)	0.94 (0.54–1.71)
PlGF < 5th percentile	81 (6.1)	3 (5.8)	174 (4.9)	0 (0.0)	185 (5.0)	13 (5.6)
sFlt-1 (MoM)	1.01 (0.74–1.42)	1.28 (0.73–1.73)	0.98 (0.74–1.37)	0.99 (0.73–1.16)	0.97 (0.73–1.36)	1.03 (0.75–1.45)
sFlt-1 > 95th percentile	80 (6.0)	7 (13.5)	181 (5.0)	2 (10.0)	183 (4.9)	15 (6.5)
Biophysical marker						
UtA-PI (MoM)	0.99 (0.84–1.17)	1.00 (0.86–1.22)	0.98 (0.83–1.17)	0.98 (0.80–1.20)	0.98 (0.84–1.17)	0.96 (0.83–1.16)
UtA-PI > 95th percentile	78 (5.8)	3 (5.8)	178 (5.0)	0 (0.0)	184 (4.9)	14 (6.0)
UA-PI (MoM)	1.00 (0.90–1.12)	1.06 (0.94–1.13)	1.01 (0.90–1.12)	1.04 (0.94–1.12)	1.01 (0.90–1.12)	1.02 (0.90–1.12)
UA-PI > 95th percentile	64 (4.8)	3 (5.8)	181 (5.0)	0 (0.0)	190 (5.1)	8 (3.4)
MCA-PI (MoM)	1.00 (0.90–1.12)	0.96 (0.86–1.07)	1.01 (0.90–1.13)	0.98 (0.86–1.08)	1.01 (0.90–1.12)	1.02 (0.92–1.13)
MCA-PI < 5th percentile	60 (4.5)	4 (7.7)	176 (4.9)	2 (10.0)	190 (5.1)	8 (3.4)
MAP (MoM)	1.01 (0.96–1.07)	1.02 (0.99–1.07)	1.01 (0.95–1.06)	1.03 (0.99–1.06)	1.00 (0.95–1.06)	1.02 (0.96–1.08)
MAP > 95th percentile	74 (5.6)	4 (7.7)	174 (5.0)	2 (10.0)	186 (5.1)	12 (5.4)
EFW percentile	54.2 (30.1–76.2)	50.2 (26.4–73.7)	49.7 (25.6–73.2)	59.1 (30.8–67.5)	49.5 (25.6–73.1)	55.5 (28.1–78.6)
EFW < 5th percentile	47 (3.5)	1 (1.9)	136 (3.8)	0 (0.0)	146 (3.9)	13 (5.6)

Data are given as median (interquartile range) or *n* (%). No significant differences found between cohort with and those without adverse outcome when adjusted for multiple comparisons with *post-hoc* Bonferroni correction. EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

36 weeks' gestation predicts the majority of pregnancies that develop PE and those that deliver SGA neonates.

Impaired placentation/placental dysfunction and fetal hypoxemia are also observed in a small group of pregnancies with SGA fetuses delivering by elective Cesarean section for evidence of fetal distress. However, combined screening at 36 weeks is not useful in predicting fetal distress in labor necessitating delivery by Cesarean section, low cord blood pH, low Apgar score or NNU admission.

Strengths and limitations of the study

The strengths of this third-trimester screening study are, first, examination of pregnant women attending for routine care, second, use of a specific methodology and appropriately-trained doctors to measure MAP and carry out the Doppler studies, third, use of automated machines to obtain prospectively reproducible measurements of serum PlGF and sFlt-1, fourth, estimation of MoM values for biophysical and biochemical markers after adjustment for factors that affect the measurements, and fifth, examination of a wide range of well-accepted indicators of adverse perinatal outcome.

The main limitation of the study is that the results of the 35–37 weeks' scan were made available to the obstetricians of the patients who would have taken specific actions of further monitoring and delivery of the cases of suspected SGA with abnormal Doppler findings. It could therefore be argued that the performance of screening for adverse perinatal outcomes by biomarkers of impaired placentation and fetal hypoxemia would have been negatively biased. It is possible that some of the cases of SGA and fetal distress that were delivered by elective Cesarean section would have resulted in stillbirth or fetal distress in labor and low cord blood pH had they not been detected by the routine assessment at 36 weeks. 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.

Comparison with findings from previous studies

The results of this study are comparable to those of the only previous screening study that examined biomarkers of impaired placentation/placental dysfunction and fetal hypoxemia at 30–34 weeks' gestation and reported that these markers are useful in the prediction of PE and birth of SGA neonates, but not of adverse events in labor or after birth[11].

Implications for clinical practice

An integrated clinical assessment at 36 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. It was previously proposed that prediction of most cases of term PE and SGA is best achieved by a third-trimester assessment at around 36 weeks' gestation. It was also suggested that such timing of the assessment would inevitably miss the cases of preterm PE and SGA and it was therefore proposed that these cases should be identified at an integrated clinic at 22 weeks and receive close monitoring for early diagnosis of PE and/or SGA, and appropriate timing of delivery[3,26].

In pregnancies without SGA or PE, combined screening at 36 weeks is not useful in the prediction of adverse events during labor or after birth. It is possible that this finding is the consequence of the long delay of about 4 weeks between assessment and delivery or the effects of the events in labor and delivery on the outcome measures that overshadow the contribution of chronic hypoxia. Alternatively, this finding provides support for the hypothesis proposed more than 20 years ago, that impaired placentation or placental dysfunction is usually not severe enough to cause fetal hypoxia until reduced nutrition has stunted the size of the fetus sufficiently to become SGA; consequently, tests of fetal hypoxia would rarely be useful when the fetus is growing normally[12]. Research into the causes of morbidity in appropriately-grown fetuses should perhaps look for pathologies other than chronic fetal hypoxia.

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REFERENCES

1. Garcia-Tizon Larroca S, Tayyar A, Poon LC, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by biophysical and biochemical markers at 30–33 weeks' gestation. *Fetal Diagn Ther* 2014; **36**: 9–17.
2. Bakalis S, Peeva G, Gonzalez R, Poon LC, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 30–34 weeks. *Ultrasound Obstet Gynecol* 2015; **46**: 446–451.
3. Poon LC, Lesmes C, Gallo DM, Akolekar R, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 19–24 weeks. *Ultrasound Obstet Gynecol* 2015; **46**: 437–445.
4. Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound guided sampling of umbilical cord and placental blood to assess fetal wellbeing. *Lancet* 1986; **1**: 1065–1067.
5. Soothill PW, Nicolaides KH, Campbell S. Prenatal asphyxia, hyperlacticaemia, hypoglycaemia and erythroblastosis in growth retarded fetuses. *BMJ* 1987; **294**: 1051–1053.
6. Economides DL, Nicolaides KH, Campbell S. Metabolic and endocrine findings in appropriate and small for gestational age fetuses. *J Perinat Med* 1991; **19**: 97–105.
7. Nicolaides KH, Bilardo KM, Soothill PW, Campbell S. Absence of end diastolic frequencies in the umbilical artery a sign of fetal hypoxia and acidosis. *BMJ* 1988; **297**: 1026–1027.
8. Soothill PW, Nicolaides K, Bilardo KM, Hackett GA, Campbell S. Uteroplacental blood velocity resistance index and umbilical venous pO2, pCO2, pH, lactate, and erythroblast count in growth retarded fetuses. *Fetal Therapy* 1986; **1**: 176–179.
9. Vyas S, Nicolaides KH, Bower S, Campbell S. Middle cerebral artery flow velocity waveforms in fetal hypoxaemia. *Br J Obstet Gynaecol* 1990; **97**: 797–803.
10. Chard T. Placental function tests. In *Antenatal and Neonatal Screening*, Wald NJ (ed). Oxford University Press: Oxford, UK, 1984; 510–522.
11. Valiño N, Giunta G, Gallo DM, Akolekar R, Nicolaides KH. Biophysical and biochemical markers at 30–34 weeks' gestation in the prediction of adverse perinatal outcome. *Ultrasound Obstet Gynecol* 2016; **47**: 194–202.
12. Soothill PW, Ajayi RA, Campbell S, Nicolaides KH. Prediction of morbidity in small and normally grown fetuses by fetal heart rate variability, biophysical profile score and umbilical artery Doppler studies. *Br J Obstet Gynaecol* 1993; **100**: 742–745.
13. Snijders RJ, Nicolaides KH. Fetal biometry at 14–40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34–48.
14. Albaiges G, Missfelder-Lobos H, Lees C, Parra M, Nicolaides KH. One-stage screening for pregnancy complications by color Doppler assessment of the uterine arteries at 23 weeks' gestation. *Obstet Gynecol* 2000; **96**: 559–564.
15. Acharya G, Wilsgaard T, Berntsen GK, Maltau JM, Kiserud T. Reference ranges for serial measurements of umbilical artery Doppler indices in the second half of pregnancy. *Am J Obstet Gynecol* 2005; **192**: 937–944.
16. Vyas S, Campbell S, Bower S, Nicolaides KH. Maternal abdominal pressure alters fetal cerebral blood flow. *Br J Obstet Gynaecol* 1990; **97**: 740–742.
17. Poon LC, Zymeri NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11–13 weeks' gestation. *Fetal Diagn Ther* 2012; **31**: 42–48.
18. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
19. Poon LCY, Volpe N, Muto B, Syngelaki A, Nicolaides KH. Birthweight with gestation and maternal characteristics in live births and stillbirths. *Fetal Diagn Ther* 2012; **32**: 156–165.
20. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX–XIV.
21. Tayyar A, Guerra L, Wright A, Wright D, Nicolaides KH. Uterine artery pulsatility index in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 689–697.
22. Akolekar R, Sarno L, Wright A, Wright D, Nicolaides KH. Fetal middle cerebral artery and umbilical artery pulsatility index: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 402–408.
23. Wright A, Wright D, Ispas A, Poon LC, Nicolaides KH. Mean arterial pressure in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 698–706.
24. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 591–598.
25. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum sFlt-1 in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 584–590.
26. Khalil AA, Morales-Rosello J, Elsaddig M, Khan N, Papageorghiou A, Bhide A, Thilaganathan B. The association between fetal Doppler and admission to neonatal unit at term. *Am J Obstet Gynecol* 2015; **213**: 57.e1–7.

