Prediction of small-for-gestational-age neonates: screening by maternal serum biochemical markers at 19–24 weeks

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KEYWORDS: α-fetoprotein; free β-human chorionic gonadotropin; placental growth factor; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of antenatal care; second-trimester screening; small-for-gestational age; soluble fms-like tyrosine kinase-1

ABSTRACT

Objective To investigate the value of maternal serum concentrations of placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG) and α-fetoprotein (AFP) at 19–24 weeks’ gestation, in combination with maternal factors and fetal biometry, in the prediction of delivery of small-for-gestational-age (SGA) neonates, in the absence of pre-eclampsia (PE) and examine the potential value of such assessment in deciding whether the third-trimester scan should be performed at 32 and/or 36 weeks’ gestation.

Methods This was a screening study in 9715 singleton pregnancies, including 481 (5.0%) that delivered SGA neonates with birth weight <5th percentile (SGA <5th), in the absence of PE. Multivariable logistic regression analysis was used to determine if screening by a combination of maternal factors, Z-scores of fetal head circumference, abdominal circumference and femur length, and log10 multiples of the median (MoM) values of PlGF, sFlt-1, PAPP-A, free β-hCG or AFP had a significant contribution to the prediction of SGA neonates. A model was developed in selecting the gestational age for third-trimester assessment, at 32 and/or 36 weeks, based on the results of screening at 19–24 weeks.

Results Compared to the normal group, the mean log10 MoM value of PlGF was lower, AFP was higher and sFlt-1, PAPP-A and free β-hCG were not significantly different in the SGA <5th group that delivered <37 weeks. The detection rate (DR) of combined screening by maternal factors, fetal biometry and serum PlGF and AFP at 19–24 weeks was 100%, 76% and 38% for SGA <5th delivering <32, 32–36 and ≥37 weeks’ gestation, respectively, at a false-positive rate (FPR) of 10%. In a hypothetical model, it was estimated that, if the desired objective of prenatal screening is to predict about 80% of the cases of SGA <5th, it would be necessary to select 11% of the population at the 19–24-week assessment to be reassessed at 32 weeks and 46% to be reassessed at 36 weeks; 54% would not require a third-trimester scan.

Conclusion Prenatal prediction of a high proportion of SGA neonates necessitates the undertaking of screening in the third trimester of pregnancy, in addition to assessment in the second trimester, and the timing of such screening, at 32 and/or 36 weeks, should be contingent on the results of the assessment at 19–24 weeks. Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

The increased risk of perinatal mortality and morbidity associated with small-for-gestational-age (SGA) neonates can be reduced substantially in cases identified prenatally, through close monitoring, timely delivery and prompt neonatal management. The traditional approach of identifying pregnancies at high risk of delivering SGA neonates is maternal abdominal palpation or measurement of the symphysis–fundus height, but the performance of such screening is poor, with detection of <30% of affected fetuses. A routine third-trimester scan is, by far, superior to abdominal palpation in identifying pregnancies at high risk of delivering SGA neonates. However, the timing of such a scan is uncertain. About 90% of SGA neonates with birth weight <5th percentile (SGA <5th) are born ≥37 weeks’ gestation.
gestation and 10% < 37 weeks. Screening at 36 weeks is superior to screening at 32 weeks in the prediction of SGA neonates, but at the inevitable expense of missing most cases delivering < 37 weeks. We have proposed that the decision on whether the third-trimester scan should be performed at 32 or 36 weeks should be based on the findings of the assessment at 19–24 weeks. It was estimated that, if the method of screening at 19–24 weeks is a combination of maternal characteristics and medical history (maternal factors) with fetal biometry, and the desired objective of prenatal screening is to predict about 80% of the cases of SGA at 5th, it would be necessary to select about 30% of the population at the 19–24-week assessment to be reassessed at 32 weeks and 40% to be reassessed at 36 weeks.

Several studies have reported on the association between low or high levels of several maternal serum biochemical markers and the birth of SGA neonates. A large screening study at 11–13 weeks’ gestation reported that, in the cases delivering SGA neonates in the absence of pre-eclampsia (PE), serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) were decreased. A meta-analysis of studies on the association between second-trimester biochemical markers of aneuploidy reported that increased risk for delivery of SGA neonates was associated with high levels of serum α-fetoprotein (AFP) and hCG. Case-control studies at 20–25 weeks’ gestation reported that, in pregnancies delivering SGA neonates in the absence of PE, maternal serum placental growth factor (PIGF) is decreased and serum soluble fms-like tyrosine kinase-1 (sFlt-1) is increased. A prospective cohort study of 3348 pregnancies reported that decreased serum PIGF at 22–26 weeks’ gestation was associated with an increased risk for delivery of SGA neonates, in the absence of PE.

The objectives of this study, in singleton pregnancies undergoing routine antenatal care, were first, to investigate the potential value of combined screening by maternal factors, fetal biometry and serum PIGF, sFlt-1, PAPP-A, free β-hCG or AFP at 19–24 weeks’ gestation in the prediction of delivery of SGA neonates, in the absence of PE, and second, to examine the potential value of such assessment in deciding whether the third-trimester scan should be at 32 or 36 weeks’ gestation.

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 second trimester of pregnancy at King’s College Hospital, London, and Medway Maritime Hospital, Kent, between October 2011 and January 2014. This visit, which is held at 19+0 to 24+6 weeks’ gestation, included the recording of maternal characteristics and medical history, calculation of estimated fetal weight from transabdominal ultrasound measurements of fetal head circumference (HC), abdominal circumference (AC) and femur length (FL), measurement of uterine artery pulsatility index and mean arterial pressure and measurement of maternal serum concentrations of PIGF, sFlt-1, PAPP-A, free β-hCG and AFP (Cobas e411, Roche Diagnostics, Penzberg, Germany). Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks or the fetal HC at this visit.

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. This study is part of a research program on the second-trimester prediction of PE and/or SGA. In this publication, we present the results on combined screening with maternal factors, fetal biometry and biochemical markers in the prediction of SGA, in the absence of PE. All pregnancies included in the study resulted in the live birth or stillbirth of phenotypically normal babies ≥ 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/assisted conception requiring the use of ovulation drugs), 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), family history of PE in the mother of the patient (yes/no) and obstetric history including parity (parous/nulliparous if no previous pregnancies ≥ 24 weeks’ gestation), previous pregnancy with PE (yes/no) or SGA (yes/no), neonatal birth weight of previous pregnancy expressed as a Z-score corrected for gestational age and the time interval between the last delivery and conception of the current pregnancy in years. The 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 primary outcome of the study was SGA without PE. The newborn was considered to be SGA if the birth weight was < 5th percentile after correcting for gestational age at delivery (SGA < 5th). The definitions of non-proteinuric gestational hypertension (GH) and PE were those of the International Society for the Study of Hypertension in Pregnancy. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to confirm if the condition was chronic hypertension, PE or GH.

Statistical analysis

The observed measurements of fetal HC, AC and FL were expressed as respective Z-scores for gestational age. The observed values of serum PIGF, sFlt-1, PAPP-A,
free β-hCG and AFP were log_{10} transformed to make their distributions Gaussian. Each measured value in the outcome groups was expressed as a multiple of the normal median (MoM) after adjustment for those characteristics found to provide a substantial contribution to the log_{10} transformed value^{18–22}. Mann–Whitney U-test was used to compare the median MoM values of each biomarker between the outcome groups and regression analysis was used to determine the significance of association between log_{10} MoM of each biomarker with gestational age at delivery and birth-weight Z-score.

The a-priori risk for SGA < 5\textsuperscript{th} delivering < 37 weeks’ gestation was determined using the algorithm derived from the multivariable logistic regression analysis of maternal factors for the prediction of SGA < 5\textsuperscript{th} delivering < 37 weeks’ gestation, as described previously\textsuperscript{7}. Multivariable logistic regression analysis was used to determine if the maternal factor-derived logit (a-priori risk), Z-scores for HC, AC and FL and log_{10} MoM values of PlGF, sFlt-1, PAPP-A, free β-hCG and AFP had significant contributions in predicting SGA < 5\textsuperscript{th} delivering < 37 weeks’ gestation. Similarly, the algorithm was used to determine the performance of screening for SGA < 5\textsuperscript{th} delivering < 32, 32–36 and ≥ 37 weeks’ gestation and for SGA defined by a birth weight < 10\textsuperscript{th} percentile (SGA < 10\textsuperscript{th}) and < 3\textsuperscript{rd} percentile (SGA < 3\textsuperscript{rd}).

The datasets from our previous studies of fetal biometry and serum metabolites at 30–34 weeks’ gestation\textsuperscript{23} and fetal biometry at 35–37 weeks\textsuperscript{6} were used to combine the maternal factor-derived logit (a-priori risk), using the algorithm derived from the previously reported multivariable logistic regression analysis\textsuperscript{7}, with fetal biometry and serum metabolites at 30–34 weeks\textsuperscript{23} and fetal biometry at 35–37 weeks\textsuperscript{6} to determine the performance of screening for SGA < 5\textsuperscript{th} delivering between 32–36 weeks and ≥ 37 weeks, respectively. At 35–37 weeks, the performance of screening by maternal factors and fetal biometry is not improved by the addition of serum metabolites\textsuperscript{6,24}.

The statistical software package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

RESULTS

The study population in which maternal factors, fetal biometry and serum biochemistry were recorded comprised 9715 pregnancies, including 9234 (95.0%) cases that were unaffected by SGA, PE or GH, and 46 and 435 that delivered SGA < 5\textsuperscript{th} neonates, in the absence of PE, < 37 and ≥ 37 weeks’ gestation, respectively. The characteristics of the study population are presented in Table 1.

Normal pregnancy outcome

In the unaffected pregnancies with a birth weight ≥ 5\textsuperscript{th} percentile, the mean ± SD and 5\textsuperscript{th}, 10\textsuperscript{th}, 90\textsuperscript{th} and 95\textsuperscript{th} percentiles of log_{10} MoM values of each biochemical marker are shown in Table S1. Correlations between log_{10} MoM values of PlGF, sFlt-1, PAPP-A, free β-hCG and AFP in the normal group are shown in Table S2 and correlations between log_{10} MoM values of each metabolite with gestational age at delivery and birth-weight Z-score are shown in Table S3.

Small-for-gestational age

Compared to the normal group, the mean log_{10} MoM value of PlGF was lower and AFP was higher in the SGA < 5\textsuperscript{th} group delivering < 37 weeks but mean log_{10} MoM values of sFlt-1, PAPP-A and free β-hCG were not significantly different (Table S4). In the group of SGA < 5\textsuperscript{th} that delivered ≥ 37 weeks, the mean log_{10} MoM values of PlGF, sFlt-1 and PAPP-A were lower, but AFP and free β-hCG were not significantly different. Correlations between log_{10} MoM values of each metabolite with gestational age at delivery and birth-weight Z-score are shown in Table S3 and Figures S1 and S2.

Performance of screening at 19–24 weeks

Multivariable logistic regression analyses demonstrated that, in the prediction of SGA < 5\textsuperscript{th} delivering < 37 weeks’ gestation, there were significant contributions from maternal factors, fetal biometry and serum PlGF and AFP (Table S5). The performance of combined screening for SGA < 10\textsuperscript{th}, SGA < 5\textsuperscript{th} and SGA < 3\textsuperscript{rd} delivering < 32, 32–36 and ≥ 37 weeks’ gestation by maternal factors, fetal biometry and serum PlGF and AFP is shown in Tables 2 and S6. The areas under the receiver–operating characteristics (ROC) curves (AUC) and detection rates (DRs), at false-positive rates (FPRs) of 5% and 10%, of SGA < 10\textsuperscript{th}, SGA < 5\textsuperscript{th} and SGA < 3\textsuperscript{rd} delivering < 37 weeks’ gestation when screening by a combination of maternal factors, fetal biometry and serum PlGF and AFP are given in Table 2 and illustrated in Figures S3–S5.

Performance of screening at 32 and 36 weeks

The fitted regression models of maternal factors, fetal biometry and serum PlGF at 30–34 weeks’ gestation for the prediction of SGA < 5\textsuperscript{th} in the absence of PE are shown in Table S7. The fitted regression model of maternal factors and fetal biometry at 35–37 weeks’ gestation for the prediction of SGA < 5\textsuperscript{th} in the absence of PE has been published previously\textsuperscript{6,24}.

The ROC curves for prediction of SGA < 5\textsuperscript{th} delivering between 32–36 weeks by combined screening in this study at 19–24 weeks and our previous study at 30–34 weeks\textsuperscript{23} are shown in Figure S5. Similarly, the ROC curves for prediction of SGA < 5\textsuperscript{th} delivering ≥ 37 weeks by combined screening in this study at 19–24 weeks and our previous studies at 30–34 weeks\textsuperscript{23} and 35–37 weeks\textsuperscript{6} are shown in Figure S5.

Selection of gestational age for third-trimester screening

In this section we develop a hypothetical model for the follow-up of pregnancies after the assessment at 22 weeks
Table 1 Characteristics of the study population of pregnant women with normal outcomes and those with small-for-gestational-age (SGA) neonates without pre-eclampsia (PE)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal (n = 9234)</th>
<th>Delivery &lt; 37 weeks (n = 46)</th>
<th>Delivery ≥ 37 weeks (n = 435)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.1 (26.6–34.8)</td>
<td>31.4 (27.4–36.1)</td>
<td>29.5 (25.2–34.0)</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>71.0 (63.5–82.0)</td>
<td>66.7 (60.1–80.0)</td>
<td>65.2 (58.0–73.9)</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>165 (160–169)</td>
<td>163 (157–167)</td>
<td>162 (157–166)</td>
</tr>
<tr>
<td>GA at screening (weeks)</td>
<td>21.9 (21.2–22.1)</td>
<td>21.9 (21.4–22.1)</td>
<td>21.9 (21.1–22.1)</td>
</tr>
<tr>
<td>Racial origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>6870 (74.4)</td>
<td>24 (52.2)*</td>
<td>280 (64.4)*</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>1611 (17.4)</td>
<td>11 (23.9)</td>
<td>101 (23.2)</td>
</tr>
<tr>
<td>South Asian</td>
<td>378 (4.1)</td>
<td>5 (10.9)</td>
<td>28 (6.4)</td>
</tr>
<tr>
<td>East Asian</td>
<td>179 (1.9)</td>
<td>3 (6.5)</td>
<td>12 (2.8)</td>
</tr>
<tr>
<td>Mixed</td>
<td>196 (2.1)</td>
<td>3 (6.5)*</td>
<td>14 (3.2)</td>
</tr>
<tr>
<td>Obstetric history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>4323 (46.8)</td>
<td>23 (50.0)</td>
<td>254 (58.4)*</td>
</tr>
<tr>
<td>Parous with no prior PE or SGA</td>
<td>4388 (47.5)</td>
<td>17 (37.0)</td>
<td>136 (31.3)*</td>
</tr>
<tr>
<td>Parous with prior PE, no SGA</td>
<td>274 (3.0)</td>
<td>1 (2.2)</td>
<td>9 (2.1)</td>
</tr>
<tr>
<td>Parous with prior SGA, no PE</td>
<td>226 (2.4)</td>
<td>5 (10.9)*</td>
<td>32 (7.4)*</td>
</tr>
<tr>
<td>Parous with prior SGA and PE</td>
<td>23 (0.2)</td>
<td>0 (0.0)</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>Interpregnancy interval (years)</td>
<td>2.9 (1.9–4.9)</td>
<td>4.5 (3.4–5.8)</td>
<td>3.5 (2.4–6.0)*</td>
</tr>
<tr>
<td>Cigarette smoker</td>
<td>873 (9.5)</td>
<td>11 (23.9)*</td>
<td>122 (28.0)*</td>
</tr>
<tr>
<td>Mode of conception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>8922 (96.6)</td>
<td>43 (93.5)</td>
<td>421 (96.8)</td>
</tr>
<tr>
<td>Ovulation drugs</td>
<td>82 (0.9)</td>
<td>1 (2.2)</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>In-vitro fertilization</td>
<td>230 (2.5)</td>
<td>2 (4.3)</td>
<td>10 (2.3)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>93 (1.0)</td>
<td>3 (6.5)*</td>
<td>8 (1.8)</td>
</tr>
<tr>
<td>Pre-existing diabetes mellitus</td>
<td>92 (1.0)</td>
<td>3 (6.5)*</td>
<td>8 (1.8)</td>
</tr>
<tr>
<td>Type 1</td>
<td>33 (0.4)</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Type 2</td>
<td>59 (0.6)</td>
<td>3 (6.5)*</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>SLE or APS</td>
<td>14 (0.2)</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>40.0 (39.0–40.9)</td>
<td>35.0 (33.2–36.0)*</td>
<td>39.9 (39.0–40.8)*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3446 (3144–3760)</td>
<td>1780 (1522–1886)*</td>
<td>2606 (2420–2762)*</td>
</tr>
<tr>
<td>Birth-weight percentile</td>
<td>51.7 (28.1–76.6)</td>
<td>2.3 (0.6–3.7)*</td>
<td>2.6 (1.4–3.8)*</td>
</tr>
</tbody>
</table>

Data are given as median (interquartile range) or n (%). Comparison with normal group by chi-square test or Fisher’s exact test for categorical variables and Mann–Whitney U-test or Student’s t-test for continuous variables, with Bonferroni correction: *P < 0.025. APS, antiphospholipid syndrome; GA, gestational age; SLE, systemic lupus erythematosus.

with the aim of detecting prenatally a high proportion of cases of SGA < 5th. The concept is illustrated in Figure 1. All pregnancies are assessed at 22 weeks and stratified into one of four groups: low risk, moderate risk, high risk and very high risk.

- The low-risk group would not require any further assessment.
- The moderate-risk group would be assessed again at 36 weeks for risk of delivery of SGA < 5th ≥ 37 weeks. On the basis of such assessment, they would be further stratified into a high-risk group in need of close monitoring and a low-risk group that would not have further assessment.
- The high-risk group would be assessed again at 32 weeks for risk of delivery of SGA < 5th between 32–36 weeks. On the basis of such assessment, they would be further stratified into a high-risk group in need of close monitoring and a low-risk group that would be reassessed at 36 weeks. The management after the assessment at 36 weeks would be the same as in the moderate-risk group above.
- The very high-risk group would require further assessment at around 26 weeks to distinguish between the SGA < 5th that would deliver < 32 weeks and the unaffected pregnancies. On the basis of such assessment, they would be further stratified into a high-risk group in need of close monitoring and a low-risk group that would be reassessed at 32 weeks. The management after the assessment at 32 weeks would be the same as in the high-risk group above.

For the model, we assumed that, if 100 000 pregnancies are examined, there will be 5000 cases of SGA < 5th, including 135, 465 and 4400 that would deliver < 32, 32–36 and ≥ 37 weeks’ gestation, respectively. The model is also based on the findings of this study that the performance of screening for SGA < 5th that deliver between 32–36 weeks is higher if screening is performed at 32, than at 22, weeks and the performance for SGA < 5th delivering ≥ 37 weeks is higher if screening is performed at 36, than at 22 or 32, weeks.
Table 2 Performance of screening for small-for-gestational-age (SGA) neonates with birth weight < 10th, < 5th and < 3rd percentiles in the absence of pre-eclampsia, delivering < 32, 32–36 and ≥ 37 weeks’ gestation, using a combination of maternal factors, fetal biometry and serum placental growth factor and α-fetoprotein at 19–24 weeks’ gestation

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Delivery &lt; 32 weeks</th>
<th>Delivery 32–36 weeks</th>
<th>Delivery ≥ 37 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SGA &lt; 10th percentile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (95% CI)</td>
<td>0.963 (0.958–0.967)</td>
<td>0.860 (0.851–0.868)</td>
<td>0.722 (0.712–0.732)</td>
</tr>
<tr>
<td>DR (% (95% CI)) at FPR of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>88.9 (51.8–99.7)</td>
<td>56.1 (42.4–69.3)</td>
<td>19.7 (16.9–22.7)</td>
</tr>
<tr>
<td>10%</td>
<td>88.9 (51.8–99.7)</td>
<td>64.9 (51.1–77.1)</td>
<td>32.2 (28.9–35.7)</td>
</tr>
<tr>
<td><strong>SGA &lt; 5th percentile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (95% CI)</td>
<td>0.999 (0.998–1.000)</td>
<td>0.931 (0.925–0.937)</td>
<td>0.752 (0.742–0.761)</td>
</tr>
<tr>
<td>DR (% (95% CI)) at FPR of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>100.0 (47.8–100.0)</td>
<td>72.7 (54.5–86.7)</td>
<td>24.6 (20.2–29.4)</td>
</tr>
<tr>
<td>10%</td>
<td>100.0 (47.8–100.0)</td>
<td>75.8 (57.7–88.9)</td>
<td>37.9 (32.8–43.1)</td>
</tr>
<tr>
<td><strong>SGA &lt; 3rd percentile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (95% CI)</td>
<td>0.999 (0.998–1.000)</td>
<td>0.966 (0.962–0.970)</td>
<td>0.760 (0.753–0.772)</td>
</tr>
<tr>
<td>DR (% (95% CI)) at FPR of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>100.0 (47.8–100.0)</td>
<td>82.4 (56.6–96.2)</td>
<td>25.2 (19.6–31.6)</td>
</tr>
<tr>
<td>10%</td>
<td>100.0 (47.8–100.0)</td>
<td>88.2 (63.6–98.5)</td>
<td>38.3 (31.8–45.2)</td>
</tr>
</tbody>
</table>

AUC, area under the receiver–operating characteristics curve; DR, detection rate; FPR, false-positive rate.

![Figure 1](https://example.com/flowchart.png)

**Figure 1** Flowchart demonstrating the potential value of the assessment at 19–24 weeks in identifying about 80% of pregnancies delivering small-for-gestational-age neonates with birth weight < 5th percentile.

**Prediction of SGA delivering < 32 weeks**

In a population of 100 000 pregnancies, there are 135 cases of SGA < 5th delivering < 32 weeks’ gestation. The estimated FPR to detect between 50% and 100% of these fetuses increases from 0.07% to 0.32% and the total number of pregnancies classified at 22 weeks as being very high risk requiring follow-up scans at around 26 weeks would vary from 134 to 439, respectively (Table S8).

**Prediction of SGA delivering between 32–36 weeks**

In a population of 100 000 pregnancies, there are 465 cases of SGA < 5th delivering between 32–36 weeks’ gestation. The estimated FPR to detect between 50% and 100% of these fetuses increases from 2.11% to 45.23% and the total number of pregnancies classified at 22 weeks as being very high risk or high risk requiring assessment at 32 weeks would vary from 2238 to 43 434, respectively (Table S8).

On the basis of the data from combined screening at 30–34 weeks, the estimated FPR to detect between 50% and 100% of the cases of SGA < 5th that deliver between 32–36 weeks would vary from 0.35% to 19.33% (Table S9). Consequently, the number of pregnancies requiring follow-up scans at around 34 weeks would vary from 383 to 8771, respectively.

In Table S9 we provide the necessary data to estimate the number of assessments at 22 and 32 weeks to achieve a desired DR of SGA < 5th delivering between 32–36 weeks. There are several approaches that can be used to achieve a desired prenatal DR. For example, one option for a DR of about 50% of SGA < 5th that deliver between 32–36 weeks is to identify at 22 weeks the pregnancies that contain 100% of this SGA group, reassess these pregnancies at 32 weeks and then define the FPR necessary to detect 50% of the affected cases. Another option is to identify at 22 weeks the pregnancies that contain 50% of the SGA group, reassess these pregnancies at 32 weeks and then define the FPR necessary to detect 100% of the affected cases. Since the performance of screening at 32 weeks is superior to that at 22 weeks, the second option would be preferable because the same overall DR can be achieved with the need for a considerably lower number of assessments at 32 weeks.

Prediction of SGA delivering ≥ 37 weeks

In a population of 100 000 pregnancies, there are 4400 cases of SGA < 5th delivering ≥ 37 weeks. The estimated FPR to detect between 50% and 100% of these fetuses increases from 17.23% to 95.98% and the total number of pregnancies classified at 22 weeks as being very high risk, high risk or moderate risk requiring assessment at 36 weeks would vary from 18 569 to 95 581, respectively (Table S8).

On the basis of the data from combined screening at 35–37 weeks by maternal factors and fetal biometry, the estimated FPR to detect between 50% and 100% of the cases of SGA < 5th that deliver ≥ 37 weeks would vary from 3.37% to 77.48% (Table S10). Consequently, the number of pregnancies requiring follow-up scans or early delivery at around 38 weeks would vary from 5273 to 75 047, respectively.

There are several approaches that can be used to achieve a desired prenatal DR of SGA < 5th delivering ≥ 37 weeks' gestation. As in the case of prediction of SGA delivering between 32–36 weeks described above, the preferred strategy would be to select, at 22 weeks, the group for assessment at 36 weeks and then define the FPR necessary to detect 100% of the affected cases. In Table S10 we provide all the necessary data to estimate the number of assessments at 22 and 36 weeks to achieve a desired DR of SGA < 5th delivering ≥ 37 weeks.

Prenatal prediction of 80% of SGA delivering at any gestational age

On the basis of the data in Tables S8–S10, it was estimated that, if the desired objective of prenatal screening is to predict about 80% of the cases of SGA < 5th in a population of 100 000 pregnancies, the following steps would be necessary (Figure 1). First, at 22 weeks, identify a very high-risk group of 412 pregnancies which would contain 80% of cases of SGA delivering < 32 weeks' gestation; these pregnancies would require monitoring which would include at least one scan at around 26 weeks. Second, at 22 weeks, identify a high-risk group of 10 965 pregnancies which would contain 80% of cases of SGA delivering at 32–36 weeks' gestation and provide combined screening at 32 weeks; such screening will identify 2420 pregnancies which would require monitoring, including at least one scan at around 34 weeks. Third, at 22 weeks, identify a moderate-risk group of 46 403 pregnancies which would contain 80% of cases of SGA delivering ≥ 37 weeks’ gestation and provide combined screening at 36 weeks; such screening will identify 33 226 pregnancies which would require monitoring, including at least one scan at around 38 weeks. Fourth, at 22 weeks, identify a low-risk group of 53 597 pregnancies which would not require any further scans.

DISCUSSION

Main findings of the study

The findings of this screening study at 19–24 weeks’ gestation demonstrate that, in pregnancies delivering SGA neonates < 37 weeks in the absence of PE, maternal serum PI GF is reduced, AFP is increased and sFlt-1, PAPP-A and free β-hCG are not significantly different from those in normal pregnancies. Significant contributions to the prediction of SGA are provided by maternal factors, fetal biometry and serum PI GF and AFP. Combined screening predicted, at 10% FPR, about 100%, 76% and 38% of SGA < 5th neonates delivering < 32, 32–36 and ≥ 37 weeks’ gestation.

The performance of combined screening for SGA by maternal factors, fetal biometry and serum metabolites is poorer in the second than in the third trimester. Thus, the DR at 10% FPR, of SGA < 5th delivering between 32–36 weeks improved from 76% with screening at 19–24 weeks to 95% with screening at 30–34 weeks. Similarly, the DR of SGA < 5th delivering ≥ 37 weeks improved from 38% with screening at 19–24 weeks, to 65% with screening at 30–34 weeks and 76% with screening at 35–37 weeks.

Prenatal detection of a high proportion of SGA neonates necessitates assessment in the third trimester and the timing of such assessment, at 32 and/or 36 weeks, could be determined from the findings of combined screening at 22 weeks.

Strengths and limitations

The strengths of this second-trimester screening study for SGA in the absence of PE are first, examination of pregnant women attending for routine care in a gestational-age
range which is widely used for the assessment of fetal anatomy and growth, second, measurement of maternal serum concentration of metabolites that have been shown to be altered in pregnancies associated with impaired placenta
centration, third, expression of the values of metabolites as MoMs after adjusting for factors that affect the mea-
measurements and fourth, use of Bayes’ theorem to combine the prior risk from maternal characteristics and medical
history with biomarkers to estimate patient-specific risks and the performance of screening for SGA of different severities delivering at selected intervals from the time of assessment.

A limitation of the study is that the diagnosis of SGA was based on the birth-weight percentile and no distinction was made between fetal growth
growth restriction (FGR) due to impaired placenta
constitutionally-small fetuses. However, the proportion of FGR to constitutional SGA is likely to be higher in SGA < 5th and SGA < 3rd than in the SGA < 10th groups and in those delivering preterm, and this is reflected in the higher performance of screening for the earlier and more severe forms of SGA. Another limitation of the study is the small number of SGA neonates that delivered < 32 weeks and this is reflected in the wide 95% CIs for the performance of screening.

Comparison with findings from previous studies

The finding in our second-trimester screening study of low serum PI GF in pregnancies that deliver SGA neonates is compatible with the results of previous screening studies in both the first and third trimesters.6,23. Similarly, the finding that serum AFP is increased in SGA pregnancies is compatible with results of previous screening studies in the early second trimester; in the third trimester, serum AFP is decreased in affected pregnancies.23 We found that in pregnancies delivering SGA neonates < 37 weeks, second-trimester serum PAPP-A and free β-hCG is not altered, but previous studies reported that levels are decreased in the first trimester of affected pregnancies; in the third-trimester of affected pregnancies, serum free β-hCG is increased and serum PAPP-A is not significantly different from normal pregnancies.23 We found that second-trimester serum sFlt-1 is decreased in pregnancies delivering SGA neonates but, in our previous screening study in the third trimester, the levels were increased in affected pregnancies.

Implications for clinical practice

In most developed countries, routine ultrasound exami
nation is carried out at 11–13 and at 19–24 weeks and, in some countries, a third scan is offered at 30–34 weeks. The implication of our findings, in the context of prenatal prediction of SGA, is that either all women should be offered two third-trimester scans at 32 and 36 weeks or the decision as to whether a third-trimester scan is neces-
sary and, if so, whether this is carried out at 32 and/or
36 weeks should be contingent on the results of the assessment at 22 weeks. The performance of screening for SGA at 22 and 32 weeks’ gestation achieved by a combination of maternal factors and fetal biometry is improved by the addition of serum biochemical testing. Serum PI GF and AFP can be measured by automated machines within 30 min of blood sampling and it is therefore possible to obtain these measurements during the routine visit for the second-trimester ultrasound scan and estimate the patient specific risks for SGA.

In a previous study, we reported that the distribution of SGA < 5th that deliver < 32, 32–36 and ≥ 37 weeks’ gestation is 3%, 9% and 88%, respectively.7 This study provides the necessary data for development of policies to achieve prenatal prediction of a desired percentage of SGA neonates. In a hypothetical model, the desired objective to predict about 80% of the cases of SGA < 5th delivering < 32, 32–36 and ≥ 37 weeks, would necessitate that the population is divided into four groups at 22 weeks (Figure 1). A very high-risk group, comprising 0.4% of pregnancies, requiring assessment at 26–28 weeks and then again at 32 and 36 weeks, a high-risk group, comprising 10.6% of pregnancies, requiring assessment at 32 and 36 weeks, a moderate-risk group, comprising 35.4% of pregnancies, requiring assessment at 36 weeks, and a low-risk group, comprising 53.6% of pregnancies in no need of further scans. In the 11.0% of the total population having assessment at 32 weeks, 22.1% (2420/10965) would require close monitoring at 32–36 weeks; monitoring would include assessment of fetal growth, biophysical profile, fetal heart-rate patterns and fetal Doppler studies. Similarly, in the 46.4% of the total population having assessment at 36 weeks, 79.2% (36746/46403) would require further monitoring ≥ 37 weeks to define the best plan for delivery.

Future studies will, first, investigate the potential improvement in performance of screening for SGA at 22, 32 and 36 weeks by combining biophysical with biochemical markers with consequent increase in DR and/or decrease in the total number of necessary scans, second, define management protocols for pregnancies identified by screening as being at high risk for SGA and third, examine whether the implementation of such protocols could reduce the high perinatal mortality and morbidity associated with SGA.

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REFERENCES


SUPPORTING INFORMATION ON THE INTERNET

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

Table S1 Log10 multiple of the median (MoM) values of maternal serum metabolite levels in pregnant women with a normal outcome

Table S2 Pearson correlation (r) between log10 multiples of the median (MoM) values of maternal serum metabolites in pregnant women delivering small-for-gestational-age (SGA) neonates and those with normal outcome

Table S3 Pearson correlation (r) between log10 multiples of the median values of maternal serum metabolites with gestational age at delivery and birth-weight Z-score in pregnant women delivering small-for-gestational-age (SGA) neonates and those with normal outcome

Table S4 Maternal serum placental growth factor (PIGF), soluble fms-like tyrosine kinase-1 (sFlt-1), pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG) and α-fetoprotein (AFP) in pregnancies delivering small-for-gestational-age (SGA) neonates and those unaffected by this outcome

Table S5 Fitted regression models with maternal factors, fetal biometry and biochemical markers for the prediction of small-for-gestational-age neonates with birth weight < 5th percentile delivering < 37 weeks’ gestation

Table S6 Performance of screening for small-for-gestational-age (SGA) neonates with birth weight < 10th, < 5th and < 3rd percentiles in the absence of pre-eclampsia, delivering < 32, 32–36 and ≥ 37 weeks’ gestation by maternal factors, serum placental growth factor (PIGF), α-fetoprotein (AFP) and their combination at 19–24 weeks’ gestation

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Table S7 Fitted regression models with maternal characteristics and history, Z-scores for fetal biometric measurements and placental growth factor (PlGF) at 30–34 weeks’ gestation, for the prediction of small-for-gestational-age neonates with birth weight < 5th percentile, in the absence of pre-eclampsia.

Table S8 Estimated number of follow-up scans in 100 000 pregnancies screened for small-for-gestational age (SGA) with birth weight < 5th percentile, in the absence of pre-eclampsia, delivering < 32, 32–36 and ≥ 37 weeks’ gestation by a combination of maternal factors, fetal biometry and serum metabolites at 19–24 weeks’ gestation.

Table S9 Estimated number of follow-up scans in 100 000 pregnancies screened for small-for-gestational age (SGA) with birth weight < 5th percentile, in the absence of pre-eclampsia, delivering between 32–36 weeks’ gestation by a combination of maternal factors, fetal biometry and serum metabolites at 19–24 weeks’ gestation and then combined screening at 30–34 weeks.

Table S10 Estimated number of follow-up scans in 100 000 pregnancies screened for small-for-gestational age (SGA) with birth weight < 5th percentile, in the absence of pre-eclampsia, delivering between 32–36 weeks’ gestation, by a combination of maternal factors, fetal biometry and serum metabolites at 19–24 weeks’ gestation and then combined screening at 35–37 weeks.

Figure S1 Log10 multiples of median (MoM) values of placental growth factor (PlGF) according to gestational age at delivery (left) and birth-weight Z-score (right) in pregnancies delivering small-for-gestational-age neonates, plotted on the 10th (—) and 50th (—) percentiles of the normal range.

Figure S2 Log10 multiples of median (MoM) values of α-fetoprotein (AFP) according to gestational age at delivery (left) and birth-weight Z-score (right) in pregnancies delivering by small-for-gestational-age neonates, plotted on the 10th (—) and 50th (—) percentiles of the normal range.

Figure S3 Receiver–operating characteristics curves for maternal factors (black line), maternal factors with fetal biometry (blue line), combination of maternal factors, fetal biometry and serum placental growth factor (green line), combination of maternal factors, fetal biometry and serum α-fetoprotein (purple line) and the combination of all (red line) in the prediction of small-for-gestational-age neonates with birth weight < 10th (left), < 5th (middle) and < 3rd (right) percentiles delivering < 37 weeks’ gestation.

Figure S4 Receiver–operating characteristics curves for maternal factors (black line), combination of maternal factors with fetal biometry (blue line) and combination of maternal factors, fetal biometry, serum placental growth factor and α-fetoprotein (red line) at 19–24 weeks in the prediction of small-for-gestational-age neonates with birth weight < 5th percentile, delivering < 32 (left), 32–36 (middle) or ≥ 37 (right) weeks’ gestation.

Figure S5 Receiver–operating characteristics curves for a combination of maternal factors, fetal biometry, serum placental growth factor and α-fetoprotein at 19–24 weeks in the prediction of small-for-gestational-age neonates with birth weight < 10th (black line), < 5th (blue line) and < 3rd (red line) percentiles, delivering < 32 (left), 32–36 (middle) or ≥ 37 (right) weeks’ gestation.

Figure S6 Receiver–operating characteristics curves for combined screening by maternal factors, fetal biometry and serum metabolites at 19–24 weeks (black line), 30–34 weeks (blue line) and 35–37 weeks (red line) in the prediction of small-for-gestational-age neonates with birth weight < 5th percentile delivering between 32–36 (left) or ≥ 37 (right) weeks’ gestation.