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Prediction of fetal growth restriction using estimated fetal weight *vs* a combined screening model in the third trimester

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KEYWORDS: estimated fetal weight; fetal biometry; fetal growth restriction; screening; small-for-gestational age; third-trimester screening

ABSTRACT

Objectives *To compare the performance of third-trimester screening, based on estimated fetal weight centile (EFWc)* vs *a combined model including maternal baseline characteristics, fetoplacental ultrasound and maternal biochemical markers, for the prediction of small-for-gestational-age (SGA) neonates and late-onset fetal growth restriction (FGR).*

Methods *This was a nested case–control study within a prospective cohort of 1590 singleton gestations undergoing third-trimester (32 + 0 to 36 + 6 weeks' gestation) evaluation. Maternal baseline characteristics, mean arterial pressure, fetoplacental ultrasound and circulating biochemical markers (placental growth factor (PlGF), lipocalin-2, unconjugated estriol and inhibin A) were assessed in all women who subsequently delivered a SGA neonate (*n = *175), defined as birth weight < 10th centile according to customized standards, and in a control group (*n = *875). Among SGA cases, those with birth weight < 3rd centile and/or abnormal uterine artery pulsatility index (UtA-PI) and/or abnormal cerebroplacental ratio (CPR) were classified as FGR. Logistic regression predictive models were developed for SGA and FGR, and their performance was compared with that obtained using EFWc alone.*

Results *In SGA cases, EFWc, CPR Z-score and maternal serum concentrations of unconjugated estriol and PlGF were significantly lower, while mean UtA-PI Z-score and lipocalin-2 and inhibin A concentrations were significantly higher, compared with controls. Using EFWc alone, 52% (area under receiver–operating characteristics curve (AUC), 0.82 (95% CI, 0.77–0.85)) of SGA and 64% (AUC, 0.86 (95% CI, 0.81–0.91)) of FGR cases were predicted at a 10% false-positive rate. A combined screening model including* a-priori *risk (maternal characteristics), EFWc, UtA-PI, PlGF and estriol (with lipocalin-2 for SGA) achieved a detection rate of 61% (AUC, 0.86 (95% CI, 0.83–0.89)) for SGA cases and 77% (AUC, 0.92 (95% CI, 0.88–0.95)) for FGR. The combined model for the prediction of SGA and FGR performed significantly better than did using EFWc alone (*P < *0.001 and* P = *0.002, respectively).*

Conclusions *A multivariable integrative model of maternal characteristics, fetoplacental ultrasound and maternal biochemical markers modestly improved the detection of SGA and FGR cases at 32–36 weeks' gestation when compared with screening based on EFWc alone. Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Fetal growth restriction (FGR), defined as failure to achieve the putative growth potential, affects 7–10% of all pregnancies[1,2]. Growth-restricted fetuses have a 5–10-fold risk of *in-utero* demise and a higher risk of perinatal morbidity and mortality[3,4]. Prenatal diagnosis of FGR has been shown to achieve a significant, 4–5-fold, reduction in perinatal morbidity and mortality[5,6]; however, identification of suboptimal fetal growth remains a challenge.

Currently, in most countries women are not routinely scanned in late pregnancy and are selected for third-trimester ultrasound assessment based on the presence of maternal risk factors or divergence in serial symphyseal–fundal height (SFH) measurements[7,8]. Nevertheless, the performance of SFH measurement

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for the identification of abnormal fetal growth has been shown to be poor[8,9]. As an alternative, universal third-trimester screening to determine estimated fetal weight centile (EFWc) is also offered in some countries, and has shown better performance than SFH[10], but still, can only identify around half or less of small fetuses[11–14].

Recently, the performance of third-trimester combined screening models, including assessment of maternal characteristics, fetal biometry, Doppler parameters and biochemical markers, in the detection of small-for-gestational-age (SGA) fetuses has been evaluated[13,15–22]. These combined models seem to improve the identification of small fetuses delivered preterm, but have limited predictive performance in identifying pathologically small fetuses near or after term, a critical period in which the majority of stillbirths and adverse outcomes occur[21,23,24]. This limited performance could be attributed partially to the manifestation of various forms of fetal smallness. Recently, we established criteria for the antenatal identification of FGR taking into account the degree of smallness and fetoplacental Doppler findings[2], demonstrating that it is possible to recognize the subgroup of small fetuses at highest perinatal risk of adverse outcome[25,26]. Yet, very little information is available on the performance of screening strategies in this high-risk group of patients. The objective of this study was to explore the potential value of third-trimester screening based on EFWc *vs* a combined screening model for the prediction of SGA and FGR fetuses.

METHODS

Study population

This was a nested case–control study drawn from a large prospective cohort of 1590 women with a singleton gestation attending their routine hospital visit in the third trimester of pregnancy (32 + 0 to 36 + 6 weeks' gestation) at the Department of Maternal-Fetal Medicine in Hospital Clinic Barcelona between January 2012 and December 2014. The assessment included recording baseline maternal characteristics, measurement of blood pressure, fetoplacental ultrasound and the collection of maternal serum. Exclusion criteria were prelabor rupture of membranes, chorioamnionitis, spontaneous preterm delivery, aneuploidy or major fetal structural abnormalities. The analysis of biomarkers was conducted in 1050 patients, including all women who subsequently delivered a neonate with birth weight (BW) $< 10^{th}$ centile ($n = 175$) and a group of controls, in a ratio of 5:1 ($n = 875$), comprising consecutive uncomplicated pregnancies in the same period, matched for gestational age (GA) at scan (± 2 weeks). All control pregnancies delivered appropriate-for-gestational-age neonates with BW $\geq 10^{th}$ centile adjusted by gender and GA at delivery according to local standards[27]. GA was calculated based on fetal crown–rump length at 11–13 weeks in all pregnancies[28]. The study protocol consisted of evaluation of maternal blood pressure, fetoplacental ultrasound and maternal biochemical markers at 32 + 0 to 36 + 6 weeks' gestation, and subsequent recording of perinatal outcome. In line with our clinical protocol, FGR cases were delivered electively at 37–38 weeks, while SGA cases were delivered at 40 weeks[2]. The remaining pregnancies were allowed to continue, but elective delivery was offered at 41 weeks. The protocol was approved by the institutional ethics committee (IRB 2012/7154), and all patients provided written informed consent.

Predictive variables

Baseline characteristics

Maternal baseline characteristics, including demographic details and obstetric and medical histories, were prospectively recorded at the time of the third-trimester visit using a patient questionnaire, and data were entered into our database. The following variables were registered: maternal age, ethnicity, nulliparity (no previous delivery after 24 weeks of pregnancy), maternal height and weight, smoking during pregnancy (yes or no), method of conception (spontaneous or use of assisted reproductive technology), medical history (including chronic hypertension, diabetes mellitus, renal disease, autoimmune disease such as systemic lupus erythematous or antiphospholipid syndrome, congenital and acquired thrombophilic conditions) and obstetric history (including previous pregnancy complicated by pre-eclampsia, SGA or stillbirth).

Third-trimester maternal blood pressure

Maternal blood pressure was measured automatically at 32 + 0 to 36 + 6 weeks' gestation with a calibrated OMRON M6 Confort device (OMRON Corporation, Kyoto, Japan), validated for use in pregnancy, according to standard procedure[29]. Blood pressure was measured in one arm without distinction, while women were seated and after a 5-min rest. Mean arterial pressure (MAP) was calculated as: diastolic blood pressure + (systolic blood pressure – diastolic blood pressure)/3.

Third-trimester fetoplacental ultrasound

Transabdominal ultrasound with Doppler evaluation was performed with a Voluson 730 Expert Machine (GE Medical Systems, Zipf, Austria) and a 6–4-MHz probe (Siemens Sonoline Antares, Siemens Medical Systems, Malvern, PA, USA), including fetal biometry and fetoplacental Doppler. Estimated fetal weight (EFW) was calculated using the Hadlock formula by ultrasound measurement of the fetal head circumference, abdominal circumference and femur length[30]. EFW centile (EFWc) was calculated using local standards[27]. Doppler recordings were performed in the absence of fetal movements and voluntarily suspended maternal breathing. The Doppler examination included: umbilical artery (UA) pulsatility index (PI) calculated from a free-floating portion of the umbilical cord, at an angle

of insonation of $< 30°$[31]; middle cerebral artery (MCA) flow velocity waveforms recorded at 1–2 cm from the circle of Willis, during absence of fetal movements at an angle of insonation of $< 30°$; and cerebroplacental ratio (CPR), calculated as the ratio of MCA-PI to UA-PI[32]. For uterine artery (UtA) evaluation, the probe was placed at the lower quadrant of the abdomen, angled medially, and color Doppler imaging was used to identify the UtA at the apparent crossover with the external iliac artery. Mean UtA-PI was calculated as the average PI of the right and left arteries[33].

Third-trimester maternal blood biomarkers

Maternal venous blood samples were collected in serum tubes and processed within 1 h. Serum was separated by centrifugation at 3000 rpm for 10 min at 4°C, and samples were immediately stored at −80°C until assayed. Serum concentrations of placental growth factor (PlGF), unconjugated estriol, inhibin A and lipocalin-2 were measured, after cases and controls were identified, using the AutoDELFIA® automated immunoanalyzer (PerkinElmer, Turku, Finland). Samples were randomly assigned to each plate, and cases and controls were always run in parallel. Biochemical markers were measured with time-resolved fluorescence immunoassays (DELFIA) on an automated platform. For PlGF, unconjugated estriol and inhibin A, commercial AutoDELFIA kits (PerkinElmer) were used, and for lipocalin-2 research reagents were prepared. The AutoDELFIA PlGF kit had a measuring range from 5.6 pg/mL to 4000 pg/mL and a run control coefficient of variation (CV) of 3.2%. The AutoDELFIA unconjugated estriol kit had a measuring range from 0.2 nmol/L to 50 nmol/L and a run control CV of $< 3.7\%$. The AutoDELFIA inhibin A kit had a measuring range from 8.0 pg/mL to 2000 pg/mL and a run control CV of 3.9%. Monoclonal capture antibody (MAB17571, R&D Systems, Abingdon, UK) and polyclonal tracer antibody (AF1757, R&D Systems) were used for the lipocalin-2 assay, which had a measuring range of 0.2 ng/mL to 6.0 ng/mL and a run control CV of 2.0%. Samples for the lipocalin-2 assay were diluted 1:100. For all assays, calibrators and quality (run) controls were run in duplicate on each plate and serum samples in singles. The laboratory personnel were blinded to the clinical results and the outcomes of the patients.

Perinatal outcome

Perinatal outcomes were recorded at delivery by reviewing medical records. The main outcomes of this study were SGA and FGR. SGA was defined as BW $< 10^{th}$ centile according to local reference customized standards[27]. Fetuses with a BW of $< 10^{th}$ centile and, additionally, suspected EFWc $< 10^{th}$ centile and either abnormal CPR ($< 5^{th}$ centile) or UtA-PI ($\geq 95^{th}$ centile) and/or a BW of $< 3^{rd}$ centile according to local standards, were classified as FGR[26]. Other perinatal characteristics such as incidence of pre-eclampsia, induction of labor, Cesarean section, as well as neonatal parameters at delivery (Apgar score, UA-pH) were also collected. Pre-eclampsia was defined as *de novo* blood pressure of $\geq 140/90$ mmHg on two occasions 4 h apart after the 20^{th} gestational week, with concurrent proteinuria (300 mg or more in a 24-h urine specimen)[34]. Neonatal metabolic acidosis was defined as a UA-pH of < 7.15 and a base excess of > 12 mEq/L in the newborn.

Statistical analysis

Student's *t*-test or the Mann-Whitney *U*-test and Pearson's chi-square test were used to perform univariate comparisons between groups of quantitative and qualitative variables, respectively. EFW and Doppler measurements were expressed as the respective percentiles and *Z*-scores, adjusted for GA. Values of MAP, PlGF, unconjugated estriol, inhibin A and lipocalin-2 were $\log_{10}$ transformed to make their distribution Gaussian, and each value was expressed as a multiple of the normal median (MoM) after adjustment for characteristics that provided a substantial contribution to the log-transformed value.

In each patient, the *a-priori* risk for SGA or FGR was calculated using multivariable logistic regression analysis with backward stepwise elimination by sequentially removing non-significant ($P > 0.05$) variables to determine which of the factors among maternal characteristics had a significant contribution to predicting SGA and late-onset FGR. The performance for the prediction of SGA and FGR by *a-priori* risk, MAP, EFWc, UtA-PI, UA-PI, MCA-PI, CPR, PlGF, unconjugated estriol, inhibin A and lipocalin-2, individually and in various combinations, was determined by receiver–operating characteristics (ROC) curve analysis. The resulting areas under the ROC curves (AUCs) were compared using the DeLong method, and $P < 0.05$ was considered statistically significant. Finally, the algorithms for SGA and FGR were applied to the whole population, and detection rates (DRs) were calculated for a 10% false-positive rate (FPR). Statistical analysis and construction of graphs were performed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) and STATA 14 (StataCorp LP, 2015, College Station, TX, USA).

RESULTS

Prediction of small-for-gestational age

Among the 1590 patients who were evaluated, 175 (11%) neonates were born with a BW $< 10^{th}$ centile and were matched with 875 controls with a normal BW. Clinical characteristics and sonographic and biochemical results were obtained for all patients and are shown in Table 1. The prevalence of smoking during pregnancy and of autoimmune disease was significantly higher in the group of women who delivered an SGA neonate than in the control group. There were no significant differences with respect to maternal age and body mass index

Table 1 Maternal baseline, pregnancy and perinatal characteristics of pregnancies with normal birth weight (controls), small-for-gestational-age (SGA) newborns and fetal growth restriction (FGR)

Characteristic	*Controls* (n = *875*)	*SGA* (n = *175*)	P*	*FGR* (n = *93*)	P†
Maternal baseline characteristics					
Age (years)	31 ± 5	32 ± 5	0.29	32.6 ± 5	0.14
BMI (kg/m^2)	22.8 ± 4.1	22.4 ± 3.7	0.16	22.3 ± 4.1	0.09
Ethnicity			0.03		0.14
White	562 (64.2)	122 (69.7)		65 (69.9)	
Other	313 (35.8)	53 (30.3)		28 (30.1)	
Smoking during pregnancy	75 (8.6)	26 (14.9)	0.009	16 (17.2)	0.007
Chronic hypertension	6 (0.7)	4 (2.3)	0.05	4 (4.3)	0.001
Maternal medical condition‡	42 (4.8)	8 (4.6)	0.1	5 (5.4)	0.84
Autoimmune disease	8 (0.9)	5 (2.9)	0.02	2 (2.2)	0.5
Nulliparous	529 (60.5)	116 (66.3)	0.14	57 (61.3)	0.87
Assisted reproductive technology	22 (2.5)	6 (3.4)	0.49	3 (3.2)	0.68
Previous history of SGA	10 (1.1)	5 (2.9)	0.08	4 (4.3)	0.01
Parameters at third-trimester evaluation					
GA at evaluation (weeks)	33.5 ± 0.9	33.8 ± 1.3	0.15	33.9 ± 1.4	0.07
Mean maternal BP (mmHg)	83 (76–88)	85 (77–97)	0.0003	92 (82–113)	< 0.001
Estimated fetal weight (g)	2223 ± 264	1926 ± 352	< 0.001	1805 ± 388	< 0.001
Estimated fetal weight centile	55 (37–74)	18 (4–36)	< 0.001	7 (1–26)	< 0.001
Mean UtA-PI *Z*-score	−0.5 ± 1.06§	0.51 ± 1.75	< 0.001	1.25 ± 2.07	< 0.001
Abnormal UtA-PI (> 95th centile)	25 (2.9)§	32 (20.4)§	< 0.001	32 (41.03)§	< 0.001
UA-PI *Z*-score	−0.20 ± 0.54§	0.46 ± 1.16	< 0.001	0.91 ± 1.38	< 0.001
Abnormal UA-PI (> 95th centile)	3 (0.3)§	20 (11.4)	< 0.001	20 (21.5)	< 0.001
MCA-PI *Z*-score	−0.10 ± 1.2§	−0.56 ± 1.0	< 0.001	−0.87 ± 1.4	< 0.001
CPR *Z*-score	−0.18 ± 1.02§	−0.89 ± 1.16	< 0.001	−1.3 ± 1.29	< 0.001
Abnormal CPR (< 5th centile)	47 (5.4)§	41 (23.4)	< 0.001	41 (44.1)	< 0.001
Maternal serum estriol MoM	1 ± 0.09	0.89 ± 0.21	0.002	0.81 ± 0.26	< 0.001
Maternal serum PlGF MoM	1.01 ± 0.15	0.86 ± 0.21	< 0.001	0.78 ± 0.22	< 0.001
Maternal serum lipocalin-2 MoM	0.99 ± 0.08	1.02 ± 0.09	< 0.001	1.03 ± 0.09	< 0.001
Maternal serum inhibin A MoM	0.99 ± 0.06	1.02 ± 0.10	< 0.001	1.03 ± 0.12	0.001
Perinatal outcome					
GA at delivery (weeks)	39.7 ± 1.3	38.4 ± 2.8	0.07	37.4 ± 3.2	< 0.001
Induction of labor	222 (25.4)	61 (34.9)	0.01	37 (39.8)	0.003
Cesarean delivery	187 (21.4)	62 (35.4)	< 0.001	46 (49.5)	< 0.001
Birth weight (g)	3381 ± 396	2421 ± 570	< 0.001	2089 ± 576	< 0.001
Birth-weight percentile	49 (27–76)	3 (0–6)	< 0.001	0 (0–2)	< 0.001
Pre-eclampsia	14 (1.6)	28 (16.0)	< 0.001	23 (24.7)	< 0.001
5-min Apgar score < 7	10 (1.1)	19 (10.9)	< 0.001	14 (15.1)	< 0.001
Neonatal acidosis	59 (6.7)	17 (9.7)	0.2	10 (10.8)	0.17

Data are presented as *n* (%), mean ± SD or median (interquartile range). *SGA pregnancies *vs* controls. †FGR pregnancies *vs* controls. ‡Including diabetes, hypothyroidism and coagulation disorder. §Missing data. BMI, body mass index; BP, blood pressure; CPR, cerebro-placental ratio; GA, gestational age; MCA, middle cerebral artery; MoM, multiples of the median of the $\log_{10}$ for the biochemical markers; PI, pulsatility index; PlGF, placental growth factor; UA, umbilical artery; UtA, uterine artery.

(BMI). In the SGA group, the median values of EFWc were significantly lower than in the control group. In addition, compared with the control group, mean UtA-PI and UA-PI *Z*-scores were significantly higher while the MCA-PI and CPR *Z*-scores were significantly lower in pregnancies that delivered a SGA neonate. Accordingly, the incidence of abnormal CPR (< 5th centile) and UtA-PI (> 95th centile) was significantly higher in SGA cases than in controls (Table 1). All controls with an abnormal CPR ($n = 47$) were delivered at term, with a median GA at delivery of 39.6 (interquartile range, 38.7–40.6) weeks, of which 12 (26%) were induced for other obstetric indications, and only five had neonatal acidosis.

The following equations best fit the prediction of the MoM ($MoM = e^y/(1 + e^y)$) for each biomarker:

- Lipocalin-2: y = 2.15 + (−0.086 × 0 if white, 1 if non-white) + (0.001 × maternal height in meters) + (0.061 × 0 if non-smoker, 1 if smoker)
- Unconjugated estriol: y = −0.456 + (0.069 × GA in weeks at blood draw) − (0.024 × maternal height in meters) + (0.070 × 0 if white, 1 if non-white) + (0.041 × 0 if parous, 1 if nulliparous)
- PlGF: y = 5.176 − (0.056 × GA in weeks at blood draw) − (0.008 × maternal age in years) − (0.107 × 0 if parous, 1 if nulliparous) + (0.211 × 0 if non-smoker, 1 if smoker) − (0.009 × maternal BMI) − (0.631 × 0 if chronic hypertension absent, 1 if present)
- Inhibin A: y = 1.783 + (0.0337 × GA in weeks at blood draw) + (0.232 × 0 if non-smoker, 1 if smoker) − (0.007 × maternal weight in kg) + (0.019 × maternal BMI).

Compared with the control group, the mean $\log_{10}$ maternal serum MoM concentrations of PlGF and estriol were significantly lower, and of lipocalin-2 and inhibin A significantly higher in the SGA cases. The incidence of pre-eclampsia and obstetric interventions, such as induction of labor and Cesarean section, was significantly higher in the group of SGA neonates; nevertheless, the rate of neonatal acidosis was not significantly different between the two groups. There was one case of antepartum fetal death at 40 weeks' gestation among the SGA neonates that was not associated with pre-eclampsia. This case had normal Doppler values at the time of assessment (33 weeks' gestation).

Adjusted odds ratios (ORs) of each maternal factor in the prediction algorithms for SGA are presented in Table S1. The likelihood of delivering a SGA neonate was higher in cigarette smokers and in women with autoimmune disease. The likelihood of SGA was not affected significantly by maternal age ($P = 0.3$), ethnicity ($P = 0.13$) or BMI ($P = 0.13$). Multivariable regression analysis demonstrated that, in the prediction of SGA, there were significant independent contributions from MAP (OR, 1.04 (95% CI, 1.03–1.06); $P < 0.001$), UA-PI *Z*-score (OR, 3.38 (95% CI, 2.57–4.43); $P < 0.001$), CPR *Z*-score (OR, 0.5 (95% CI, 0.41–0.6); $P < 0.001$) and maternal serum biochemical markers (all $P \leq 0.005$). However, the best prediction model for SGA was provided by a combination of *a-priori* risk (including maternal age and height, BMI, smoking, previous history of SGA neonate and chronic hypertension), EFWc, mean UtA-PI and biochemical markers (including PlGF, estriol and lipocalin-2, but not inhibin A). AUCs and DRs at a FPR of 10% for the prediction of SGA, when screening with EFWc alone and when using the combined model, are shown in Table 2 and in Figure 1. At a FPR of 10%, the DR of EFWc for the prediction of SGA was 52% (AUC, 0.82 (95% CI, 0.77–0.85)), while the combined screening model including maternal characteristics, EFWc, UtA-PI, PlGF, lipocalin-2 and estriol achieved a DR of 61% (AUC, 0.86 (95% CI, 0.83–0.89)). The addition of maternal characteristics and biophysical and biochemical markers to EFWc improved the prediction of SGA by 9% ($P < 0.001$).

Table 2 Screening performance for detection of small-for-gestational age (SGA) newborns in third trimester

	Prediction of SGA	
Screening test	*AUC (95% CI)*	*DR (%) at 10% FPR*
Maternal *a-priori* risk*	0.660 (0.61–0.70)	25
EFWc	0.815 (0.77–0.85)	52
EFWc + PlGF + lipocalin-2 + estriol	0.837 (0.80–0.87)	58
Maternal *a-priori* risk* plus:		
MAP	0.680 (0.62–0.74)	35
EFWc	0.827 (0.79–0.87)	56
CPR	0.716 (0.67–0.76)	33
Inhibin A	0.666 (0.62–0.71)	27
Lipocalin-2	0.678 (0.63–0.73)	32
Estriol	0.716 (0.67–0.76)	34
PlGF	0.739 (0.69–0.78)	42
PlGF + lipocalin-2 + estriol	0.770 (0.73–0.81)	47
EFWc + UtA-PI	0.815 (0.77–0.86)	53
EFWc + CPR + UtA-PI	0.831 (0.79–0.87)	58
EFWc + UtA-PI + PlGF + lipocalin-2 + estriol	0.860 (0.83–0.89)	61

*Including maternal age and height, body mass index, smoking, previous history of SGA and chronic hypertension. AUC, area under receiver–operating characteristics curve; CPR, cerebroplacental ratio; DR, detection rate; EFWc, estimated fetal weight centile; FPR, false-positive rate; MAP, mean arterial pressure; PI, pulsatility index; PlGF, placental growth factor; UtA, uterine artery.

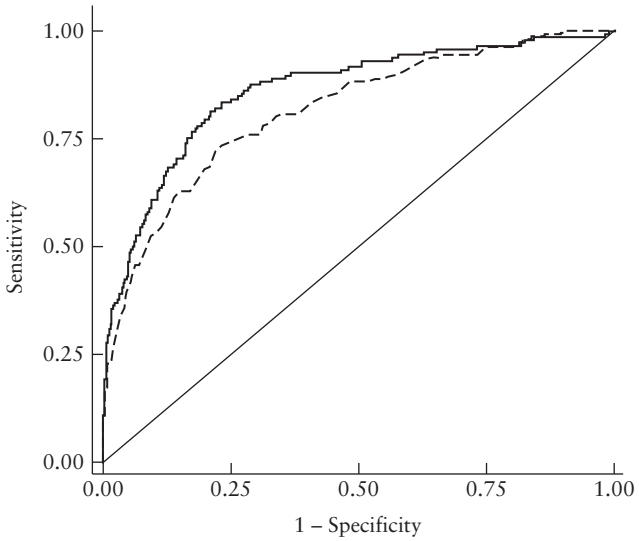

Figure 1 Receiver–operating characteristics curves for prediction of small-for-gestational-age (SGA) neonate by estimated fetal weight centile (EFWc) alone (– – – -) and combination of maternal baseline characteristics (age, height, body mass index, smoking, chronic hypertension and previous SGA), EFWc, uterine artery pulsatility index, placental growth factor, lipocalin-2 and unconjugated estriol (——).

Prediction of fetal growth restriction

Among the 175 SGA neonates, 93 (53%) cases were antenatally categorized as FGR. Epidemiological and clinical characteristics of the FGR cases are shown in Table 1. The prevalences of smoking during pregnancy, chronic hypertension and previous history of SGA were significantly higher in the FGR group than in the control group. In accordance with the findings in the SGA neonates, in the late-onset FGR group, mean UtA-PI and UA-PI *Z*-scores were significantly higher, while CPR *Z*-score was significantly lower compared with the control group. The rate of obstetric intervention (induction of labor and Cesarean section) and the prevalence of pre-eclampsia or adverse perinatal outcomes, such as low Apgar score at birth, were significantly higher in the FGR cases.

Multivariable regression analysis demonstrated that, in the prediction of late-onset FGR, there were significant independent contributions to the *a-priori* risk from

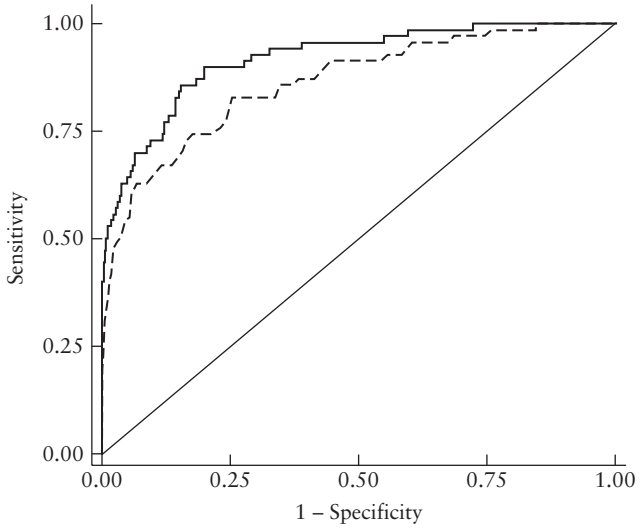

Figure 2 Receiver–operating characteristics curves for prediction of fetal growth restriction by estimated fetal weight centile (EFWc) alone (– – – -) and combination of maternal baseline characteristics (maternal race, height, smoking, obesity, previous history of small-for-gestational age, autoimmune disease), EFWc, uterine artery pulsatility index, placental growth factor and unconjugated estriol (——).

maternal characteristics such as smoking (OR, 2.12 (95% CI, 1.19–3.81); $P = 0.01$), previous history of SGA (OR, 3.86 (95% CI, 1.23–12.30); $P = 0.02$) and chronic hypertension (OR, 7.12 (95% CI, 1.97–25.70); $P = 0.003$) (Table S1). Furthermore, MAP (OR, 1.09 (95% CI, 1.07–1.11); $P < 0.001$), UA-PI *Z*-score (OR, 5.56 (95% CI, 3.87–7.97); $P < 0.001$), CPR *Z*-score (OR, 0.32 (95% CI, 0.24–0.41); $P < 0.001$), mean UtA-PI *Z*-score (OR, 2.46 (95% CI, 2.05–2.96); $P < 0.001$) and biochemical markers (lipocalin-2, estriol, PlGF, inhibin A; all $P < 0.001$) were independent significant contributors to the prediction of FGR. The best prediction model for FGR was provided by a combination of *a-priori* risk (including maternal race and height, smoking, obesity, previous history of SGA and autoimmune disease), EFWc, mean UtA-PI *Z*-score and biochemical markers (unconjugated estriol and PlGF). AUCs at a FPR of 10% for the prediction of FGR when screening with EFWc alone and using the combined model are given in Figure 2, with AUC and DR results summarized in Table 3. At a FPR of 10%, the DR of EFWc for the prediction of FGR was 64% (AUC, 0.86 (95% CI, 0.81–0.91)), while combined screening by maternal characteristics, EFWc, UtA-PI, PlGF and estriol predicted 77% (AUC, 0.92 (95% CI, 0.88–0.95)) of FGR cases.

On pairwise comparison, there was no difference in the predictive ability of EFWc alone compared with the combination of EFWc and biochemical markers (PlGF and estriol) in the third trimester, while the combination of maternal *a-priori* risk with EFWc, UtA-PI and biochemical markers (PlGF and estriol) improved the prediction of FGR by 13% ($P = 0.002$). Bootstrap resampling did not show a significant change in the model. The performance of the models was also tested excluding patients with pre-eclampsia, and the performance was not significantly different (Tables S2 and S3).

Table 3 Screening performance for detection of fetal growth restriction (FGR) in third trimester

	Prediction of FGR	
Screening test	*AUC (95% CI)*	*DR (%) at 10% FPR*
Maternal *a-priori* risk*	0.681 (0.62–0.74)	29
EFWc	0.862 (0.81–0.91)	64
EFWc + PlGF + estriol	0.897 (0.85–0.93)	71
Maternal *a-priori* risk*plus:		
MAP	0.757 (0.68–0.83)	42
EFWc	0.869 (0.82–0.92)	64
UA-PI	0.784 (0.72–0.85)	58
UtA-PI	0.788 (0.72–0.85)	56
Inhibin A	0.696 (0.63–0.76)	36
Lipocalin-2	0.709 (0.65–0.77)	31
Estriol	0.795 (0.74–0.84)	45
PlGF	0.830 (0.77–0.88)	56
PlGF + lipocalin-2 + estriol	0.870 (0.82–0.91)	64
EFWc + UtA-PI	0.881 (0.83–0.93)	71
EFWc + UA-PI + UtA-PI	0.893 (0.85–0.94)	70
EFWc + UtA-PI + PlGF + estriol	0.917 (0.88–0.95)	77

*Including maternal race, height, smoking, obesity, previous history of small-for-gestational age, autoimmune disease. AUC, area under receiver–operating characteristics curve; DR, detection rate; EFWc, estimated fetal weight centile; FPR, false-positive rate; MAP, mean arterial pressure; MoM, multiples of the median; PI, pulsatility index; PlGF, placental growth factor; UA, umbilical artery; UtA, uterine artery.

DISCUSSION

The results of this study showed that a combined screening model including maternal characteristics, fetoplacental Doppler and biochemical markers has a better performance than EFWc alone in predicting SGA and FGR. When using a standard definition of fetal smallness (BW < 10th centile), the use of EFWc at 32–36 weeks' gestation could identify only half of the SGA newborns (DR, 52%), which is in agreement with previous studies reporting a DR of between 54% and 63% in the third trimester[11,12,16,17,35]. The combination of maternal characteristics, EFWc, UtA-PI and biochemical markers (PlGF, lipocalin-2 and estriol) improved the prediction of SGA, achieving a DR of 61% at a FPR of 10%. The improvement is still limited and the result is comparable with those of other studies predicting neonates with a BW < 10th centile at, or after, term using combined models, with DRs between 51% and 74% at a 10% FPR[19,20,23]. It is probable that a definition of fetal smallness using the 10th centile lacks sensitivity, as cases of true growth restriction that do not fall below the 10th centile can be missed, but it also includes 'constitutionally small' fetuses. Thus, we hypothesized that using a more specific definition of FGR could improve the predictive

value. By using a prenatal stringent definition of FGR[2], EFWc measurement identified 64% of FGR cases at a FPR of 10%. This performance was improved to a DR of 77% by using a combined screening model including maternal characteristics, EFWc, UtA-PI and biochemical markers (PlGF and estriol). To our knowledge, this is the first report that distinguishes the performance of a combined screening model in pregnancies with antenatal suspicion of FGR from those with SGA, demonstrating that a combined screening model was able to identify most of the fetuses at higher risk of perinatal morbidity and mortality.

It is noteworthy that mean UtA-PI improved the performance of the predictive models of both SGA and FGR. Similar studies have also shown that, among Doppler parameters, mean UtA-PI is a consistent significant contributor to the prediction of SGA in the third trimester[19,23], while UA-PI, MCA-PI and CPR seem to have a limited performance when evaluated at these gestational ages[15,19]. It is also worth mentioning that, in the prediction of FGR, third-trimester biochemical markers alone had a similar performance to that obtained by EFWc alone. This result is important as, in centers in which third-trimester screening is not routine practice, the use of a set of biomarkers may select a population at a higher risk of FGR that may benefit from more intensive follow-up. Our group has previously reported a similar predictive ability between angiogenic factors and Doppler indices for adverse perinatal outcome in a cohort of growth-restricted fetuses[36]. Limited availability of trained sonographers may justify additional research on the use of predictive markers such as maternal risk factors and biochemical markers as contingency screening strategies for the prediction of FGR.

While all biochemical markers showed significantly different concentrations between SGA and FGR cases and controls, PlGF and estriol were the only biochemical markers that were consistently included in the two combined predictive models in addition to maternal factors and ultrasonographic data. These results are consistent with those of previous studies proposing angiogenic factors and estriol for the prediction and diagnosis of fetal smallness[22,36–43]. Angiogenic imbalance has emerged as a valuable parameter for identifying placenta-related disorders. Cross-sectional and longitudinal studies have reported that, compared with controls, PlGF serum concentrations are significantly lower in women who subsequently deliver a SGA neonate[23,37,38]. Furthermore, our group recently reported that lower maternal serum PlGF concentration in cases diagnosed with FGR is associated with subsequent adverse perinatal outcome and histologic evidence of placental underperfusion[36,39]. Thus, it has been proposed that PlGF alone would be enough to differentiate growth-restricted fetuses from constitutionally small fetuses[44].

As part of the Quad screen, inhibin A and estriol were initially considered as a screening method for trisomy 21 and subsequently were used to predict the occurrence of adverse perinatal outcome[43]. Our results are in agreement with those of previous studies showing lower maternal serum concentration of estriol and higher concentration of inhibin A in small fetuses with placental compromise identified by abnormal UA Doppler[45–47]. Similar to other reports, we also found significantly higher serum concentrations of inhibin A and lipocalin-2 in pregnancies with SGA fetuses[46,48]. Yet, neither inhibin A nor lipocalin-2 was of value in the combined screening model for FGR presented herein. It is possible that the limited value of the biochemical markers in the prediction of late-onset FGR is related to limited knowledge of the multifactorial components of growth restriction and the broad definitions used in the literature.

We acknowledge that, although the design of this study is efficient in exploring potential predictors, nested case–control studies are susceptible to bias, and the performance of the combined screening model presented here should be validated in other populations. We also acknowledge that, in the prediction of FGR, the model oversized the rule of 10 events per predictive variable, thus we had to use bootstrap analysis to validate the model-based inferences. Other limitations of the present study include the lack of information from histological examination reports of the placenta and the long period between the time of screening and the time of onset of the condition. The scan-to-delivery interval is likely to attenuate the predictive value of the sonographic and biochemical markers in the analysis, resulting in a conservative bias.

In conclusion, in comparison with screening based on EFWc alone, a combined screening model of maternal characteristics, fetoplacental ultrasound and maternal biochemical markers modestly improves the detection of SGA and/or FGR cases at 32–36 weeks' gestation. The concept that FGR may involve several different subtypes is now emerging in the literature[25]. Our findings support the idea that SGA and FGR are two different entities, as the baseline maternal characteristics, biophysical and biochemical parameters that were included in the predictive algorithms were different for the two groups. If late-onset FGR represents a heterogeneous disease with minimal or no placental involvement, tests based on the identification of placental dysfunction may continue to be of limited value in the prediction of this condition. It is possible that, owing to their clinical heterogeneity, SGA and late-onset FGR require different predictive algorithms, because of the relevant differences in the degree of placental and fetal compromise in these two clinical forms.

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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 Univariate regression analysis to determine significant contributors to the prediction of small-for-gestational-age (SGA) and fetal growth-restricted (FGR) cases

Table S2 Third-trimester screening performance for detection of small-for-gestational-age newborns in pregnancies without pre-eclampsia ($n = 1008$)

Table S3 Third-trimester screening performance for prediction of fetal growth restriction in pregnancies without pre-eclampsia ($n = 1008$)