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Prediction of stillbirth from placental growth factor at 11–13 weeks

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KEYWORDS: first-trimester screening; placental growth factor; pyramid of pregnancy care; stillbirth

ABSTRACT

Objectives *To investigate whether the addition of maternal serum placental growth factor (PlGF) measured at 11–13 weeks' gestation improves the performance of screening for stillbirths that is achieved by a combination of maternal factors and first-trimester biomarkers such as maternal serum pregnancy-associated plasma protein-A (PAPP-A), fetal ductus venosus pulsatility index for veins (DV-PIV) and uterine artery pulsatility index (UtA-PI) and to evaluate the performance of screening with this model for all stillbirths and those due to impaired placentation and unexplained causes.*

Methods *This was a prospective screening study of 45 452 singleton pregnancies including 45 225 live births and 227 (0.49%) antepartum stillbirths; 131 (58%) were secondary to impaired placentation and 96 (42%) were due to other or unexplained causes. Multivariable logistic regression analysis was used to determine whether the addition of maternal serum PlGF improved the performance of screening that was achieved by a combination of maternal factors and PAPP-A, DV-PIV and UtA-PI.*

Results *Significant contribution to the prediction of stillbirth was provided by maternal factor-derived* a-priori *risk and multiples of the median values of PlGF, DV-PIV and UtA-PI but not of serum PAPP-A. A model combining these variables predicted 42% of all stillbirths and 61% of those due to impaired placentation, at a false-positive rate of 10%; within the impaired placentation group the detection rate of stillbirth < 32 weeks' gestation was higher than that of stillbirth ≥ 37 weeks (71%* vs *46%; P = 0.031).*

Conclusions *A high proportion of stillbirths due to impaired placentation can be identified effectively in the first trimester of pregnancy. Addition of PlGF improves the performance of screening achieved by other maternal factors and biomarkers.*

INTRODUCTION

Antepartum stillbirths can be classified broadly into those thought to be the consequence of impaired placentation, including those associated with pre-eclampsia (PE), birth of a small-for-gestational-age (SGA) neonate or placental abruption, and those due to other or unexplained causes; the rationale of categorizing stillbirths according to the likely underlying cause is that antenatal interventions and preventive strategies could potentially be undertaken more effectively[1–3]. Screening for stillbirth in early pregnancy by a combination of maternal factors, including weight, racial origin, method of conception, cigarette smoking and history of diabetes mellitus, chronic hypertension, systemic lupus erythematosus and antiphospholipid syndrome predicted about 30% of stillbirths, at a false-positive rate (FPR) of 10%[4]. A first-trimester screening study combining maternal factors with measurements of uterine artery pulsatility index (UtA-PI), fetal ductus venosus pulsatility index for veins (DV-PIV) and maternal serum pregnancy-associated plasma protein-A (PAPP-A), which provide indirect information on placentation, reported that, at a FPR of 10%, the detection rate (DR) of stillbirth due to impaired placentation improved to 55%, whereas the DR of stillbirths due to other causes or were unexplained was only 24%[5].

Placental growth factor (PlGF) is an angiogenic protein produced by the placenta and is implicated in trophoblastic invasion of the maternal spiral arteries[6–8]. Maternal serum levels at 11–13 weeks' gestation are decreased in pregnancies with impaired placentation that develop PE and in those that deliver SGA neonates[9–11].

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The objective of this study was to investigate whether measurement of maternal serum PlGF at 11–13 weeks' gestation improves the performance of screening for stillbirths that is achieved by a combination of maternal factors and PAPP-A, DV-PIV and UtA-PI, and to evaluate the performance of screening with this model for all stillbirths and those due to impaired placentation and to unexplained or other causes.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for routine pregnancy care at 11 + 0 to 13 + 6 weeks' gestation at King's College Hospital and Medway Maritime Hospital, UK, between March 2006 and October 2015. We recorded maternal characteristics and medical history and performed combined screening to estimate the risk for fetal aneuploidy based on maternal age, fetal nuchal translucency thickness and measurement of maternal serum PAPP-A and free β-human chorionic gonadotropin (β-hCG)[12]. Transabdominal color Doppler ultrasound was performed to measure fetal DV-PIV and UtA-PI[13,14]. Maternal serum concentrations of free β-hCG, PAPP-A and PlGF were measured using automated analyzers which provide reproducible results within 10 min of blood sampling (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA or Cobas e411 system, Roche Diagnostics, Penzberg, Germany). Gestational age was determined from measurement of fetal crown–rump length[15]. The study was approved by the ethics committee and all participating women gave written informed consent.

The inclusion criteria were women with a singleton pregnancy who delivered a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. Pregnancies with aneuploidy or major fetal abnormality, and those ending in a miscarriage, termination of pregnancy or stillbirth due to intrapartum causes were excluded.

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 that required the use of ovulation drugs), cigarette smoking during pregnancy (yes/no), history of chronic hypertension (yes/no), history of systemic lupus erythematosus or antiphospholipid syndrome (SLE/APS), history of pre-existing diabetes mellitus (yes/no), and obstetric history that included parity (parous/nulliparous if no previous pregnancy ≥ 24 weeks' gestation), previous pregnancy with miscarriage between 16 and 23 weeks, previous pregnancy with stillbirth, previous pregnancy with a small-for-gestational-age neonate, gestational age at delivery and birth weight of the neonate in the last pregnancy, interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal weight and height were measured.

Outcome measures

Data on pregnancy outcome were obtained from the maternity hospital records or the general practitioners of the women. The hospital maternity records of all women with antepartum stillbirth were reviewed to determine whether the death was associated with PE, placental abruption, a birth weight < 10th percentile for gestational age[16] or it was due to other or unexplained reasons.

Statistical analysis

Data from continuous variables were expressed as median (interquartile range) and from categorical variables as *n* (%). Comparison of the maternal characteristics between the outcome groups was by the chi-square test or Fisher's exact test for categorical variables and Kruskal-Wallis or Mann–Whitney *U*-test for continuous variables. A P-value < 0.05 was considered significant. *Post-hoc* Bonferroni correction was used for multiple comparisons.

The measured values of PAPP-A, PlGF, UtA-PI and DV-PIV were $\log_{10}$ transformed to make their distributions Gaussian and each value was expressed as a multiple of the normal median (MoM) after adjustment for characteristics that provide a substantial contribution to the $\log_{10}$ transformed value[13,17–19]. The *a-priori* risk for stillbirth was estimated from the algorithm derived from multivariable logistic regression analysis of maternal characteristics and history as described previously[4]. Univariable and multivariable logistic regression analyses were then used to determine if the maternal factor-derived logit (*a-priori* risk), and the $\log_{10}$ MoM value of each biochemical and biophysical marker had a significant contribution to stillbirth and whether the addition of serum PlGF ($\log_{10}$ MoM) improved the performance of screening that was achieved by a combination of maternal factors and PAPP-A, DV-PIV and UtA-PI. The significant predictors in the multivariate analysis were used to determine the patient-specific risk of stillbirth using the equation odds/(1 + odds), where odds = e^Y and Y was estimated from the coefficients of variables in the logistic regression analysis. The distribution of risks was used to determine the performance of screening by receiver–operating characteristics (ROC) curve analysis and the DR and FPR were estimated. The significance of improvement in performance of screening by the addition of maternal serum PlGF was assessed by comparison of the areas under the ROC curves (AUC)[20].

The statistical software package SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for the data analyses.

RESULTS

Study population

The 45 452 singleton pregnancies that fulfilled the study entry criteria included 45 225 live births and 227 (0.49%) antepartum stillbirths; 131 (58%) were secondary to impaired placentation and 96 (42%) were due to other or unexplained causes. The maternal and pregnancy characteristics of the outcome groups are compared in Table 1.

Biomarkers in outcome groups

In pregnancies with stillbirth, compared to live births, there was lower serum PAPP-A MoM (0.85 *vs* 1.00; $P < 0.0001$) and PlGF MoM (0.78 *vs* 1.00; $P < 0.0001$) and higher UtA-PI MoM (1.23 *vs* 1.00; $P < 0.0001$), but there was no significant difference in DV-PIV MoM. In pregnancies with stillbirth due to impaired placentation, compared to live births, there was lower serum PAPP-A MoM (0.69 *vs* 1.00; $P < 0.0001$) and PlGF MoM (0.63 *vs* 1.00; $P < 0.0001$) and higher DV-PIV MoM (1.04 *vs* 1.00; $P < 0.0001$) and UtA-PI MoM (1.39 *vs* 1.00, $P < 0.0001$); in stillbirths due to unexplained or other causes there were no significant differences from live births for any of the biomarkers (Table S1 and Figure 1).

Prediction of stillbirth and performance of screening

The results of univariable and multivariable regression analyses are shown in Table S2. In the multivariable

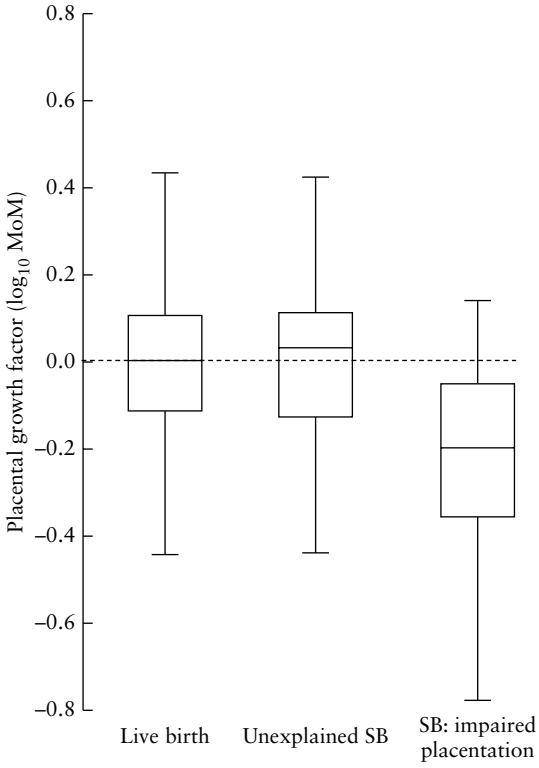

Figure 1 Box-and-whiskers plot of multiples of the median (MoM) of placental growth factor (PlGF) in live births, unexplained stillbirths (SB) and SB due to impaired placentation. Boxes with internal lines represent median and interquartile range and whiskers are range. MoM, multiples of the median.

Table 1 Maternal and pregnancy characteristics in pregnancies that resulted in stillbirth, stratified according to whether this was unexplained or due to impaired placentation, compared with pregnancies that resulted in live birth

		Stillbirth		
Characteristic	*Live birth* (n = *45 225*)	*All* (n = *227*)	*Unexplained* (n = *96*)	*Impaired placentation* (n = *131*)
Age (years)	31.1 (26.5–34.8)	32.6 (26.7–36.1)	32.5 (25.9–36.2)	32.7 (27.0–36.0)
Weight (kg)	67.0 (59.3–78.0)	73.6 (63.6–83.4)‡	71.3 (62.9–82.8)*	74.0 (63.6–85.0)†
Height (m)	1.65 (1.60–1.69)	1.65 (1.60–1.68)	1.66 (1.60–1.68)	1.65 (1.60–1.68)
Racial origin				
Caucasian	32 834 (72.6)	122 (53.7)	55 (57.3)	67 (51.1)
Afro-Caribbean	8359 (18.5)	86 (37.9)‡	34 (35.4)†	52 (39.7)†
South Asian	1927 (4.3)	8 (3.5)	3 (3.1)	5 (3.8)
East Asian	929 (2.1)	4 (1.8)	1 (1.0)	3 (2.3)
Mixed	1176 (2.6)	7 (3.1)	3 (3.1)	4 (3.1)
Mode of conception				
Spontaneous	43 877 (97.0)	214 (94.3)	91 (94.8)	123 (93.9)
Assisted	1348 (3.0)	13 (5.7)	5 (5.2)	8 (6.1)
Cigarette smoker	4335 (9.6)	25 (11.0)	9 (9.4)	16 (12.2)
Chronic hypertension	699 (1.5)	15 (6.6)‡	1 (1.0)	14 (10.7)†
APS/SLE	97 (0.2)	2 (0.9)	0 (0)	2 (1.5)*
Pre-existing diabetes mellitus	435 (1.0)	9 (4.0)†	5 (5.2)†	4 (3.1)*
Nulliparous	21 266 (47.0)	109 (48.0)	46 (47.9)	63 (48.1)
Previous miscarriage	574 (1.3)	2 (0.9)	1 (1.0)	1 (0.8)
Previous stillbirth	360 (0.8)	10 (4.4)‡	3 (3.1)*	7 (5.3)†
Previous SGA	1494 (3.3)	9 (4.0)	2 (2.1)	7 (5.3)
Interpregnancy interval (years)	3.0 (2.0–5.0)	4.3 (2.5–7.2)	3.6 (2.1–6.4)	4.5 (2.9–8.3)

Data are given as median (interquartile range) or *n* (%). Comparison of stillbirth groups with live-birth group by chi-square test and Mann–Whitney *U*-test with *post-hoc* Bonferroni correction for multiple comparisons: *$P < 0.025$; †$P < 0.01$; ‡$P < 0.001$. APS, antiphospholipid syndrome; SGA, small-for-gestational age; SLE, systemic lupus erythematosus.

Table 2 Performance of screening for all stillbirths, unexplained stillbirths and those due to imparied placentation, and at various gestational ages, based on maternal factors and a combination of maternal factors with biochemical and biophysical markers at 11–13 weeks' gestation, at a fixed false-positive rate (FPR)

			Detection rate (% (95% CI))	
Outcome	n	*AUC (95% CI)*	*5% FPR*	*10% FPR*
All stillbirths	227			
Maternal factors		0.647 (0.607–0.686)	19.4 (14.3–24.5)	30.4 (24.4–36.4)
Maternal factors + UtA-PI + DV-PIV + PlGF		0.731 (0.693–0.769)	31.7 (25.7–37.8)	41.9 (35.5–48.3)
Unexplained stillbirth	96			
Maternal factors		0.625 (0.567–0.683)	14.6 (7.5–21.7)	26.0 (17.2–34.8)
Stillbirths from impaired placentation	131			
Maternal factors		0.663 (0.610–0.716)	22.9 (15.7–30.1)	33.6 (25.5–41.7)
Maternal factors plus:				
PlGF		0.814 (0.777–0.852)	40.5 (32.1–48.9)	51.1 (42.5–59.7)
DV-PIV		0.684 (0.631–0.737)	28.2 (20.5–35.9)	37.4 (29.1–45.7)
UtA-PI		0.790 (0.748–0.832)	35.1 (26.9–43.3)	45.8 (37.3–54.3)
PlGF + DV-PIV		0.820 (0.782–0.858)	42.0 (33.6–50.5)	51.4 (42.8–60.0)
UtA-PI + DV-PIV		0.795 (0.754–0.836)	36.6 (28.1–44.5)	48.1 (39.5–56.7)
PlGF + UtA-PI		0.848 (0.811–0.884)	47.9 (39.4–56.5)	60.8 (52.4–69.2)
UtA-PI + DV-PIV + PlGF		0.852 (0.816–0.888)	48.1 (36.2–60.0)	61.1 (49.5–72.7)
Stillbirth < 32 weeks	68			
Maternal factors		0.664 (0.587–0.741)	29.4 (18.6–40.2)	36.8 (25.3–48.3)
Maternal factors + UtA-PI + DV-PIV + PlGF		0.900 (0.859–0.940)	61.8 (50.3–73.4)	70.6 (59.8–81.4)
Stillbirth < 37 weeks	98			
Maternal factors		0.666 (0.604–0.727)	24.5 (16.0–33.1)	32.7 (23.4–42.0)
Maternal factors + UtA-PI + DV-PIV + PlGF		0.875 (0.835–0.915)	54.1 (44.2–64.0)	66.3 (56.9–75.7)
Stillbirth ≥ 37 weeks	33			
Maternal factors		0.655 (0.551–0.758)	18.2 (5.1–31.4)	36.4 (20.0–52.8)
Maternal factors + UtA-PI + DV-PIV + PlGF		0.785 (0.710–0.860)	30.3 (14.6–45.9)	45.5 (28.5–62.5)

AUC, area under receiver–operating characteristics curve; DV-PIV, ductus venosus pulsatility index for veins; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

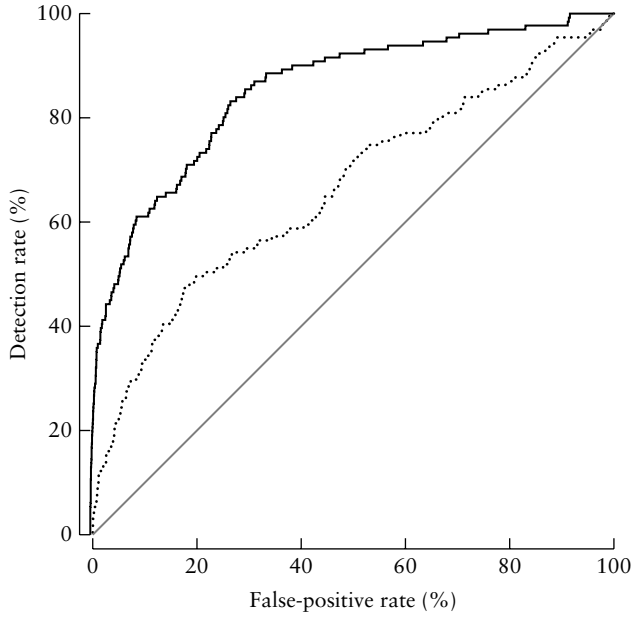

Figure 2 Receiver–operating characteristics curves for prediction of stillbirth due to impaired placentation from maternal factors (·····) and from a combination of maternal factors and uterine artery pulsatility index, ductus venosus pulsatility index for veins and placental growth factor (——).

regression analysis, there was a significant contribution to the prediction of stillbirth due to impaired placentation from maternal factor-derived *a-priori* risk and MoM values of PlGF, DV-PIV and UtA-PI, but not of serum PAPP-A ($R^2 = 0.193$; $P < 0.0001$). Multivariable regression analysis demonstrated that the odds ratios (ORs) for maternal factors, DV-PIV, UtA-PI and PlGF were 9.0 (95% CI, 5.3–15.3), 2.7 (95% CI, 1.8–4.0), 7.7 (95% CI, 4.8–12.3) and 0.04 (95% CI, 0.02–0.08), respectively. In a model without PlGF, a change in each unit of PAPP-A MoM was associated with an OR for stillbirth of 0.46.

The performance of screening for stillbirth with maternal factors and biomarkers is shown in Table 2. Combined screening by maternal factors, PAPP-A, DV-PIV and UtA-PI detected 50% of all stillbirths due to impaired placentation, at a 10% FPR (AUC of 0.809 (95% CI, 0.767–0.850)). The addition of serum PlGF, which resulted in the contribution of serum PAPP-A becoming non-significant, improved the DR to 61% (AUC of 0.852 (95% CI, 0.816–0.888)) (Figure 2). In the impaired-placentation group, screening by maternal factors, PlGF, DV-PIV and UtA-PI, the DR of stillbirth < 32 weeks' gestation was higher than that of stillbirth ≥ 37 weeks (71% *vs* 46%; $P = 0.031$).

DISCUSSION

Main findings of the study

The findings of the study demonstrate that, in our large study population from hospitals in the UK, 58% of antepartum stillbirths are due to impaired placentation

and 42% are unexplained or due to other causes. A model which combines maternal factors, serum PlGF, UtA-PI and fetal DV-PIV at 11–13 weeks' gestation can potentially predict about 60% of stillbirths due to impaired placentation, at a 10% FPR; the performance of screening is better for stillbirth < 32 weeks' gestation (71%) compared to those that occur at term (46%). Although serum PAPP-A on its own or in combination with UtA-PI and DV-PIV is useful in the early prediction of stillbirth, its contribution is not significant once serum PlGF is added to the model. In the multivariable regression model in this study, the OR for stillbirth with each unit of change in serum metabolite MoM was 0.46 for PAPP-A and 0.04 for PlGF, with the implication that the contribution of PlGF was 10-fold higher than that of PAPP-A.

Strengths and limitations

The strengths of this screening study are first, examination of a large population of pregnant women attending for routine assessment at 11–13 weeks' gestation, second, systematic recording of data on maternal characteristics and medical history to identify known risk factors associated with stillbirth, third, use of a specific methodology and appropriately trained doctors to measure fetal DV-PIV and UtA-PI, fourth, use of automated machines to provide accurate measurement within 40 min of sampling of maternal serum metabolites that have been shown to be altered in pregnancies associated with impaired placentation, fifth, expression of the values of biomarkers as MoMs after adjustment for factors that affect the measurements, and sixth, use of multivariable regression analysis to take into account possible interrelations between the different variables to define the relative predictive value of each factor.

A potential limitation of the study is that the performance of screening by a model derived and tested using the same dataset is overestimated; consequently, the model needs validation from prospective studies.

Comparison with other studies

Previous studies reported that, at 11–13 weeks' gestation, serum PlGF is reduced in pregnancies with impaired placentation resulting in PE or the birth of an SGA neonate[9–11,21]. The performance of early screening for stillbirth due to impaired placentation by an algorithm that incorporates serum PlGF is superior to that relying on maternal factors alone or a combination of maternal factors with UtA-PI, DV-PIV and serum PAPP-A[4,5]. This is not surprising since we have reported previously that, in early screening for PE by a combination of maternal factors and biomarkers, serum PlGF had a better performance than did serum PAPP-A[11].

Clinical implications of the study

The results of our study demonstrate that a high proportion of stillbirths due to impaired placentation can be identified effectively in the first trimester of pregnancy. In the case of PE there is a widely accepted process of screening which is based on a combination of maternal factors; we have demonstrated that the performance of screening by a combination of maternal factors with biomarkers including UtA-PI and PlGF, is by far superior to that which relies on maternal factors alone[11,22]. It is therefore likely that combined screening will be widely adopted, especially because there is evidence that pharmacological interventions in the high-risk group, by drugs such as low-dose aspirin starting < 16 weeks' gestation, could potentially improve placentation and reduce the associated risk of PE, SGA and stillbirth by more than 50%[23].

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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 Biochemical and biophysical markers at 11–13 weeks' gestation in pregnancies with live birth and those that resulted in stillbirth due to abnormal placentation or unexplained causes
Table S2 Univariable and multivariable logistic regression analyses for prediction of stillbirth due to impaired placentation by maternal factors and biomarkers at 11–13 weeks' gestation

