Ultrasound Obstet Gynecol 2016; **47**: 194–202
Published online 7 January 2016 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.14928

Biophysical and biochemical markers at 30–34 weeks' gestation in the prediction of adverse perinatal outcome

N. VALIÑO*, G. GIUNTA*, D. M. GALLO*, R. AKOLEKAR*† and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, Kent, UK*

KEYWORDS: mean arterial pressure; middle cerebral artery Doppler; perinatal outcome; placental growth factor; pyramid of antenatal care; sFlt-1; third-trimester screening; umbilical artery Doppler; uterine artery Doppler

ABSTRACT

Objective *To investigate the potential value of biophysical and biochemical markers at 30–34 weeks' gestation in the prediction of adverse perinatal outcome.*

Methods *This was a screening study in 8268 singleton pregnancies at 30–34 weeks' gestation. Estimated fetal weight (EFW), uterine artery (UtA) pulsatility index (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 stillbirth, pre-eclampsia, delivery of small-for-gestational-age (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 admission to the neonatal unit (NNU).*

Results *Multivariable regression analysis demonstrated that significant prediction of PE was provided by PlGF, sFlt-1, MAP and MCA-PI, with a DR of 98% for PE delivering < 37 weeks' gestation and 56% for those delivering ≥ 37 weeks, at a 10% FPR. Prediction of SGA was provided by EFW, PlGF, sFlt-1, UtA-PI, UA-PI and MCA-PI, with a DR of 88% for SGA delivering < 37 and 51% for those delivering ≥ 37 weeks' gestation, at a 10% FPR. Prediction of stillbirth was provided by EFW, UtA-PI and MCA-PI, with DR of 30% at 10% FPR. Prediction of Cesarean section for fetal distress before labor was provided by EFW, sFlt-1, UtA-PI and UA-PI, with a DR of 90% at a 10% FPR. Prediction of fetal distress in labor was provided by EFW and sFlt-1, with a DR of 16% at a 10% FPR. There were no significant differences from the normal outcome group in any of the biomarkers for low cord blood pH, low Apgar score or NNU admission for cases other than those with PE and/or SGA.*

Conclusion *At 30–34 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 stillbirth and adverse events in labor or after birth.* *Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Impaired placentation, reflected in 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) is associated with subsequent development of pre-eclampsia (PE) and delivery of a small-for-gestational-age (SGA) neonate[1,2]. Another marker of development of PE is increased mean arterial pressure (MAP)[1,2]. Impaired placentation is also associated with fetal hypoxemia and consequent redistribution in the fetal circulation, reflected in reduced fetal middle cerebral artery (MCA) PI and increased UA-PI[3–10].

A screening study at 30–34 weeks' gestation, involving more than 30 000 singleton pregnancies, reported that the incidence of adverse perinatal outcome is higher in SGA than in non-SGA fetuses, but the majority of cases for each adverse event are in the non-SGA group, including about 70% of stillbirths and more than 80% of cases of Cesarean section for fetal distress, low cord blood pH and low 5-min Apgar score[11]. This is analogous to screening for Down syndrome in which the risk in women aged ≥ 35 years is substantially higher than in younger women, but the overall contribution from the latter group is more than twice as high as that from the older group. It could therefore be argued that the objective of prenatal

Correspondence to: Dr R. Akolekar, Department of Fetal Medicine, Medway Maritime Hospital, Windmill Road, Gillingham, Kent, ME7 5NY, UK (e-mail: ranjit.akolekar@nhs.net)

Accepted: 9 June 2015

screening should be identification of pregnancies with impaired placentation and fetal hypoxemia, irrespective of fetal size.

The objective of this screening study was to investigate the potential value of UtA-PI, UA-PI, MCA-PI, MAP and serum levels of PlGF and sFlt-1 at 30–34 weeks' gestation in the prediction of adverse perinatal outcome, including development of PE, delivery of a SGA neonate, stillbirth, 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).

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 and University College London Hospital, London, UK, and Medway Maritime Hospital, Kent, UK, between May 2011 and August 2014. This visit, which is attended at 30 + 0 to 34 + 6 weeks' gestation, included the recording of maternal characteristics and medical history, and estimation of fetal size from transabdominal ultrasound measurement of fetal head circumference, abdominal circumference and femur length. 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[12,13]. Transabdominal color-flow mapping was used to visualize the UtA, UA and fetal MCA[14–16]. Pulsed-wave Doppler was then used to obtain waveforms; when three similar waveforms were obtained consecutively the PI was measured.

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 (yes/no), medical history of chronic hypertension (yes/no), diabetes mellitus (yes/no), systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS) and parity (parous/nulliparous if no previous pregnancy ≥ 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 stillbirth, Cesarean section for fetal distress before or during labor, umbilical arterial cord blood pH ≤7.0 or 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[17]. The birth-weight *Z*-score was also derived from the normal range for gestational age[17]. The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy[18].

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 adjusting for variables from maternal characteristics and medical history that affect these measurements[9–23]. Univariable and multivariable logistic regression analysis was 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) curves 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, 8268 singleton pregnancies were examined at 30–34 weeks' gestation. The characteristics of the study population and the various subgroups according to adverse perinatal outcome are given and compared in Table 1.

Biomarkers in pregnancies with stillbirth, PE or delivery of SGA neonate

For pregnancies resulting in stillbirth, PE or delivery of a SGA neonate with birth weight < 10th percentile, the median MoM values of each biomarker and the proportion of pregnancies with values above or below a specified percentile are compared to pregnancies unaffected by any one of these adverse outcomes (Table 2).

Of the 8268 pregnancies, stillbirth occurred in 23 (0.28%). In eight (34.8%) the birth weight was < 10th percentile and in seven (30.4%) death occurred < 37 weeks' gestation. Compared to the unaffected group, there were no significant differences in median MoM values of any of

Table 1 Maternal and pregnancy characteristics of study population of 8268 women with singleton pregnancy and those subgroups with adverse perinatal outcome of stillbirth, pre-eclampsia (PE), small-for-gestational-age (SGA) neonate, fetal distress in 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 = 8268)*	*Stillbirth (n = 23)*	*PE (n = 223)*	*SGA (n = 822)*	*Fetal distress (n = 512)*	*Low cord pH (n = 84)*	*Low Apgar score (n = 70)*	*NNU admission (n = 524)*
Maternal age (years)	31.0 (26.6–34.7)	32.9 (23.2–37.2)	31.5 (26.8–34.8)	29.7 (25.2–34.2)†	31.2 (27.0–35.9)	32.1 (27.1–35.5)	31.9 (26.5–35.6)	30.9 (26.4–35.0)
Maternal weight (kg)	77.0 (68.7–87.8)	89.0 (66.2–100.5)	83.8 (72.0–97.5)†	71.8 (64.0–81.0)†	81.2 (72.0–93.0)†	78.8 (73.3–88.7)	81.9 (71.0–91.1)	79.2 (70.8–92.0)†
Maternal height (m)	1.65 (1.60–1.69)	1.65 (1.61–1.68)	1.64 (1.59–1.69)	1.63 (1.58–1.67)†	1.63 (1.59–1.68)†	1.64 (1.59–1.68)	1.62 (1.60–1.67)	1.64 (1.60–1.69)
Cigarette smoker	827 (10.0)	3 (13.0)	13 (5.8)	148 (18.0)†	46 (9.0)	7 (8.3)	8 (11.4)	67 (12.8)
Racial origin								
Caucasian	6185 (74.8)	16 (69.6)	138 (61.9)	530 (64.5)	337 (65.8)	67 (79.8)	41 (58.6)	396 (75.6)
Afro-Caribbean	1437 (17.4)	5 (21.7)	69 (30.9)†	191 (23.2)†	130 (25.4)†	11 (13.1)	23 (32.9)*	101 (19.3)
South Asian	312 (3.8)	1 (4.3)	10 (4.5)	56 (6.8)†	27 (5.3)	4 (4.8)	2 (2.9)	12 (2.3)
East Asian	150 (1.8)	1 (4.3)	4 (1.8)	13 (1.6)	11 (2.1)	0 (0.0)	1 (1.4)	6 (1.1)
Mixed	184 (2.2)	0 (0.0)	2 (0.9)	32 (3.9)*	7 (1.4)	2 (2.4)	3 (4.3)	9 (1.7)
Mode of conception								
Spontaneous	7996 (96.7)	22 (95.7)	215 (96.4)	799 (97.2)	485 (94.7)	83 (98.8)	68 (97.1)	500 (95.4)
Assisted	272 (3.3)	1 (4.3)	8 (3.6)	23 (2.8)	27 (5.3)*	1 (1.2)	2 (2.9)	24 (4.6)
Medical disorder								
Chronic hypertension	121 (1.5)	1 (4.3)	30 (13.5)†	10 (1.2)	13 (2.5)	1 (1.2)	3 (4.3)	13 (2.5)
SLE/APS	15 (0.2)	0 (0.0)	0 (0.0)	1 (0.1)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Diabetes mellitus	80 (1.0)	0 (0.0)	2 (0.9)	7 (0.9)	8 (1.6)	0 (0.0)	1 (1.4)	19 (3.6)†
Obstetric history								
Parous	4198 (50.8)	10 (43.5)	91 (40.8)	324 (39.4)	146 (28.5)	39 (46.4)	36 (51.4)	216 (41.2)
Nulliparous	4070 (49.2)	13 (56.5)	132 (59.2)*	498 (60.6)†	366 (71.5)†	45 (53.6)	34 (48.6)	308 (58.8)†
Pregnancy complication								
Gestational diabetes	215 (2.6)	2 (8.7)	13 (5.8)*	19 (2.3)	19 (3.7)	7 (8.3)*	3 (4.3)	32 (6.1)†
SROM	455 (5.5)	0 (0.0)	4 (1.8)	52 (6.3)	69 (13.5)†	4 (4.8)	3 (4.3)	53 (10.1)†
Onset of labor and mode of delivery								
Spontaneous labor								
Vaginal delivery	5353 (64.7)	8 (34.8)	37 (16.6)	514 (62.5)	0 (0.0)	41 (48.8)	26 (37.1)	246 (46.9)
Cesarean section	765 (9.3)	0 (0.0)	12 (5.4)	65 (7.9)	335 (65.4)†	19 (22.6)	12 (17.1)	94 (17.9)†
Induced labor								
Vaginal delivery	931 (11.3)	13 (56.5)†	89 (39.9)†	127 (15.5)†	0 (0.0)	9 (10.7)	9 (12.9)	60 (11.5)
Cesarean section	344 (4.2)	0 (0.0)	38 (17.0)†	46 (5.6)*	177 (34.6)†	9 (10.7)	12 (17.1)†	53 (10.1)†
Elective Cesarean section	875 (10.6)	2 (8.7)	47 (21.1)†	70 (8.5)	0 (0.0)	6 (7.1)	11 (15.7)	71 (13.5)
Assessment								
GA at assessment (weeks)	32.2 (32.0–32.5)	32.2 (32.0–32.4)	32.1 (32.0–32.4)	32.1 (32.0–32.5)	32.2 (32.0–32.6)	32.1 (32.0–32.4)	32.2 (32.0–32.5)	32.2 (32.0–32.5)
EFW percentile	53.3 (28.9–77.6)	42.5 (16.5–71.9)	48.9 (20.9–75.7)†	18.1 (6.9–37.0)†	56.0 (31.7–80.4)	55.1 (29.6–73.6)	56.8 (23.5–86.5)	54.9 (24.7–78.3)
Outcome								
GA at delivery (weeks)	40.0 (39.0–40.9)	38.3 (36.9–40.7)*	38.6 (37.3–40.2)†	39.9 (38.9–40.9)	40.6 (39.4–41.4)†	40.0 (38.5–40.9)	39.9 (38.4–41.2)	39.0 (36.8–40.6)†
Birth-weight percentile	48.5 (23.7–74.4)	32.7 (4.3–75.2)†	35.0 (9.8–65.8)†	5.3 (2.7–7.8)†	41.9 (16.2–71.5)†	49.8 (19.0–78.4)	52.5 (15.4–83.0)	44.5 (18.4–77.8)

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 stillbirth, pre-eclampsia (PE) delivering < 37 or ≥ 37 weeks, delivery of small-for-gestational-age (SGA) neonate < 37 or ≥ 37 weeks' gestation and those unaffected by any of these adverse outcomes

			PE		*SGA*	
Biomarker	*Unaffected* (n = *7207*)	*Stillbirth* (n = *23*)	*Delivery < 37 weeks* (n = *40*)	*Delivery ≥ 37 weeks* (n = *183*)	*Delivery < 37 weeks* (n = *60*)	*Delivery ≥ 37 weeks* (n = *762*)
Biochemical marker						
PlGF (MoM)	1.01 (0.63–1.57)	0.63 (0.45–1.31)	0.13 (0.02–0.22)†	0.41 (0.28–0.78)†	0.33 (0.16–0.61)†	0.64 (0.38–1.06)†
PlGF < 5th p	224 (3.1)	1 (4.3)	31 (77.5)†	41 (22.4)†	25 (41.7)†	93 (12.2)†
PlGF < 10th p	534 (7.4)	2 (8.7)	36 (90.0)†	69 (37.7)†	31 (51.7)†	156 (20.5)†
sFlt-1 (MoM)	0.99 (0.74–1.34)	1.21 (0.64–1.95)	4.54 (3.05–6.47)†	1.43 (0.94–2.20)†	1.43 (1.06–3.13)†	1.10 (0.78–1.52)†
sFlt-1 >95th p	265 (3.7)	2 (8.7)	32 (80.0)†	45 (24.6)†	19 (31.7)†	52 (6.8)†
sFlt-1 >90th p	602 (8.4)	6 (26.1)*	35 (87.5)†	62 (33.9)†	22 (36.7)†	102 (13.4)†
Biophysical marker						
UtA-PI (MoM)	0.99 (0.85–1.16)	1.13 (1.00–1.31)	1.60 (1.19–2.10)†	1.02 (0.85–1.28)	1.08 (0.97–1.49)†	1.04 (0.91–1.28)†
UtA-PI > 95th p	294 (4.1)	3 (13.0)	20 (50.0)†	19 (10.4)†	12 (20.0)†	68 (8.9)*
UtA-PI > 90th p	618 (8.6)	4 (17.4)	24 (60.0)†	33 (18.0)†	19 (31.7)†	131 (17.2)†
UA-PI (MoM)	1.01 (0.91–1.12)	1.02 (0.86–1.13)	1.08 (0.88–1.17)	0.99 (0.92–1.10)	1.16 (1.01–1.23)†	1.06 (0.95–1.17)†
UA-PI > 95th p	317 (4.4)	1 (4.3)	4 (10.0)	6 (3.3)	14 (23.3)†	72 (9.4)†
UA-PI > 90th p	664 (9.2)	1 (4.3)	5 (12.5)	17 (9.3)	15 (25.0)†	125 (16.4)†
MCA-PI (MoM)	1.02 (0.92–1.12)	0.95 (0.86–1.01)	1.01 (0.93–1.12)	0.99 (0.87–1.09)*	0.96 (0.87–1.06)*	1.00 (0.90–1.10)*
MCA-PI < 5th p	339 (4.7)	2 (8.7)	2 (5.0)	18 (9.8)*	5 (8.3)	48 (6.3)
MCA-PI < 10th p	668 (9.5)	4 (17.4)	4 (10.0)	30 (16.4)*	8 (13.3)	95 (12.5)
MAP (MoM)	1.00 (0.94–1.05)	1.01 (0.97–1.09)	1.16 (1.10–1.26)†	1.08 (1.00–1.13)†	1.03 (0.97–1.11)†	1.01 (0.96–1.06)†
MAP > 95th p	279 (3.9)	1 (4.3)	26 (65.0)†	40 (21.9)†	11 (18.3)†	46 (6.0)*
MAP > 90th p	606 (8.4)	2 (8.7)	29 (72.5)†	63 (34.4)†	14 (23.3)†	90 (11.8)*
EFW p	57.3 (33.5–79.4)	42.5 (16.5–71.9)	15.6 (1.0–52.2)†	55.0 (29.0–77.5)	4.5 (0.2–18.2)†	19.1 (8.0–38.1)†
EFW < 5th p	144 (2.0)	2 (8.7)	12 (30.0)†	7 (3.8)	31 (51.7)†	135 (17.7)
EFW < 10th p	331 (4.6)	4 (17.4)	17 (42.5)†	13 (7.1)	38 (63.3)†	228 (29.9)†

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; p, percentile; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

the seven biomarkers in pregnancies resulting in stillbirth (Table 2). However, there was a non-significant tendency for lower PlGF, MCA-PI and EFW and higher sFlt-1 and UtA-PI. Multivariable logistic regression analysis demonstrated that significant contributions for prediction of stillbirth were provided by EFW, UtA-PI and MCA-PI (area under ROC curve (AUC), 0.683 (95% CI, 0.568–0.797); Table 3). The DR of stillbirth by combined screening, at a FPR of 10%, was 30.4% (Figure 1a).

PE occurred in 223 (2.7%) pregnancies, including 40 (17.9%) in which delivery was < 37 weeks. Compared to the unaffected group, there was lower PlGF, MCA-PI and EFW, and higher sFlt-1, UtA-PI and MAP in pregnancies that developed PE; in general, the differences from normal were more marked in the group of PE delivering < 37 weeks' gestation than in those delivering ≥ 37 weeks (Table 2). Multivariable logistic regression analysis demonstrated that significant contributions for prediction of PE delivering < 37 weeks were provided by PlGF, sFlt-1, UtA-PI and MAP (AUC, 0.992 (95% CI, 0.985–1.000)) and contributions for prediction of PE delivering ≥ 37 weeks were provided by PlGF, sFlt-1, MCA-PI and MAP (AUC, 0.808 (95% CI, 0.772–0.844); Table 3). The DR of combined screening by all significant contributors, at a FPR of 10%, was 97.5% for PE delivering < 37 weeks and 55.8% for PE delivering ≥ 37 weeks (Figure 1b).

In the pregnancies resulting in livebirth, the birth weight was < 10th percentile in 822 (9.9%) cases. Compared to the unaffected group, there was lower PlGF, MCA-PI and EFW and higher sFlt-1, UtA-PI, UA-PI and MAP in pregnancies with a SGA neonate; the differences from normal were more marked in the group of SGA delivering < 37 weeks' gestation than in those delivering ≥ 37 weeks (Table 2). Multivariable logistic regression analysis demonstrated that significant contributions for prediction of SGA delivering < 37 weeks were provided by EFW, PlGF, sFlt-1 and UtA-PI (AUC, 0.954 (95% CI, 0.930–0.978)) and contributions for prediction of SGA delivering ≥ 37 weeks were provided by EFW, PlGF, sFlt-1, UtA-PI, UA-PI and MCA-PI (AUC, 0.829 (95% CI, 0.813–0.844); Table 3). The DR of combined screening by all significant contributors, at a FPR of 10%, was 88.3% for SGA delivering < 37 weeks and 51.0% for SGA delivering ≥ 37 weeks (Figure 1c).

Biomarkers in pregnancies delivered by Cesarean section for fetal distress

In the 8245 pregnancies with a livebirth, 6263 were delivered vaginally following spontaneous or induced labor, 873 by elective Cesarean section for a variety of indications and 1109 by Cesarean section following

Table 3 Uni- and multivariable logistic regression analyses in prediction of stillbirth, pre-eclampsia and delivery of small-for-gestational-age neonate using biochemical and biophysical markers

	Stillbirth				*Pre-eclampsia**				*Small-for-gestational age**			
	Univariable analysis		*Multivariable analysis*		*Univariable analysis*		*Multivariable analysis*		*Univariable analysis*		*Multivariable analysis*	
Biomarker	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P
EFW Z-score	0.63 (0.42 to 0.95)	0.028	0.66 (0.44 to 0.99)	0.037	0.70 (0.61 to 0.81)	< 0.0001	—	—	0.28 (0.25–0.30)	< 0.0001	0.29 (0.26–0.32)	< 0.0001
PlGF ($\log_{10}$ MoM)	0.32 (0.09 to 1.15)	0.080	—	—	0.02 (0.01 to 0.03)	< 0.0001	0.10 (0.06 to 0.15)	< 0.0001	0.13 (0.10–0.16)	< 0.0001	0.20 (0.15–0.26)	< 0.0001
sFlt-1 ($\log_{10}$ MoM)	3.04 (0.43 to 21.68)	0.267	—	—	123.8 (70.1 to 218.8)	< 0.0001	17.42 (9.0 to 33.72)	< 0.0001	3.80 (2.70–5.36)	< 0.0001	1.73 (1.18–2.56)	0.005
UtA-PI ($\log_{10}$ MoM)	75.41 (2.79 to 2.0E3)	0.010	51.89 (2.10 to 12.8E2)	0.016	40.8 (13.6 to 122.4)	< 0.0001	—	—	18.48 (9.88–34.56)	< 0.0001	8.39 (4.17–16.91)	< 0.0001
UA-PI ($\log_{10}$ MoM)	0.13 (0.00 to 67.44)	0.520	—	—	0.85 (0.11 to 6.81)	0.880	—	—	160.1 (49.3–520.2)	< 0.0001	24.28 (6.51–90.60)	< 0.0001
MCA-PI ($\log_{10}$ MoM)	3.1E−4 (4.4E−7 to 0.21)	0.015	0.001 (9.6E−7 to 0.29)	0.019	0.05 (0.01 to 0.41)	0.006	0.05 (0.01 to 0.58)	0.017	0.11 (0.03–0.36)	< 0.0001	0.11 (0.03–0.43)	0.001
MAP ($\log_{10}$ MoM)	4.29E3 (0.03 to 73.5E7)	0.174	—	—	8.6E12 (1.7E11 to 4.5E14)	< 0.0001	2.1E8 (2.7E6 to 1.6E10)	< 0.0001	160.1 (18.7–1367.9)	< 0.0001	—	—

*In pregnancies delivering ≥ 37 weeks. EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; OR, odds ratio; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

spontaneous or induced labor; in the latter group the indication for Cesarean section was fetal distress in 512 cases. In the elective Cesarean section group ($n = 873$), there were a variety of indications including breech or transverse lie ($n = 191$), placenta previa ($n = 41$), previous Cesarean section, traumatic birth or maternal request ($n = 567$), maternal medical disorder ($n = 44$) and SGA fetuses with fetal compromise diagnosed by abnormal fetal heart rate patterns or fetal Doppler indices ($n = 30$).

For pregnancies resulting in delivery by Cesarean section for fetal distress before ($n = 30$) or during ($n = 512$) labor and those delivering vaginally, the median MoM values of each biomarker and the proportion of pregnancies with values above or below a specified percentile are shown in Table 4.

Compared to those delivering vaginally, there was lower EFW, PlGF and MCA-PI and higher sFlt-1, UtA-PI, UA-PI and MAP in the group with Cesarean section for fetal distress before labor; the values were $>90^{th}$ or $<10^{th}$ percentile, depending on the biomarker, in 13–80% of the cases (Table 4). Multivariable logistic regression analysis demonstrated that significant contribution to the prediction of elective Cesarean section for fetal distress before labor was provided by EFW, sFlt-1, UtA-PI and UA-PI (AUC, 0.972 (95% CI, 0.944–0.999)); the DR at a 10% FPR was 90.0% (Table 5 and Figure 1d).

Compared to those delivering vaginally there was higher sFlt-1 in the group with Cesarean section for fetal distress in labor, however no significant differences were seen in the median values of the other biomarkers. Multivariable logistic regression analysis demonstrated that significant contribution to prediction of Cesarean section for fetal distress in labor was provided by EFW and sFlt-1 (AUC, 0.556 (95% CI, 0.530–0.582)); the DR at a 10% FPR was 16.2% (Table 5 and Figure 1d).

Biomarkers in pregnancies with adverse outcome after delivery

The median MoM values of each biochemical and biophysical marker and the proportion of pregnancies with biomarker values above or below a specified percentile in pregnancies with and without low cord blood pH, low 5-min Apgar score and admission to NNU are shown in Table 6.

No significant differences in the median MoM values of any of the biomarkers were seen in the group with low cord blood pH compared to those with normal pH and in those with low 5-min Apgar score compared to those with normal score. In the group with NNU admission, there was lower PlGF and higher sFlt-1 and UtA-PI compared to those not admitted. Multivariable logistic regression analysis demonstrated that significant contribution to prediction of NNU admission was provided by all three biomarkers (AUC, 0.592 (95% CI, 0.566–0.619); Table 7); the DR was 24.8%, at a 10% FPR. However, when the cases of PE and those with SGA neonates were excluded, none of the biomarkers provided significant contribution in the prediction of NNU admission.

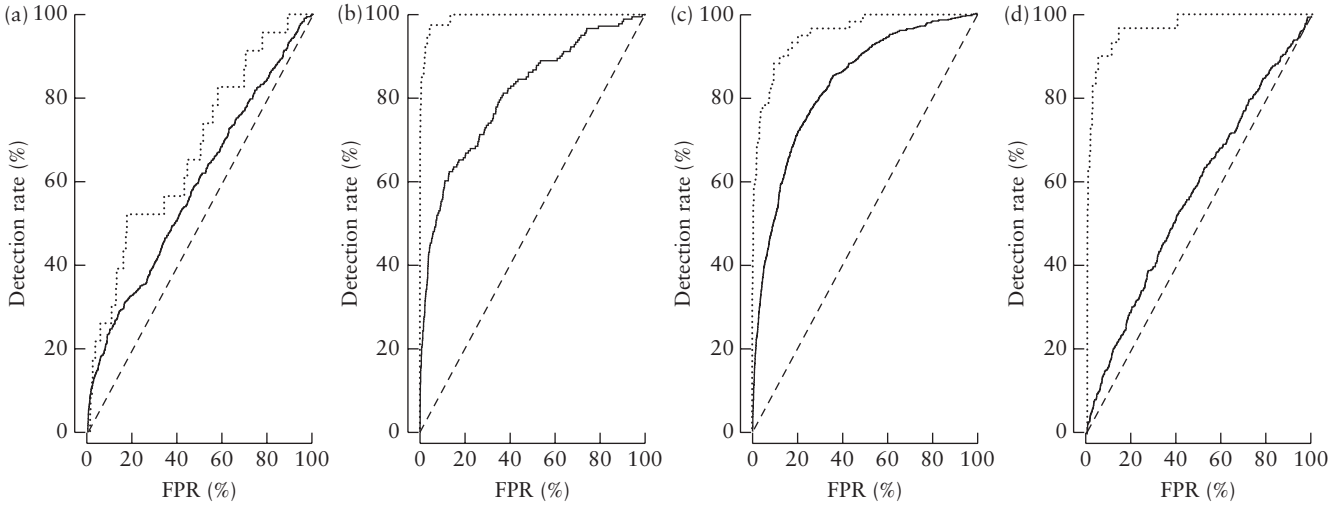

Figure 1 Receiver–operating characteristics curves for prediction of: (a) stillbirth (·····) and neonatal admission (——); (b) pre-eclamptic pregnancy delivering < 37 (·····) and ≥ 37 (——) weeks' gestation; (c) small-for-gestational-age neonate delivered < 37 (·····) and ≥ 37 (——) weeks' gestation; and (d) fetal distress before (·····) and during (——) labor resulting in delivery by Cesarean section. FPR, false-positive rate.

Table 4 Biochemical and biophysical markers in pregnancies delivering by Cesarean section for fetal distress and those delivering vaginally

		Cesarean section for fetal distress	
Biomarker	*Vaginal delivery (n = 6263)*	*During labor (n = 512)*	*Before labor (n = 30)*
Biochemical marker			
PlGF (MoM)	0.95 (0.58–1.51)	0.94 (0.52–1.58)	0.24 (0.11–0.42)‡
PlGF < 5th percentile	348 (4.6)	29 (5.7)	15 (50.0)‡
PlGF < 10th percentile	711 (9.4)	60 (11.7)	19 (63.3)‡
sFlt-1 (MoM)	1.01 (0.74–1.39)	1.05 (0.79–1.52)†	3.55 (1.47–5.43)‡
sFlt-1 > 95th percentile	328 (4.3)	42 (8.2)‡	18 (60.0)‡
sFlt-1 > 90th percentile	693 (9.1)	77 (15.0)‡	21 (70.0)‡
Biophysical marker			
UtA-PI (MoM)	1.00 (0.85–1.17)	1.00 (0.86–1.18)	1.44 (1.01–2.14)‡
UtA-PI > 95th percentile	355 (4.7)	33 (6.4)	12 (40.0)‡
UtA-PI > 90th percentile	726 (9.0)	66 (12.9)	16 (53.3)†
UA-PI (MoM)	1.02 (0.92–1.12)	1.03 (0.92–1.13)	1.14 (1.06–1.43)‡
UA-PI > 95th percentile	370 (4.9)	26 (5.1)	10 (33.3)‡
UA-PI > 90th percentile	746 (9.8)	56 (10.9)	12 (40.0)‡
MCA-PI (MoM)	1.02 (0.91–1.12)	1.04 (0.92–1.13)	0.96 (0.89–1.03)*
MCA-PI < 5th percentile	379 (5.0)	27 (5.3)	3 (10.0)
MCA-PI < 10th percentile	761 (10.0)	49 (9.6)	4 (13.3)
MAP (MoM)	1.00 (0.95–1.05)	1.01 (0.95–1.06)	1.10 (0.99–1.21)‡
MAP > 95th percentile	331 (4.5)	34 (6.8)	14 (46.7)‡
MAP > 90th percentile	692 (9.4)	65 (13.0)	14 (46.7)‡
EFW percentile	51.9 (28.3–75.8)	56.0 (31.7–80.4)	4.16 (0.16–8.96)‡
EFW < 5th percentile	279 (3.7)	19 (3.7)	15 (50.0)‡
EFW < 10th percentile	541 (7.1)	40 (7.8)	24 (80.0)‡

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.

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 and/or placental dysfunction reflected in increased MAP, UtA-PI and serum sFlt-1 and reduced serum PlGF. In such cases, the EFW is reduced and there is also evidence of fetal hypoxemia reflected in low fetal MCA-PI and high UA-PI. The deviation from normal for the biophysical and biochemical markers is more marked in the most severe cases of PE and SGA requiring iatrogenic preterm delivery. Screening by a combination of biomarkers at 32 weeks' gestation predicted 98% of preterm PE and 56% of term PE, at a

Table 5 Univariable and multivariable logistic regression analyses in prediction of Cesarean section for fetal distress before and during labor

	Cesarean section for fetal distress during labor				*Cesarean section for fetal distress before labor*			
	Univariable analysis		*Multivariable analysis*		*Univariable analysis*		*Multivariable analysis*	
Variable	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P
EFW *Z*-score	1.12 (1.02–1.22)	0.022	1.21 (1.02–1.23)	0.015	0.21 (0.16 to 0.29)	< 0.0001	0.26 (0.17–0.39)	< 0.0001
PlGF ($\log_{10}$ MoM)	0.83 (0.63–1.10)	0.201	—	—	0.01 (0.003 to 0.02)	< 0.0001	—	—
sFlt-1 ($\log_{10}$ MoM)	2.31 (1.52–3.51)	< 0.0001	2.36 (1.55–3.60)	< 0.0001	13.0E2 (3.3E2 to 51.5E2)	< 0.0001	2.1E2 (40.5 to 1.07E3)	< 0.0001
UtA-PI ($\log_{10}$ MoM)	1.60 (0.72–3.57)	0.252	—	—	19.5E3 (16.2E2 to 23.4E4)	< 0.0001	1.2E3 (43.7 to 3.0E4)	< 0.0001
UA-PI ($\log_{10}$ MoM)	1.57 (0.38–6.44)	0.534	—	—	11.6E8 (2.4E6 to 5.5E11)	< 0.0001	1.2E4 (14.3 to 10.5E6)	0.006
MCA-PI ($\log_{10}$ MoM)	3.00 (0.66–13.72)	0.156	—	—	0.001 (4.0E–6 to 0.40)	0.023	—	—
MAP ($\log_{10}$ MoM)	20.13 (1.44–282.4)	0.026	—	—	1.05E15 (6.49E10 to 1.69E19)	< 0.0001	—	—

EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; OR, odds ratio; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

Table 6 Biochemical and biophysical markers in pregnancies with and without low cord blood pH, 5-min Apgar score < 7 and admission to neonatal unit (NNU)

	Cord blood pH		*5-min Apgar score*		*NNU admission*	
Biomarker	*Normal (n = 3244)*	*Low (n = 84)*	*Normal (n = 7054)*	*Low (n = 70)*	*No (n = 7721)*	*Yes (n = 524)*
Biochemical marker						
PlGF (MoM)	0.94 (0.57–1.52)	1.01 (0.49–1.49)	0.94 (0.57–1.50)	1.13 (0.56–1.71)	0.95 (0.58–1.51)	0.83 (0.40–1.47)†
PlGF < 5th p	172 (5.3)	9 (10.7)	363 (5.1)	3 (4.3)	341 (4.4)	72 (13.7)†
PlGF < 10th p	338 (10.4)	13 (15.5)	720 (10.2)	6 (8.6)	717 (9.3)	108 (20.6)†
sFlt-1 (MoM)	1.03 (0.75–1.44)	0.97 (0.73–1.27)	1.02 (0.75–1.40)	1.16 (0.78–1.57)	1.00 (0.74–1.38)	1.16 (0.85–1.71)†
sFlt-1 > 95th p	195 (6.0)	4 (4.8)	356 (5.0)	5 (7.1)	340 (4.4)	72 (13.7)†
sFlt-1 > 90th p	367 (11.3)	9 (10.7)	698 (9.9)	12 (17.1)	709 (9.2)	112 (21.4)†
Biophysical marker						
UtA-PI (MoM)	1.00 (0.86–1.18)	1.01 (0.87–1.20)	1.00 (0.85–1.18)	0.98 (0.86–1.09)	1.00 (0.85–1.17)	1.02 (0.87–1.23)*
UtA-PI > 95th p	187 (5.8)	6 (7.1)	370 (5.2)	2 (2.9)	361 (4.7)	50 (9.5)†
UtA-PI > 90th p	348 (10.7)	9 (10.7)	733 (10.4)	2 (2.9)	748 (9.7)	75 (14.3)*
UA-PI (MoM)	1.02 (0.91–1.13)	0.99 (0.90–1.11)	1.02 (0.91–1.12)	1.01 (0.89–1.12)	1.02 (0.91–1.12)	1.03 (0.92–1.15)
UA-PI > 95th p	165 (5.1)	7 (8.3)	346 (4.9)	0 (0.0)	378 (4.9)	35 (6.7)
UA-PI > 90th p	327 (10.1)	10 (11.9)	700 (9.9)	3 (4.3)	762 (9.9)	64 (12.2)
MCA-PI (MoM)	1.02 (0.92–1.12)	1.00 (0.92–1.09)	1.02 (0.91–1.12)	1.03 (0.90–1.13)	1.02 (0.91–1.12)	1.01 (0.91–1.11)
MCA-PI < 5th p	170 (5.2)	5 (6.0)	356 (5.0)	3 (4.3)	388 (5.0)	23 (4.4)
MCA-PI < 10th p	326 (10.0)	8 (9.5)	718 (10.2)	6 (8.6)	777 (10.1)	46 (8.8)
MAP (MoM)	1.00 (0.95–1.06)	1.01 (0.94–1.06)	1.00 (0.95–1.06)	1.00 (0.92–1.04)	1.00 (0.95–1.05)	1.01 (0.95–1.07)*
MAP > 95th p	172 (5.5)	2 (2.4)	339 (4.9)	3 (4.4)	350 (4.7)	51 (10.0)†
MAP > 90th p	328 (10.4)	8 (9.6)	685 (10.0)	6 (8.8)	725 (9.7)	75 (14.9)†
EFW p	55.3 (30.5–78.6)	55.1 (29.6–73.6)	53.3 (29.0–77.6)	56.7 (23.5–86.5)	53.3 (29.1–77.5)	54.9 (24.7–78.3)
EFW < 5th p	127 (3.9)	3 (3.6)	272 (3.9)	3 (4.3)	284 (3.7)	43 (8.2)†
EFW < 10th p	224 (6.9)	9 (10.7)	514 (7.3)	8 (11.4)	567 (7.3)	56 (10.7)*

Data are given as median (interquartile range) or *n* (%). Records of cord blood pH and 5-min Apgar score were not available for all pregnancies resulting in livebirth. 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; p, percentile; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

FPR of 10%; the respective values for SGA in the absence of PE were 88% and 51%.

Impaired placentation/placental dysfunction and fetal hypoxemia were also observed in some of the pregnancies resulting in stillbirth, in those developing fetal distress in labor necessitating delivery by Cesarean section and in those requiring admission to NNU. However, the performance of screening with biomarkers at 32 weeks' gestation for these complications is poor with respective DRs of 30%, 16% and 25%, at a FPR of 10%. Biomarker

Table 7 Univariable logistic regression analysis in prediction of umbilical cord blood pH, low 5-min Apgar score and univariable and multivariable logistic regression analysis in prediction of admission to neonatal unit

	Low cord blood pH		*Low 5-min Apgar score*		*Neonatal unit admission*			
	Univariable analysis		*Univariable analysis*		*Univariable analysis*		*Multivariable analysis*	
Variable	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P
EFW *Z*-score	0.92 (0.75–1.14)	0.450	1.09 (0.86–1.38)	0.481	0.96 (0.88 to 1.04)	0.316	—	—
PlGF (log_{10} MoM)	0.69 (0.37–1.32)	0.265	1.33 (0.63–2.80)	0.457	0.39 (0.30 to 0.51)	< 0.0001	0.58 (0.44–0.76)	< 0.0001
sFlt-1 (log_{10} MoM)	0.60 (0.22–1.64)	0.322	2.22 (0.77–6.40)	0.142	6.37 (4.32 to 9.39)	< 0.0001	4.77 (3.19–7.14)	< 0.0001
UtA-PI (log_{10} MoM)	1.95 (0.30–12.49)	0.482	0.33 (0.04–2.79)	0.308	5.25 (2.46 to 11.22)	< 0.0001	3.37 (1.58–7.18)	0.002
UA-PI (log_{10} MoM)	0.82 (0.03–22.72)	0.909	0.09 (0.003–3.36)	0.194	4.69 (1.17 to 18.82)	0.029	—	—
MCA-PI (log_{10} MoM)	0.16 (0.01–5.39)	0.306	4.10 (0.08–219.22)	0.487	0.61 (0.14 to 2.66)	0.513	—	—
MAP (log_{10} MoM)	2.68 (0.01–13.26)	0.755	0.01 (0.00–9.63)	0.190	103.8 (8.02 to 13.43E3)	< 0.0001	—	—

EFW, estimated fetal weight; MAP, mean arterial pressure; MCA, middle cerebral artery; MoM, multiples of the median; OR, odds ratio; PI, pulsatility index; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UA, umbilical artery; UtA, uterine artery.

testing at 32 weeks was not useful in the prediction of low cord blood pH, low Apgar score or NNU admission for cases other than those with PE and/or SGA.

Strengths and limitations of the study

The strengths of this third-trimester screening study are, first, examination of a large population of pregnant women attending for routine care at a gestational-age range which is widely used for the assessment of fetal growth and wellbeing, 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 prospectively obtain 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 30–34 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 with suspected SGA and those with abnormal Doppler findings. Consequently, the performance of screening for adverse perinatal outcomes by biomarkers of impaired placentation and fetal hypoxemia would have been negatively biased. It is likely that at least some of the cases of SGA and fetal distress that were delivered by elective Cesarean section would have resulted in stillbirth, fetal distress in labor and low cord blood pH had they not been detected by the routine assessment at 32 weeks. In our study, there were 30 pregnancies with SGA fetuses that were delivered by elective Cesarean section because of abnormal fetal heart rate patterns or fetal Doppler indices and this number is not negligible in comparison to the number of pregnancies with stillbirths and a birth weight < 10^{th} percentile ($n = 8$).

Comparison with findings from previous studies

There are no previous third-trimester screening studies of a routine population for adverse perinatal events. In previous screening studies at 30–34 weeks' gestation, we used Bayes' theorem to combine maternal characteristics and medical history with biophysical and biochemical markers and reported that, at a 10% FPR, the DR was 99% for preterm PE, 89% for preterm SGA, 75% for term PE and 57% for term SGA[21,22].

Implications for clinical practice

An integrated clinical assessment at 32 weeks' gestation, which includes measurement of biomarkers, would identify nearly all cases that will develop PE and those that will deliver SGA neonates < 37 weeks' gestation and the majority of cases with these complications that deliver at term. Performance of screening by combining biophysical and biochemical markers is superior to screening by either group of markers alone. The performance of screening for stillbirth is poor but this is likely to be underestimated because identification of cases with impaired placentation/placental dysfunction and fetal hypoxemia and their timely delivery would have prevented some of the stillbirths.

In pregnancies without SGA or PE, combined screening at 32 weeks is not useful in the prediction of adverse events during labor or after birth. The extent to which the performance of screening is improved by assessment at 36 weeks, rather than 32 weeks, remains to be determined.

ACKNOWLEDGMENTS

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116). This study is part of the PhD thesis of Nuria Valiño for Universidad de A Coruña, Spain.

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. 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.
4. Soothill PW, Nicolaides KH, Campbell S. Prenatal asphyxia, hyperlacticaemia, hypoglycaemia and erythroblastosis in growth retarded fetuses. *BMJ* 1987; **294**: 1051–1053.
5. 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.
6. 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.
7. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. *Obstet Gynecol* 1992; **79**: 416–420.
8. Odibo AO, Riddick C, Pare E, Stamilio DM, Macones GA. Cerebroplacental Doppler ratio and adverse perinatal outcomes in intrauterine growth restriction: evaluating the impact of using gestational age-specific reference values. *J Ultrasound Med* 2005; **24**: 1223–1228.
9. Bahado-Singh RO, Kovanci E, Jeffres A, Oz U, Deren O, Copel J, Mari G. The Doppler cerebroplacental ratio and perinatal outcome in intrauterine growth restriction. *Am J Obstet Gynecol* 1999; **180**: 750–756.
10. Morales-Roselló J, Khalil A, Morlando M, Bhide A, Papageorghiou A, Thilaganathan B. Poor neonatal acid-base status in term fetuses with low cerebroplacental ratio. *Ultrasound Obstet Gynecol* 2015; **45**: 156–161.
11. Bakalis S, Akolekar R, Gallo DM, Poon LC, Nicolaides KH. Umbilical and fetal middle cerebral artery Doppler at 30–34 weeks' gestation in the prediction of adverse perinatal outcome. *Ultrasound Obstet Gynecol* 2015; **45**: 409–420.
12. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
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 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.
18. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.

