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

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Abstract

Objective: To investigate the potential 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 maternal factors and fetal biometry for prediction of delivery of small for gestational age (SGA) neonates in the absence of preeclampsia (PE) and examine the potential value of such assessment in deciding whether the third-trimester scan should be at 32 and / or 36 weeks' gestation.

Methods: Screening study in 9,715 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, fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) and log_{10} multiple of the median (MoM) values of PLGF, sFlt-1, PAPP-A, free ß-hCG or AFP had significant contribution in predicting 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: In the SGA <5th group delivering at <37 weeks, compared to the normal group, the mean log_{10} MoM value of PlGF was lower, AFP was higher and sFlt-1, PAPP-A and free β-hCG were not significantly different. 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 at <32, 32-36 and at ≥37 weeks' gestation, respectively, at 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 at the 19-24 weeks assessment to select 11% of the population to be reassessed at 32 weeks, 46% to be reassessed at 36 weeks and 54% that do 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.

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Introduction

Small for gestational age (SGA) neonates are at increased risk of perinatal mortality and morbidity, but the risks can be substantially reduced if the condition is identified prenatally, because in such cases close monitoring and appropriate timing of delivery and prompt neonatal care can be undertaken [1]. The traditional approach of identifying pregnancies at high-risk of delivering SGA neonates is maternal abdominal palpation or measurement of the symphysial-fundal height, but the performance of such screening is poor with a detection rate (DR) of less than 30% [2,3]. A routine third-trimester scan is by far superior to abdominal palpation in identifying pregnancies at high-risk of delivering SGA neonates [4-7]. However, the timing of such scan is uncertain. About 90% of SGA neonates with birth weight below the 5th percentile (SGA <5th), are born at ≥37 weeks' gestation and 10% at <37 weeks [7]. Screening at 36 weeks is superior to screening at 32 weeks in the prediction of SGA delivering at ≥37 weeks, but at the inevitable expense of missing most cases delivering at <37 weeks [5-7]. We have proposed that the decision on whether the third-trimester scan should be at 32 or 36 weeks should be based on the findings of the assessment at 19-24 weeks [7]. 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 <5th, it would be necessary at the 19-24 weeks assessment to select about 50% of the population to be reassessed at 32 weeks and 60% to be reassessed at 36 weeks [7].

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 PE serum pregnancy associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) were decreased [8]. 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 [9]. Case control studies at 20-25 weeks' gestation have reported that in pregnancies delivering SGA neonates in the absence of preeclampsia (PE) maternal placental growth factor (PlGF) is decreased and serum soluble fms-like tyrosine kinase-1 (sFlt-1) is increased [10-12]. A prospective cohort study of 3,348 pregnancies reported that decreased plasma PLGF at 22-26 weeks' gestation was associated with increased risk for delivery of SGA neonates in the absence of PE [13].

The objectives of this study in singleton pregnancies undergoing routine antenatal care are firstly, to investigate the potential value of combined screening by maternal factors, fetal biometry and serum PlGF, 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 secondly, 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 the 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 was held at 19^{+0}-24^{+6} weeks' gestation, included recording of maternal characteristics and medical history, estimation of fetal size from transabdominal ultrasound measurement of fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) [14], measurement of uterine artery pulsatility index and mean arterial pressure and measurement of serum concentrations of PlGF, sFlt-1, PAPP-A, free β-hCG and AFP (Cobas e411, Roche Diagnostics, Penzberg,

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Germany). Gestational age was determined by the measurement of fetal crown-rump length at 11-13 weeks or the fetal HC at this visit [14,15].

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 programme 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. The pregnancies included in the study were those resulting in live birth or stillbirth of phenotypically normal babies at ≥24 weeks' gestation.

Patient characteristics

Patient characteristics recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), medical history of chronic hypertension (yes or no), diabetes mellitus (yes or no), systemic lupus erythematosus (SLE) or anti-phospholipid syndrome (APS), family history of PE in the mother of the patient (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at ≥ 24 weeks' gestation), previous pregnancy with PE (yes or no), neonatal birth weight of previous pregnancy expressed as a Z-score corrected for gestational age [16] and the time interval between the last delivery and conception of the current pregnancy in years. The maternal weight and height were 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 less than the 5th percentile after correction for gestational age at delivery (SGA<5th) [16]. The definitions of non-proteinuric gestational hypertension (GH) and PE were those of the International Society for the Study of Hypertension in Pregnancy [17]. 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 the respective Z-score for gestational age [14]. The observed values of serum PlGF, 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<5th delivering at <37 weeks' gestation was determined using the algorithm derived from the multivariable logistic regression analysis of maternal factors for the prediction of SGA <5th delivering at <37 weeks' gestation [7].

Multivariable logistic regression analysis was used to determine if the maternal factor-derived logit (*a priori* risk), the 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 <5th

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delivering at <37 weeks' gestation. The algorithm was used to determine the performance of screening for SGA <5th delivering at <32, 32-36 and at ≥37 weeks' gestation and SGA defined by birth weight <10th percentile (SGA <10th) and SGA with birth weight <3rd percentile (SGA <3rd).

The datasets from our previous studies of fetal biometry and serum metabolites at 30-34 weeks' gestation [24] and fetal biometry at 35-37 weeks [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 [7], with fetal biometry and serum metabolites at 30-34 [7,24] and fetal biometry at 35-37 weeks [6,7] to determine the performance of screening for SGA <5th delivering at 32-36 weeks and at ≥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 [6,25].

The statistical software package SPSS 22.0 (SPSS Inc.,Chicago, IL) 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 of 9,715 pregnancies, including 9,234 (95.0%) cases that were unaffected by SGA, PE or GH, and 46 and 435 delivering SGA <5th neonates in the absence of PE at <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 birth weight >5th percentile, the mean, standard deviation and 5th, 10th, 90th and 95th percentiles of $\log_{10}$ MoM values of each biochemical marker are shown in sTable 1.

Correlations between $\log_{10}$ MoM values of PlGF, sFlt-1, PAPP-A, free β-hCG and AFP in the normal group are shown in sTable 2 and correlations between $\log_{10}$ MoM values of each metabolite with gestational age at delivery and birth weight Z-score are shown in sTable 3.

Small for gestational age

In the SGA <5th group delivering at <37 weeks, compared to the normal group, the mean $\log_{10}$ MoM value of PlGF was lower, AFP was higher and sFlt-1, PAPP-A and free β-hCG were not significantly different (sTable 4). In the group of SGA <5th delivery at ≥37 weeks, the mean $\log_{10}$ MoM values of PlGF, sFlt-1 and PAPP-A were lower, AFP and free β-hCG were not significantly different (Table 2). Correlations between $\log_{10}$ MoM values of each metabolite with gestational age at delivery, assessment to delivery interval and birth weight Z-score are shown in sTable 3 and sFigures 1-2.

Performance of screening at 19-24 weeks

Multivariable logistic regression analyses demonstrated that in the prediction of SGA<5th delivering at <37 weeks' gestation there were significant contributions from maternal factors, fetal biometry and serum PlGF and AFP (sTable 5). The performance of combined screening for SGA <10th, SGA <5th and SGA <3rd delivering at <32, 32-36 and ≥37 weeks' gestation by maternal factors, fetal biometry and serum PlGF and AFP is shown in sTable 6 and Table 2. The areas under the receiver operating characteristic curves (AUROC) and the DRs, at FPRs of 5% and 10%, of SGA <10th, SGA <5th and SGA <3rd delivering at <37 weeks'

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gestation in screening by a combination of maternal factors, fetal biometry and serum PlGF and AFP are given in Table 2 and illustrated in sFigures 3-5.

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 <5th in the absence of PE are shown in sTable 7. The fitted regression model of maternal factors and fetal biometry at 35-37 weeks' gestation for the prediction of SGA <5th in the absence of PE has been previously published [6,25].

The ROC curves for prediction of SGA <5th delivering at 32-36 weeks by combined screening from this study at 19-24 weeks and our previous study at 30-34 weeks [24] are shown in sFigure 5. Similarly, the ROC curves for prediction of SGA <5th delivering at ≥37 weeks by combined screening from this study at 19-24 weeks and our previous studies at 30-34 weeks [24] and at 35-37 weeks [6] are shown in sFigure 5.

Selection of the gestation for third-trimester screening

In this section we develop a hypothetical model for the follow up of pregnancies after the assessment at 22 weeks 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 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 at 36 weeks for risk of delivery of SGA <5th at ≥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 at 32 weeks for risk of delivery of SGA <5th at 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 at <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 5,000 cases of SGA <5th, including 135, 465 and 4,400 that would deliver at <32, at 32-36 and at ≥37 weeks' gestation, respectively [7]. The model is also based on the findings of this study that the performance of screening for SGA <5th delivering at 32-36 weeks is higher if screening is at 32 than at 22 weeks and the performance for SGA <5th delivering at ≥37 weeks is higher if screening is at 36 than at 22 or 32 weeks.

Prediction of SGA delivering at <32 weeks

In a population of 100,000 pregnancies there are 135 cases of SGA <5th delivering at <32 weeks. 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 (sTable 8).

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Prediction of SGA delivering at 32-36 weeks

In a population of 100,000 pregnancies there are 465 cases of SGA <5th delivering at 32-36 weeks. 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 2,237 to 43,434, respectively (sTable 9).

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 at 32-36 weeks would vary from 0.35 to 19.33% (sTable 9). Consequently, the number of pregnancies requiring follow-up scans at around 34 weeks would vary from 383 to 8,771, respectively.

In sTable 9 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 at 32-36 weeks. There are several approaches that can be used to achieve a desired prenatal DR. For example, one option for DR of about 50% of SGA <5th that deliver at 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 at ≥37 weeks

In a population of 100,000 pregnancies there are 4,400 cases of SGA <5th delivering at ≥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 (sTable 10).

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 at ≥*37* weeks would vary from 3.37% to 77.48% (sTable 10). Consequently, the number of pregnancies requiring follow-up scans or early delivery at around 38 weeks would vary from 5,273 to 75,047, respectively.

There are several approaches that can be used to achieve a desired prenatal DR of SGA <5th delivering at ≥*37* weeks' gestation. As in the case of prediction of SGA delivering at 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 sTable 10 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 at ≥*37* weeks.

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

On the basis of data in sTables 8-10, 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 at <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 group of

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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 2,420 of pregnancies which would require monitoring, including at least one scan at around 34 weeks. Third, at 22 weeks identify a group of 46,403 pregnancies which would contain 80% of cases of SGA delivering at ≥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 at <37 weeks in the absence of PE, maternal serum PlGF is reduced, AFP is increased and sFlt-1, PAPP-A and free β-hCG are not significantly different from normal pregnancies. Significant contributions to the prediction of SGA are provided by maternal factors, fetal biometry and serum PlGF and AFP. Combined screening predicted, at 10% FPR, about 100%, 76% and 38% of SGA <5th neonates delivering at <32, 32-36 and at >37 weeks' gestation.

The performance of combined screening for SGA with 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 at 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 at ≥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 firstly, 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, secondly, measurement of maternal serum concentration of metabolites that have been shown to be altered in pregnancies associated with impaired placentation, thirdly, expression of the values of metabolites as MoMs after adjustment for factors that affect the measurements and fourthly, 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 restriction (FGR) due to impaired placentation and 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 at <32 weeks and this is reflected in the wide 95% CIs for the performance of screening.

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Comparison with findings from previous studies

The finding of our second-trimester screening study of low serum PlGF in pregnancies that deliver SGA neonates, is compatible with the results of previous screening studies in both the first- and third-trimesters [8,24]. Similarly, the finding that serum AFP is increased in SGA pregnancies is compatible with results of previous screening studies in the early second-trimester [9]; in the third-trimester, serum AFP in affected pregnancies is decreased [24]. We found that in pregnancies delivering SGA neonates at <37 weeks, second-trimester serum PAPP-A and free ß-hCG is not altered, but previous studies reported that in affected pregnancies the levels are decreased in the first-trimester [9]; in the third-trimester, in affected pregnancies serum free ß-hCG is increased and serum PAPP-A is not significantly different from normal [24]. We found that in pregnancies delivering SGA neonates, second-trimester serum sFlt-1 is decreased, but in our previous screening study in the third-trimester the levels in affected pregnancies were increased [24].

Implications for clinical practice

In most developed countries routine ultrasound examination 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 necessary 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 PlGF and AFP can be measured by automated machines within 30 minutes 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 at <32, 32-36 and at ≥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 at <32, 32-36 and at ≥37 weeks, would necessitate that at 22 weeks the population is divided into four groups (Figure 1). A very high-risk group, comprising of 0.4% of pregnancies, requiring assessment at 26-28 weeks and then again at 32 and 36 weeks, a high-risk group, comprising of 10.6% of pregnancies, requiring assessment at 32 and 36 weeks, a moderate-risk group, comprising of 35.4% of pregnancies, requiring assessment at 36 weeks, and a low-risk group, comprising of 53.6% of pregnancies in no need for further scans. In the 11.0% of the total population having assessment at 32 weeks, 22.1% (2,420 of 10,965) 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 35.4% of the total population having assessment at 36 weeks, 79.2% (36,746 of 46,403) would require further monitoring at ≥37 weeks to define the best plan for delivery.

Future studies will firstly, 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, secondly, define management protocols for pregnancies identified by screening as being at high-risk for SGA and thirdly, examine whether the implementation of such protocols could reduce the high perinatal mortality and morbidity associated with SGA.

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Figure legends

sFigure 1. Placental growth factor (PlGF) $\log_{10}$ multiple of median (MoM) with assessment to delivery interval (left) and birth weight Z-score (right) in pregnancies complicated by small for gestational age neonates, plotted on the 10th and 50th percentile of the normal range.

sFigure 2. α-fetoprotein (AFP) $\log_{10}$ multiple of median (MoM) with assessment to delivery interval (left) and birth weight Z-score (right) in pregnancies complicated by small for gestational age neonates, plotted on the 5th, 10th, 50th, 90th and 95th percentile of the normal range.

sFigure 3. Receiver operating characteristics curves of maternal factors (black line), maternal factors with fetal biometry (blue line), maternal factors, fetal biometry and serum placental growth factor (green line), 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) percentile delivering at <37 weeks' gestation.

sFigure 4. Receiver operating characteristics curves of maternal factors (black line), maternal factors with fetal biometry (blue line) and maternal factors, fetal biometry and 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 below the 5th percentile, delivering at <32 (left), 32-36 (middle) and at $\geq$37 (right) weeks' gestation.

sFigure 5. Receiver operating characteristics curves of maternal factors, fetal biometry and serum placental growth factor and α-fetoprotein at 19-24 weeks in the prediction of small for gestational age neonates with birth weight below the 10th (black line), 5th (blue line) and 3rd (red line) percentile, delivering at <32 (left), 32-36 (middle) and at $\geq$37 (right) weeks' gestation.

sFigure 6. Receiver operating characteristics curves of combined screening with maternal factors, fetal biometry and serum metabolites at 19-24 weeks (black line) and 30-34 weeks (blue line) in the prediction of small for gestational age neonates with birth weight below the 5th percentile (SGA <5th) delivering at 32-36 weeks' gestation (left) and at 19-24 weeks (black), 30-34 weeks (blue line) and 35-37 weeks (red line) in the prediction of SGA <5th delivering at $\geq$37 weeks' gestation (right).

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

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Table 1. Characteristics of the study population.

Characteristic	Normal (n=9,234)	SGA <37w (n=46)	SGA ≥37 w (n=435)
Maternal age in years, median (IQR)	31.1 (26.6-34.8)	31.4 (27.4-36.1)	29.5 (25.2-34.0)
Maternal weight in Kg, median (IQR)	71.0 (63.5-82.0)	66.7 (60.1-80.0)	65.2 (58.0-73.9)*
Maternal height in cm, median (IQR)	165 (160-169)	163 (157-167)	162 (157-166)*
Gestation at screening in weeks, median (IQR)	21.9 (21.2-22.1)	21.9 (21.4-22.1)	21.9 (21.1-22.1)
Racial origin			
Caucasian, n (%)	6,870 (74.4)	24 (52.2)*	280 (64.4)*
Afro-Caribbean, n (%)	1,611 (17.4)	11 (23.9)	101 (23.2)*
South Asian, n (%)	378 (4.1)	5 (10.9)	28 (6.4)
East Asian, n (%)	179 (1.9)	3 (6.5)	12 (2.8)
Mixed, n (%)	196 (2.1)	3 (6.5)*	14 (3.2)
Past obstetric history			
Nulliparous, n (%)	4,323 (46.8)	23 (50.0)	254 (58.4)*
Parous with no prior PE and SGA, n (%)	4,388 (47.5)	17 (37.0)	136 (31.3)*
Parous with prior PE no SGA, n (%)	274 (3.0)	1 (2.2)	9 (2.1)
Parous with prior SGA no PE, n (%)	226 (2.4)	5 (10.9)*	32 (7.4)*
Parous with prior SGA and PE, n (%)	23 (0.2)	0 (0.0)	4 (0.9)
Inter-pregnancy interval in years, median (IQR)	2.9 (1.9-4.9)	4.5 (3.4-5.8)	3.5 (2.4-6.0)*
Cigarette smoker, n (%)	873 (9.5)	11 (23.9)*	122 (28.0)*
Conception			
Spontaneous, n (%)	8,922 (96.6)	43 (93.5)	421 (96.8)
Ovulation drugs, n (%)	82 (0.9)	1 (2.2)	4 (0.9)
In vitro fertilization, n (%)	230 (2.5)	2 (4.3)	10 (2.3)
Chronic hypertension	93 (1.0)	3 (6.5)*	8 (1.8)
Pre-existing diabetes mellitus, n (%)	92 (1.0)	3 (6.5)*	4 (1.0)
Type 1, n (%)	33 (0.4)	0 (0.0)	2 (0.5)
Type 2, n (%)	59 (0.6)	3 (6.5)*	2 (0.5)
SLE / APS, n (%)	14 (0.2)	0 (0.0)	2 (0.5)
Gestation at delivery in weeks, median (IQR)	40.0 (39.0-40.9)	35.0 (33.2-36.0)*	39.9 (39.0-40.8)*
Birth weight in grams, median (IQR)	3,446 (3,144-3,760)	1,780 (1,522-1,886)*	2,606 (2,420-2,762)*
Birth weight in percentile, median (IQR)	51.7 (28.1-76.6)	2.3 (0.6-3.7)*	2.6 (1.4-3.8)*

SLE = systemic lupus erythematosus; APS = antiphospholipid syndrome; IQR = interquartile range; PE = preeclampsia; SGA = small for gestational age

Comparisons between outcome groups: Chi square test or Fisher exact test for categorical variables and Mann Whitney-U test or student t-test for continuous variables, with Bonferroni correction: * P<0.025

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Table 2. Performance of screening for small for gestational age with birth weight <10th, <5th and <3rd percentile in the absence of preeclampsia, delivering at <32, at <32-36 and at ≥37 weeks' gestation with a combination of maternal factors, fetal biometry and serum placental growth factor and α-fetoprotein at 19-24 weeks' gestation.

Screening test	Small for gestational age without preeclampsia		
	<32 weeks	32-36 weeks	≥37 weeks
SGA <10th percentile			
AUROC (95% CI)	0.963 (0.958-0.967)	0.860 (0.851-0.868)	0.722 (0.712-0.732)
DR, % (95% CI) at FPR of:			
5%	88.9 (51.8-99.7)	56.1 (42.4-69.3)	19.7 (16.9-22.7)
10%	88.9 (51.8-99.7)	64.9 (51.1-77.1)	32.2 (28.9-35.7)
SGA <5th percentile			
AUROC (95% CI)	0.999 (0.998-1.000)	0.931 (0.925-0.937)	0.752 (0.742-0.761)
DR, % (95% CI) at FPR of:			
5%	100.0 (47.8-100.0)	72.7 (54.5-86.7)	24.6 (20.2-29.4)
10%	100.0 (47.8-100.0)	75.8 (57.7-88.9)	37.9 (32.8-43.1)
SGA <3rd percentile			
AUROC (95% CI)	0.999 (0.998-1.000)	0.966 (0.962-0.970)	0.760 (0.753-0.772)
DR, % (95% CI) at FPR of:			
5%	100.0 (47.8-100.0)	82.4 (56.6-96.2)	25.2 (19.6-31.6)
10%	100.0 (47.8-100.0)	88.2 (63.6-98.5)	38.3 (31.8-45.2)

SGA = small for gestational age; AUROC = area under receiver operating characteristics curve; CI = confidence intervals; DR = detection rate; FPR = false positive rate

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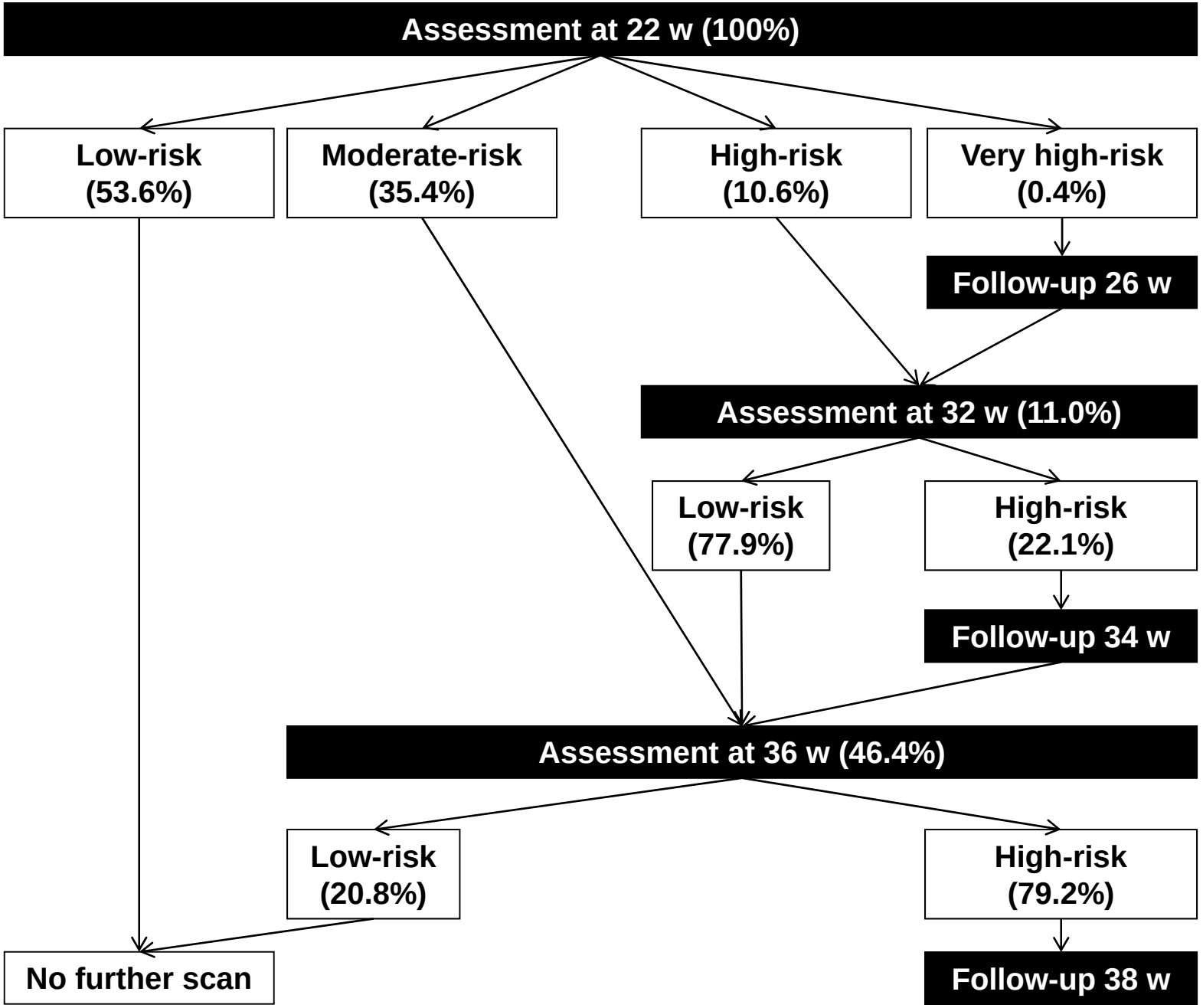

Figure 1

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