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

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Short title: Prediction of stillbirth from biomarkers

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Abstract

Objectives: 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 of 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. Multivariate 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 (NT), ductus venosus pulsatility index for veins (DV-PIV), uterine artery pulsatility index (UT-PI) and maternal serum free ß-human chorionic gonadotrophin ((ß-hCG) 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, UT-PI and DV-PIV. A model combining these variables predicted 40% of all stillbirths and 55% of those due to impaired placentation, at false positive rate of 10%; within the impaired placentation group the detection rate of stillbirth at <32 weeks' gestation was higher than that of stillbirth at ≥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 due to impaired placentation. The extent to which such stillbirths could be prevented remains to be determined.

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 26% of unexplained stillbirths and 31% of those due to impaired placentation, at false positive rate (FPR) of 10%; within the impaired placentation group the detection rate (DR) of stillbirth at <32 weeks' gestation was higher than that of stillbirth at ≥37 weeks (38% vs 28%).[1]

The objective of this study is 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 of this model for all stillbirths and those due to impaired placentation and unexplained 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}-13^{+6} weeks' gestation at King's College Hospital and Medway Maritime Hospital, United Kingdom. We recorded maternal characteristics and medical history and performed combined screening to estimate risks for fetal aneuploidies based on maternal age, fetal nuchal translucency (NT) thickness and measurement of maternal serum pregnancy associated plasma protein-A (PAPP-A) and free ß-human chorionic gonadotropin (hCG).[2] Transabdominal colour Doppler ultrasound was performed to measure ductus venosus pulsatility index for veins (DV-PIV) and uterine artery pulsatility index (UT-PI).[3,4] Gestational age was determined from measurement of fetal crown-rump length (CRL).[5] The women were screened between March 2006 and October 2015 and gave written informed consent to participate in the study, which was approved by the Ethics Committee.

The inclusion criteria for the study were singleton pregnancies that delivered a phenotypically normal live birth or stillbirth at ≥24 weeks' gestation. We excluded pregnancies with aneuploidies, major fetal abnormalities, those ending in a miscarriage, termination of pregnancy or stillbirths due to intrapartum causes. Data on pregnancy outcome were obtained from the maternity hospital records or the general practitioners of women. The hospital maternity records of all women with antepartum stillbirths were reviewed to determine if the death was associated with preeclampsia, abruption or the birthweight was <10^{th} percentile for gestational age [6] or it was unexplained.

Statistical analysis

Data from continuous variables were expressed as medians and interquartile ranges and from categorical data as n (%). Comparison of the maternal characteristics between the outcome groups was by the $\chi 2$-square test or Fisher's exact test for categorical variables and Kruskal-Wallis or Mann-Whitney U-test for continuous variables, respectively. A p value of < 0.05 was considered significant. *Post-hoc* Bonferroni correction was used for multiple comparisons.

The measured fetal NT 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 those characteristics found to provide a substantial contribution to the log_{10} transformed value.[8,9] Similarly, the observed measurements of ductus venosus PIV and uterine artery 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 multivariate logistic regression analysis of maternal characteristics and history as previously described.[R] Univariate and multivariate logistic regression analysis was 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 in stillbirths. The variables which provided a significant contribution 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 patient-specific risks was used to determine the performance of screening by receiver operating characteristic (ROC) curves 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: IBM Corp, 2013) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for the data analyses.

Results

Study population

The 76,897 singleton pregnancies fulfilling the 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 sTable 1.

Biomarkers in outcome groups

In the stillbirth group, compared to live births, 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 UT-PI MoM (1.25 vs 1.00, p<0.0001), but there were no significant differences in serum free ß-hCG MoM or delta NT (sTable 2). Similarly, in the stillbirths due to impaired placentation, compared to live births, 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 UT-PI MoM (1.41 vs 1.00, p<0.0001); in the stillbirths due to unexplained causes there were no significant differences from live births in any of the biomarkers (Figure 1).

Prediction of stillbirth and performance of screening

The results of univariate and multivariate regression analysis are shown in sTable 3. In the multivariate regression analysis, there was a significant contribution to the prediction of stillbirths due to impaired placentation from maternal factor derived priori risk and MoM values of PAPP-A, DV-PIV and UT-PI (R^2=0.152; p<0.0001).

The performance of screening for stillbirth is shown in Table 1. The DR for all stillbirths, at FPR of 10%, increased from 31% for maternal factors to 40% with addition of biomarkers (p=0.008). Within the impaired placentation group, the DR increased from 36% for maternal factors to 55% with addition of biomarkers (p<0.0001). The DR of stillbirth based on

maternal factors and biomarkers at <32 weeks' gestation was higher than that of stillbirths at ≥37 weeks (64% vs 41%; p=0.023).

Conclusions

Main findings of the study

The findings of the study demonstrate that more than half of stillbirths due to impaired placentation can be predicted in the first trimester of pregnancy from a combination of maternal factors and biomarkers. The performance of screening is better for stillbirths secondary to impaired placentation compared to those that are unexplained and in the impaired placentation group, the DR is higher for stillbirths 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, 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 UT-PI, fourth, expression of the values of biomarkers as MoMs after adjustment for factors that affect the measurements, and fifth, use of multivariate 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.

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 10% FPR.[11] In this study we used a quantitative rather than qualitative assessment of waveforms from the DV and in addition included measurement of UT-PI to improve the DR to 40%.

Previous studies have reported that low serum PAPP-A is associated with increased risk of stillbirth. In a screening study of 54,722 singleton pregnancies, including 225 stillbirths, we found that for PAPP-A ≤ 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 ≤ 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 previously examined the contribution of reversed a-wave in the DV and reported that this finding is associated with a 2-fold increase in risk of stillbirth.[14] We found that the risk of stillbirth increased 18-fold for every unit increase in UT-PI MoM. A previous study in 9,859 pregnancies, including 62 stillbirths, reported that if impedance to flow in the uterine arteries was ≥ 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 the stillbirths due to impaired placentation can be effectively identified in the first trimester of pregnancy. Pharmacological interventions in the high-risk group, by such drugs as low-dose aspirin starting at <16 weeks'

gestation, could potentially improve placentation and reduce the associated stillbirths by more than 50%.[17]

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Table 1. Performance of screening for stillbirths at various cut-offs of gestational age based on maternal factors and combination of maternal factors with biochemical and biophysical markers at 11-13 weeks' gestation at fixed false positive rates (FPR).

Outcome	N	AUROC (95% CI)	Detection rates (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)
+ PAPP-A + Ut PI + DV PIV		0.724 (0.690-0.758)	32.5 (26.9-38.1)	39.9 (34.0-45.8)
Unexplained				
Maternal factors	111	0.623 (0.569-0.677)	14.4 (7.9-20.9)	24.3 (16.3-32.3)
Abnormal placentation				
All stillbirths	157			
Maternal factors		0.681 (0.633-0.728)	23.6 (17.0-30.2)	36.3 (28.8-43.8)
+ 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)
+ Ut 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)
+ Ut PI + DV PIV		0.814 (0.777-0.850)	40.8 (33.1-48.5)	52.2 (44.4-60.1)
+ PAPP-A + Ut PI		0.818 (0.781-0.856)	45.9 (38.1-53.7)	54.1 (46.3-61.9)
+ PAPP-A + Ut PI + DV PIV		0.825 (0.788-0.861)	47.8 (40.0-55.6)	54.8 (47.0-62.6)
< 32 weeks	90			
Maternal factors		0.700 (0.637-0.764)	31.1 (21.5-40.7)	40.0 (29.9-50.1)
+ PAPP-A + Ut PI + DV PIV		0.870 (0.827-0.912)	57.8 (47.6-68.0)	64.4 (54.5-74.3)
< 37 weeks	121			
Maternal factors		0.694 (0.641-0.748)	26.4 (18.6-34.3)	36.4 (27.8-45.0)
+ PAPP-A + Ut PI + DV PIV		0.842 (0.801-0.883)	52.9 (44.0-61.8)	59.7 (51.0-68.4)
≥ 37 weeks	36			
Maternal factors		0.634 (0.530-0.737)	13.9 (2.6-25.2)	30.1 (15.1-45.0)
+ PAPP-A + Ut PI + DV PIV		0.766 (0.690-0.841)	30.6 (15.6-45.7)	41.7 (25.6-57.8)

AUROC=area under receiver operating characteristic curves; CI= confidence interval; FPR = False positive rates; PAPP-A= pregnancy associated plasma protein-A; Ut PI=Uterine artery pulsatility index; DV PIV = ductus venosus pulsatility index for veins

Supplementary Table 1. Maternal and pregnancy characteristics in pregnancies that had a stillbirth, stratified according to sub-groups, compared with pregnancies that had a live births

Maternal characteristics	Live births (n=76,629)	All stillbirths (n=268)	Unexplained (n=111)	Impaired placentation (n=157)
Age, median (IQR)	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, median (IQR)	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, median (IQR)	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, n (%)	54,664 (71.3)	143 (53.4)	64 (57.7)	79 (50.3)
Afro-Caribbean, n (%)	15,088 (19.7)	103 (38.4)*	39 (35.1)*	64 (40.8)*
South Asian, n (%)	3,256 (4.2)	10 (3.7)	4 (3.6)	6 (3.8)
East Asian, n (%)	1,576 (2.1)	4 (1.5)	1 (0.9)	3 (1.9)
Mixed, n (%)	2,045 (2.7)	8 (3.0)	3 (2.7)	5 (3.2)
Method of conception				
Spontaneous, n (%)	74,173 (96.8)	251 (93.7)	104 (93.7)	147 (93.6)
Assisted conception, n (%)	2,456 (3.2)	17 (6.3)†	7 (6.3)	10 (6.4)
Cigarette smoking, n (%)	7,125 (9.3)	31 (11.6)	10 (9.0)	21 (13.4)
Chronic hypertension, n (%)	1,075 (1.4)	18 (6.7)*	1 (0.9)	17 (10.8)*
SLE / APS, n (%)	154 (0.2)	4 (1.5) †	0	4 (2.5)*
Diabetes mellitus, n (%)	695 (0.9)	10 (3.7)*	5 (4.5)*	5 (3.2) †
Parity				
Nulliparous, n (%)	36,320 (47.4)	132 (49.3)	55 (49.5)	77 (49.0)
Previous miscarriage, n (%)	1,004 (1.3)	5 (1.9)	3 (2.7)	2 (1.3)
Previous stillbirth, n (%)	617 (0.8)	13 (4.9)*	4 (3.6) †	9 (5.7)*
Previous SGA, n (%)	2,486 (3.2)	13 (4.9)	3 (2.7)	10 (6.4)
Inter-pregnancy interval, median (IQR)[a]	1.0 (0.0-3.1)	0.8 (0.0-4.4)	0.9 (0.0-4.0)	0.7 (0.0-4.9)

Post hoc Bonferroni correction for multiple comparisons; † = p< 0.01; * = p< 0.001

IQR=interquartile range; SLE=systemic lupus erythematosus; APS=anti-phospholipid syndrome; SGA= small for gestational age

[a] Inter-pregnancy interval median (IQR) reported for parous women

Supplementary Table 2. Median and interquartile range of biochemical and biophysical markers in pregnancies with livebirths compared to those that had a stillbirth

Biomarker	Livebirths (n=76,629)	All stillbirths (n=268)	Unexplained (n=111)	Abnormal placentation (n=157)
Pregnancy associated plasma protein-A (MoM)	1.00 (0.69-1.43)	0.85 (0.53-1.24)***	1.04 (0.71-1.44)	0.70 (0.46-1.13)***
Free β-human chorionic gonadotropin (MoM)	1.00 (0.68-1.52)	1.06 (0.63-1.50)	1.10 (0.67-1.49)	0.98 (0.61-1.59)
Ductus venosus pulsatility index (MoM)	1.00 (0.90-1.10)	1.02 (0.92-1.15)**	1.02 (0.91-1.11)	1.05 (0.94-1.19)***
Delta fetal nuchal translucency	0.00 (-0.19-0.22)	-0.01 (-0.18-0.23)	0.07 (-0.16-0.25)	-0.03 (-0.19-0.19)
Uterine artery pulsatility index (MoM)	1.00 (0.81-1.21)	1.25 (0.97-1.55)***	1.02 (0.83-1.28)	1.41 (1.16-1.66)***

MoM= multiple of the median

Supplementary Table 3. Univariate and multivariate logistic regression analysis for the prediction of stillbirths due to impaired placentation by maternal factors and biomarkers at 11-13 weeks' gestation

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Maternal factor derived logit (*a priori* risk)	16.27 (10.46-25.31)	<0.0001	12.93 (8.12-20.57)	<0.0001
Log_{10} PAPP-A MoM	0.18 (0.13-0.27)	<0.0001	0.25 (0.16-0.40)	<0.0001
Log_{10} free β-hCG MoM	0.93 (0.51-1.69)	0.814		
Delta fetal nuchal translucency	0.89 (0.58-1.35)	0.575		
Log_{10} ductus venosus PIV MoM	193.15 (41.73-894.00)	<0.0001	75.73 (15.65-366.48)	<0.0001
Log_{10} uterine artery PI MoM	$14.13e^{03}$ ($3.53e^{03}$-$56.51e^{03}$)	<0.0001	$5.13e^{03}$ ($1.26e^{03}$-$20.91e^{03}$)	<0.0001

PAPP-A = pregnancy associated plasma protein-A; hCG = human chorionic gonadotropin; PI = pulsatility index; PIV = pulsatility index for veins; MoM= multiple of the median; OR = odds ratio; CI = confidence interval

Figure 1. Box and whiskers plot of pregnancy associated plasma protein-A multiple of the median (MoM), uterine artery pulsatility index (PI) MoM and ductus venosus (DV) PIV MoM in live births (a), unexplained stillbirths (b) and stillbirths due to impaired placentation (c). The bottom and top edges of each box represent the first and third quartiles, respectively; the band within the box represents the median value.

Figure 2. Receiver–operating characteristics curves for prediction of stillbirth due to impaired placentation from maternal factors and maternal factors with biomarkers.