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Prediction of stillbirth from biochemical and biophysical markers at 11–13 weeks

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KEYWORDS: ductus venosus Doppler; first-trimester screening; pyramid of pregnancy care; serum PAPP-A; stillbirth; uterine artery Doppler

ABSTRACT

Objectives *To develop a model for the prediction of stillbirth that is based on a combination of maternal characteristics and medical history with first-trimester biochemical and biophysical markers 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 76 897 singleton pregnancies, including 76 629 live births and 268 (0.35%) antepartum stillbirths; 157 (59%) were secondary to impaired placentation and 111 (41%) were due to other or unexplained causes. Multivariable logistic regression analysis was used to determine if there was a significant contribution to prediction of stillbirth from the maternal factor-derived* a-priori *risk, fetal nuchal translucency thickness, ductus venosus pulsatility index for veins (DV-PIV), uterine artery pulsatility index (UtA-PI) and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A (PAPP-A). The significant contributors were used to derive a model for first-trimester prediction of stillbirth.*

Results *Significant contribution to prediction of stillbirth was provided by maternal factors, PAPP-A, UtA-PI and DV-PIV. A model combining these variables predicted 40% of all stillbirths and 55% 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 (64%* vs *42%).*

Conclusions *A model based on maternal factors and first-trimester biomarkers can potentially predict more than half of subsequent stillbirths that occur due to impaired placentation. The extent to which such stillbirths could be prevented remains to be determined. Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Risk factors for antepartum stillbirth include increasing maternal weight, Afro-Caribbean racial origin, assisted conception, cigarette smoking, diabetes mellitus, chronic hypertension, systemic lupus erythematosus and antiphospholipid syndrome[1]. In a prospectively screened population of 113 415 singleton pregnancies, including 396 (0.35%) antepartum stillbirths, multiple regression analysis was used to combine these risk factors into a model that predicted 31% of those that were due to impaired placentation and 26% of unexplained stillbirths or those due to other causes, at a false-positive rate (FPR) of 10%; within the impaired-placentation group, the detection rate (DR) of stillbirth < 32 weeks' gestation was higher than that of stillbirth ≥ 37 weeks (38% *vs* 28%)[1]. Antepartum stillbirths attributed to impaired placentation include those that are associated with pre-eclampsia, birth of a small-for-gestational-age neonate or placental abruption.

The objective of this study was to develop a model for prediction of stillbirth based on a combination of maternal characteristics and medical history with first-trimester biochemical and biophysical markers and evaluate the performance of screening with this model for all stillbirths and those due to impaired placentation and 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

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age, fetal nuchal translucency (NT) thickness and measurement of maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG)[2]. Transabdominal color Doppler ultrasound was performed to measure ductus venosus pulsatility index for veins (DV-PIV) and uterine artery pulsatility index (UtA-PI)[3,4]. Gestational age was determined from measurement of fetal crown–rump length (CRL)[5]. 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 stillbirths were reviewed to determine whether the death was associated with pre-eclampsia, placental abruption, a birth weight < 10th percentile for gestational age[6] or it was due to other or unexplained causes.

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 fetal NT thickness was expressed as a difference from the expected normal mean for fetal CRL (delta value)[7]. The values of serum PAPP-A and free β-hCG were $\log_{10}$-transformed to make their distributions Gaussian and each measured value was expressed as a multiple of the normal median (MoM) after adjustment for characteristics that were found to provide a substantial contribution to the $\log_{10}$-transformed value[8,9]. Similarly, the observed measurements of DV-PIV and UtA-PI were expressed as a MoM after adjustment for maternal characteristics[3,10].

The *a-priori* risk for stillbirths was estimated from the algorithm derived from multivariable logistic regression analysis of maternal characteristics and history as described previously[1]. Univariable and multivariable logistic regression analyses were then used to determine if the maternal factor-derived logit (*a-priori* risk), $\log_{10}$ MoM value of each biochemical and biophysical marker had a significant contribution to prediction of stillbirth. The variables which provided a significant contribution in the multivariable 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 patient-specific risks was used to determine the performance of screening by receiver–operating characteristics (ROC) curve analysis and the DR and FPR were estimated.

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 76 897 singleton pregnancies that fulfilled the study entry criteria included 76 629 live births and 268 (0.35%) antepartum stillbirths; 157 (59%) were secondary to impaired placentation and 111 (41%) 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 birth there was lower serum PAPP-A MoM (0.85 *vs* 1.00; $P < 0.0001$), and higher DV-PIV MoM (1.02 *vs* 1.00; $P < 0.01$) and UtA-PI MoM (1.25 *vs* 1.00, $P < 0.0001$), but there were no significant differences in serum free β-hCG MoM or delta NT (Table S1). Similarly, in pregnancies with a stillbirth due to impaired placentation, there was lower serum PAPP-A MoM (0.70 *vs* 1.00; $P < 0.0001$), and higher DV-PIV MoM (1.05 *vs* 1.00; $P < 0.0001$) and UtA-PI MoM (1.41 *vs* 1.00; $P < 0.0001$). In stillbirth due to unexplained or other causes there were no significant differences in any of the biomarkers when compared to live births (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 regression analysis, there was a significant contribution to

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 = *76 629*)	*All* (n = *268*)	*Unexplained* (n = *111*)	*Impaired placentation* (n = *157*)
Age (years)	31.3 (26.7–35.1)	31.6 (26.4–35.6)	32.6 (26.1–36.3)	30.8 (26.4–35.5)
Weight (kg)	67.0 (59.2–77.2)	73.0 (63.0–84.0)†	71.0 (62.9–83.3)*	74.0 (63.2–85.1)†
Height (m)	1.65 (1.60–1.69)	1.65 (1.60–1.68)	1.65 (1.61–1.68)	1.64 (0.60–1.68)
Racial origin				
Caucasian	54 664 (71.3)	143 (53.4)	64 (57.7)	79 (50.3)
Afro-Caribbean	15 088 (19.7)	103 (38.4)†	39 (35.1)†	64 (40.8)†
South Asian	3256 (4.2)	10 (3.7)	4 (3.6)	6 (3.8)
East Asian	1576 (2.1)	4 (1.5)	1 (0.9)	3 (1.9)
Mixed	2045 (2.7)	8 (3.0)	3 (2.7)	5 (3.2)
Mode of conception				
Spontaneous	74 173 (96.8)	251 (93.7)	104 (93.7)	147 (93.6)
Assisted	2456 (3.2)	17 (6.3)*	7 (6.3)	10 (6.4)
Cigarette smoker	7125 (9.3)	31 (11.6)	10 (9.0)	21 (13.4)
Chronic hypertension	1075 (1.4)	18 (6.7)†	1 (0.9)	17 (10.8)†
APS/SLE	154 (0.2)	4 (1.5)*	0 (0)	4 (2.5)†
Pre-existing diabetes mellitus	695 (0.9)	10 (3.7)†	5 (4.5)†	5 (3.2)*
Nulliparous	36 320 (47.4)	132 (49.3)	55 (49.5)	77 (49.0)
Previous miscarriage	1004 (1.3)	5 (1.9)	3 (2.7)	2 (1.3)
Previous stillbirth	617 (0.8)	13 (4.9)†	4 (3.6)*	9 (5.7)†
Previous SGA	2486 (3.2)	13 (4.9)	3 (2.7)	10 (6.4)
Interpregnancy interval (years)	3.0 (2.0–5.1)	4.4 (2.5–7.7)*	4.0 (2.1–7.1)	4.5 (2.9–8.4)†

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: *$P < 0.01$; †$P < 0.001$. APS, antiphospholipid syndrome; SGA, small-for-gestational age; SLE, systemic lupus erythematosus.

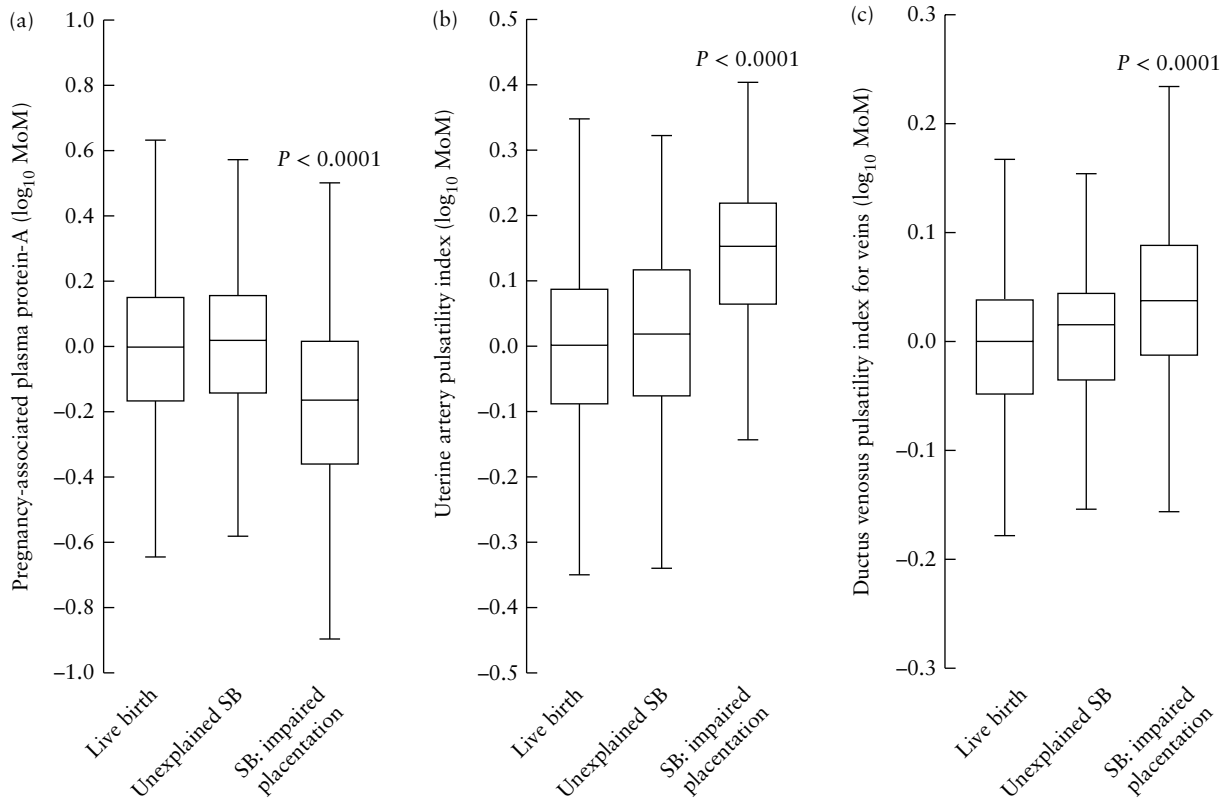

Figure 1 Box-and-whisker plot of multiples of the median (MoM) values of pregnancy-associated plasma protein-A (a), uterine artery pulsatility index (b) and ductus venosus pulsatility index for veins (c) in livebirths, unexplained stillbirths (SB) and SB due to impaired placentation. Boxes with internal lines represent median and interquartile range and whiskers are range.

the prediction of stillbirth due to impaired placentation from maternal factor-derived *a-priori* risk and MoM values of PAPP-A, DV-PIV and UtA-PI ($R^2 = 0.152$; $P < 0.0001$).

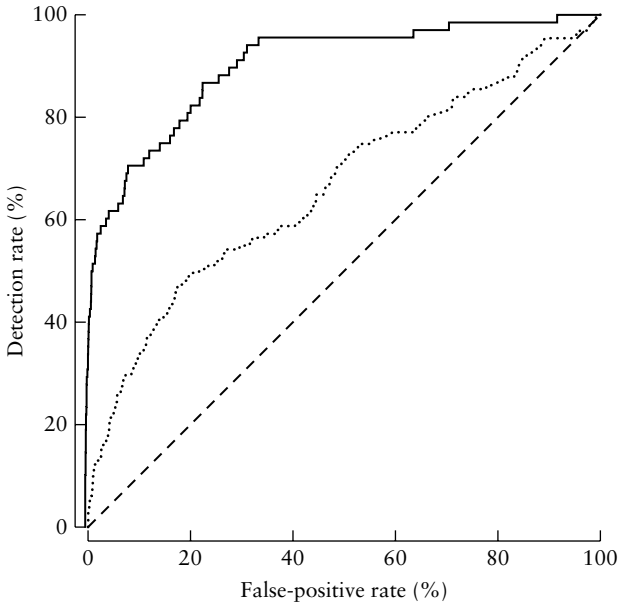

Figure 2 Receiver–operating characteristics curves for prediction of stillbirth due to impaired placentation from maternal factors (·····) and from a combination of maternal factors and biomarkers (——).

The performance of screening for stillbirth is shown in Figure 2 and Table 2. The DR for all stillbirths, at a FPR of 10%, increased from 31% when using maternal factors alone to 40% with the addition of biomarkers ($P = 0.008$). Within the impaired-placentation group, the DR increased from 36% when using maternal factors alone to up to 55% with the addition of biomarkers ($P < 0.0001$). The DR of stillbirth < 32 weeks' gestation based on maternal factors and biomarkers was higher than that of stillbirths ≥ 37 weeks (64% *vs* 42%; $P = 0.023$).

DISCUSSION

Main findings of the study

The findings of this study demonstrate that more than half of stillbirths that are due to impaired placentation can be predicted in the first trimester of pregnancy by a combination of maternal factors and biomarkers. The performance of screening is better for stillbirths that are secondary to impaired placentation than for those that are unexplained. Among stillbirths due to impaired placentation, the DR is higher for those that occur preterm than at term.

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,

Table 2 Performance of screening for all stillbirths, unexplained stillbirths and those due to impaired 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))	
Screening test	n	*AUC (95% CI)*	*5% FPR*	*10% FPR*
All stillbirths	268			
Maternal factors		0.657 (0.621–0.693)	19.8 (15.0–24.6)	31.3 (25.6–36.9)
Maternal factors + PAPP-A + UtA-PI + DV-PIV		0.724 (0.690–0.758)	32.5 (26.9–38.1)	39.9 (34.0–45.8)
Unexplained stillbirth	111			
Maternal factors		0.623 (0.569–0.677)	14.4 (7.9–20.9)	24.3 (16.3–32.3)
Stillbirth from impaired placentation	157			
Maternal factors		0.681 (0.633–0.728)	23.6 (17.0–30.2)	36.3 (28.8–43.8)
Maternal factors plus:				
PAPP-A		0.732 (0.687–0.777)	32.5 (25.2–39.8)	42.0 (34.3–49.7)
DV-PIV		0.700 (0.654–0.747)	28.7 (21.6–35.8)	38.3 (30.7–45.9)
UtA-PI		0.808 (0.771–0.845)	39.5 (31.9–47.2)	50.3 (42.5–58.1)
PAPP-A + DV-PIV		0.745 (0.701–0.789)	36.3 (28.8–43.8)	44.6 (36.8–52.4)
UtA-PI + DV-PIV		0.814 (0.777–0.850)	40.8 (33.1–48.5)	52.2 (44.4–60.1)
PAPP-A + UtA-PI		0.818 (0.781–0.856)	45.9 (38.1–53.7)	54.1 (46.3–61.9)
PAPP-A + UtA-PI + DV-PIV		0.825 (0.788–0.861)	47.8 (40.0–55.6)	54.8 (47.0–62.6)
Stillbirth < 32 weeks	90			
Maternal factors		0.700 (0.637–0.764)	31.1 (21.5–40.7)	40.0 (29.9–50.1)
Maternal factors + PAPP-A + UtA-PI + DV-PIV		0.870 (0.827–0.912)	57.8 (47.6–68.0)	64.4 (54.5–74.3)
Stillbirth < 37 weeks	121			
Maternal factors		0.694 (0.641–0.748)	26.4 (18.6–34.3)	36.4 (27.8–45.0)
Maternal factors + PAPP-A + UtA-PI + DV-PIV		0.842 (0.801–0.883)	52.9 (44.0–61.8)	59.7 (51.0–68.4)
Stillbirth ≥ 37 weeks	36			
Maternal factors		0.634 (0.530–0.737)	13.9 (2.6–25.2)	30.1 (15.1–45.0)
Maternal factors + PAPP-A + UtA-PI + DV-PIV		0.766 (0.690–0.841)	30.6 (15.6–45.7)	41.7 (25.6–57.8)

AUC, area under receiver–operating characteristics curve; DV-PIV, ductus venosus pulsatility index for veins; PAPP-A, pregnancy-associated plasma protein-A; UtA-PI, uterine artery pulsatility index.

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 DV-PIV and UtA-PI, fourth, expression of the values of biomarkers as MoMs after adjustment for factors that affect the measurements, and fifth, 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 may be overestimated.

Comparison with other studies

In a previous study in 33 452 pregnancies, which included 142 stillbirths, we demonstrated that significant contributors to stillbirth were maternal factors, serum PAPP-A and reversed flow in the DV; the DR of all stillbirths was 35%, at a 10% FPR[11]. In the present study we used a quantitative rather than qualitative assessment of DV waveforms and, in addition, included measurement of UtA-PI to improve the DR to 40%.

Previous studies have reported that low serum PAPP-A is associated with an increased risk of stillbirth. In a screening study of 54 722 singleton pregnancies, including 225 stillbirths, we found that for PAPP-A $\leq$ 0.42 MoM, which corresponds to the 5th percentile, the odds of having a stillbirth were increased by a factor of 1.94[12]. Another screening study of 33 395 pregnancies, including 95 stillbirths, found that for PAPP-A $\leq$ 0.42 MoM, the odds of stillbirth were increased 2.15-fold[13].

We found that the risk of stillbirth increased 2.9-fold for every unit increase in DV-PIV MoM. We examined previously the contribution of reversed a-wave in the DV and reported that this finding is associated with a 2-fold increase in the risk of stillbirth[14]. We found that the risk of stillbirth increased 18-fold for every unit increase in UtA-PI MoM. A previous study in 9859 pregnancies, including 62 stillbirths, reported that if impedance to flow in the UtAs was $\geq$ 90th percentile, the odds of stillbirth increased by a factor of 2.13[15].

Clinical implications of the study

The results of our study demonstrate that more than half of stillbirths due to impaired placentation can be identified effectively in the first trimester of pregnancy. 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 stillbirths by more than 50%[16].

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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 from maternal factors and biomarkers at 11–13 weeks' gestation

