Prediction of stillbirth by placental growth factor at 19-24 weeks' gestation

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Short title: Placental growth factor and prediction of stillbirths

Key words: Stillbirth, Pyramid of care, Uterine artery Doppler, Placental growth factor

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Abstract

Objectives: To investigate whether measurement of maternal serum placental growth factor (PLGF) at 19-24 weeks' gestation improves the performance of screening for stillbirths that is achieved by a combination of maternal factors, fetal biometry and uterine artery pulsatility index (UT-PI) and evaluate the performance of screening of this model for all stillbirths and those due to impaired placentation and unexplained or other causes.

Methods: This was a prospective screening study of 70,003 singleton pregnancies including 268 stillbirths, carried out in two phases. The first phase, which included prospective measurements of UT-PI and fetal biometry were available in all cases. The second phase included prospective measurements of maternal serum PLGF which were available for 9,870 live births and 86 antepartum stillbirths. The values of PLGF obtained from this screening study were simulated in the remaining cases based on bivariate Gaussian distributions, defined by the mean and standard deviations. Multivariate 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, fetal biometry and UT-PI.

Results: Significant contribution to the prediction of stillbirths was provided by maternal factor derived *a priori* risk, MoM values of PLGF, UT-PI and fetal biometry Z-scores. A model combining these variables predicted 58% of all stillbirths and 84% 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 (97% vs 61%; $p<0.01$).

Conclusions: A high proportion of stillbirths due to impaired placentation can be effectively identified in the second trimester of pregnancy.

Introduction

A screening study of 70,003 singleton pregnancies at 19-24 weeks' gestation, including 268 stillbirths, reported that 59% of antepartum stillbirths were associated with preeclampsia (PE), birth of small for gestational age (SGA) neonates or placental abruption and these were attributed to impaired placentation; 41% were due to other or unexplained causes.[1] Screening for stillbirth by a combination of factors from maternal characteristics and medical history predicted 34% of stillbirths due to impaired placentation and 23% of those that were due to other or unexplained causes, at false positive rate (FPR) of 10%.[1,2] Prediction of stillbirth due to impaired placentation was substantially improved by a model combining maternal factors, fetal biometry and uterine artery pulsatility index (UT-PI) with DR of 75% at FPR of 10%.[1]

Placental growth factor (PLGF) is an angiogenic protein produced by the placenta and is implicated in trophoblastic invasion of maternal spiral arteries.[3,4] Maternal serum levels in the first, second and third trimesters are decreased in pregnancies with impaired placentation that develop PE and those that deliver SGA neonates.[5-15] There is also evidence that measurement of serum PLGF at 11-13 weeks' gestation is useful in predicting stillbirth.[16]

The objective of this study was to investigate whether measurement of maternal serum placental growth factor (PLGF) at 19-24 weeks' gestation improves the performance of screening for stillbirths that is achieved by a combination of maternal factors, fetal biometry and UT-PI and evaluate the performance of screening of 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 19^{+0}-24^{+6} weeks' gestation at King's College Hospital and Medway Maritime Hospital, United Kingdom between March 2006 and October 2015.[1] Gestational age was determined form the measurement of fetal head circumference (HC) in the second-trimester or crown-rump length in the first-trimester.[17,18] The study was carried out in two phases: in the first phase, we recorded maternal characteristics and medical history, performed ultrasound examination for measurement of fetal HC, abdominal circumference (AC) and femur length (FL) and measured UT-PI by transvaginal color Doppler.[17,19] In the second phase, we also measured maternal serum concentration of PLGF using automated analysers which provide reproducible results within 40 minutes of blood sampling (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA or Cobas e411 system, Roche Diagnostics, Penzberg, Germany).

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. The inclusion criteria for this study were singleton pregnancies that delivered a phenotypically normal live birth or stillbirth at $\geq$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 [20] or it was due to other causes or was unexplained.

Statistical analysis

The observed measurements of PLGF and UT-PI were log_{10} transformed to ensure homogeneity of variance and make the distribution 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.[21,22] The observed measurements of fetal HC, AC and FL were expressed as the respective Z-score corrected for gestational age.[17] The measured values of UT-PI and fetal biometry were available in all cases in the study population of 70,003 singleton pregnancies. Maternal serum PLGF measurements were available in 9,956 pregnancies, including 86 with stillbirth. In all stillbirths and subgroups of stillbirths, mean and standard deviations (SDs) of log_{10}MoM PLGF values were estimated; the values for PLGF were then simulated in the remaining cases in study population, based on the bivariate Gaussian distributions of the marker in stillbirths and live births, defined by the mean and SD (log_{10}MoM). 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.[2] Univariate and multivariate logistic regression analysis was used to determine significance of contribution of these biomarkers in prediction of 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, Z-scores of fetal biometry and Mom values of UT-PI.[1] 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 maternal and pregnancy characteristics of the total study population of 70,003 singleton pregnancies is as previously described (sTable 1)[1]. Maternal serum PLGF values were available in 9,956 singleton pregnancies, including 9,870 live births and 86 antepartum stillbirths; maternal and pregnancy characteristics of this group are shown in sTables 2.

Biomarkers in outcome groups

In the stillbirth group, compared to live births, the PLGF MoM was lower (0.65 vs 1.00, p<0.0001), UT-PI MoM was higher (1.37 vs 1.00, p<0.0001) and the Z-scores of HC, AC and FL were lower (-0.20 vs -0.01, p<0.0001; -0.29 vs. 0.0, p<0.0001; -0.12 vs -0.01, p=0.012, respectively). In the stillbirths due to impaired placentation, compared to live births, the PLGF MoM was lower (0.42 vs 1.00, p<0.0001), UT-PI MoM was higher (1.68 vs 1.00, p<0.0001) and the Z-scores of HC, AC and FL were lower (-0.38 vs -0.01, p<0.0001; -0.66 vs. 0.0, p<0.0001; -0.46 vs -0.01, p<0.0001, respectively); in the stillbirths due to unexplained causes there were no significant differences from live births in any of the biomarkers (sTable 3 and Figure 1).

Prediction of stillbirth and performance of combined screening

The results of univariate and multivariate regression analysis are shown in sTable 4. In the multivariate regression analysis, there was a significant contribution to the prediction of stillbirths due to impaired placentation from maternal factor derived *a priori* risk, PLGF MoM, UT-PI MoM and Z-scores of HC and AC but not FL (R^2=0.482; p<0.0001). The performance of screening for stillbirth is shown in Table 1 and Figure 2.

The DR for all stillbirths, at FPR of 10%, increased from 55% in screening by a combination of maternal factors, fetal biometry and UT-PI to 58% with the addition of serum PLGF; the respective values for the impaired placentation group were 75% and 84%. Within the impaired placentation group the DR with the combined model was higher for stillbirths at <32 weeks' gestation than those at ≥37 weeks (97% vs 61%; p<0.01).

Discussion

Main findings of the study

The findings of the study demonstrate that a high proportion of stillbirths due to impaired placentation can be effectively identified by second trimester screening by a combination of maternal factors, serum PLGF, fetal biometry and UT-PI. Such combined screening at 19-24 weeks can potentially predict 84% all stillbirths due to impaired placentation, at a 10% FPR; the performance of screening is better for stillbirths <32 weeks' gestation (97%), compared to those at term (61%).

Strengths and limitations

The strengths of this screening study are first, examination of a large population of pregnant women attending for routine assessment at 19-24 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 UT-PI, fourth, use of automated machines to provide accurate measurement of maternal serum PLGF concentration within 40 minutes of sampling, fifth, expression of the values of biomarkers as MoMs after adjustment for factors that affect the measurements, and sixth, use of multivariate regression analysis to take into account possible interrelations between the different variables to define the relative predictive value of each factor.

Potential limitations of the study include estimation of performance based on simulation of PLGF values; however, there was no significant difference between the bivariate Gaussian distributions of the measured and simulated values. Another potential limitation 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 in the second trimester have reported the benefit of incorporating measurement of serum PLGF in models of screening for PE and SGA.[7,10,13] Previous second-trimester studies have highlighted the value of uterine artery Doppler in screening for stillbirth.[1,23-25] Our study demonstrated the value of combining maternal factors, fetal biometry, UT-PI and PLGF in screening for stillbirth.

Clinical implications of the study

Prevention of impaired placentation related stillbirth could potentially be achieved by a two stage screening and intervention strategy. The first stage, at 11-13 weeks, would aim at improving placentation through such pharmacological interventions as low-dose aspirin and pravastatin in the high-risk group;[26,27] first-trimester screening by a combination of maternal factors, UT-PI, fetal ductus venosus pulsatility index for veins and maternal serum PLGF could detect about 60% of stillbirths due to impaired placentation, at FPR of 10%.[16] The second stage, at 19-24 weeks would identify a high-risk group that could benefit from close monitoring for early diagnosis of PE and SGA and appropriate management to prevent stillbirth in such pregnancies; as demonstrated in this study about 85% of stillbirths could be predicted from combined screening at 20 weeks' gestation.

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Table 1. Performance of screening for stillbirths by maternal factors, uterine artery pulsatility index and fetal biometry, without and with placental growth factor at 19-24 weeks' gestation at fixed false positive rates of 5% and 10%.

Outcome	N	AUROC (95% CI)	Detection rates (95% CI)	
			5% FPR	10% FPR
All stillbirths	268			
Maternal factors, UT-PI, biometry		0.748 (0.711-0.785)	45.1 (39.1-51.0)	54.7 (48.7-60.6)
+ PLGF		0.781 (0.746-0.817)	50.7 (44.7-56.7)	57.6 (51.7-63.5)
Abnormal placentation				
All stillbirths	159			
Maternal factors, UT-PI, biometry		0.904 (0.875-0.933)	69.8 (62.7-76.9)	74.8 (68.1-81.6)
+ PLGF		0.950 (0.932-0.967)	76.1 (69.5-82.7)	83.6 (77.8-89.4)
< 32 weeks	90			
Maternal factors, UT-PI, biometry		0.952 (0.921-0.982)	85.6 (78.4-92.9)	87.8 (81.0-94.6)
+ PLGF		0.990 (0.983-0.998)	94.4 (89.7-99.2)	96.7 (93.1-100.0)
< 37 weeks	126			
Maternal factors, UT-PI, biometry		0.929 (0.899-0.959)	79.4 (72.3-86.5)	82.5 (75.9-89.1)
+ PLGF		0.973 (0.960-0.985)	84.1 (77.7-90.5)	89.7 (84.5-95.0)
≥ 37 weeks	33			
Maternal factors, UT-PI, biometry		0.810 (0.743-0.877)	33.3 (17.2-49.4)	45.5 (28.4-62.4)
+ PLGF		0.863 (0.802-0.923)	45.5 (28.5-62.5)	60.6 (43.9-77.3)

AUROC = area under receiver operating characteristic curves; CI = confidence interval; UT-PI = uterine artery pulsatility index; PLGF = placental growth factor; FPR = false positive rate

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

Maternal characteristics	Live births (n=69,735)	Stillbirths		
		All cases (n=268)	Unexplained (n=109)	Impaired placentation (n=159)
Age, median (IQR)	30.5 (25.8-34.5)	30.5 (25.8-35.4)	30.9 (26.1-35.5)	30.4 (25.5-35.4)
Weight, median (IQR)	67.0 (59.2-78.0)	73.4 (63.7-85.2)*	71.6 (64.2-84.0)*	74.0 (63.5-85.8)*
Height, median (IQR)	1.64 (1.60-1.69)	1.65 (1.60-1.68)	1.65 (1.62-1.68)	1.63 (1.60-1.68)
Racial origin				
Caucasian, n (%)	48,794 (70.0)	144 (53.7)	65 (59.6)	79 (49.7)
Afro-Caribbean, n (%)	15,053 (21.6)	103 (38.4)	39 (35.8)*	64 (40.3)*
South Asian, n (%)	2,775 (4.0)	9 (3.4)	1 (0.9)	8 (5.0)
East Asian, n (%)	1,363 (2.0)	5 (1.9)	1 (0.9)	4 (2.5)
Mixed, n (%)	1,750 (2.5)	7 (2.6)	3 (2.8)	4 (2.5)
Method of conception				
Spontaneous, n (%)	67,777 (97.2)	255 (95.1)	105 (96.3)	150 (94.3)
Assisted conception, n (%)	1,958 (2.8)	13 (4.9)	4 (3.7)	9 (5.7)
Cigarette smoking, n (%)	7,478 (10.7)	35 (13.1)	14 (12.8)	21 (13.2)
Chronic hypertension, n (%)	1,031 (1.5)	17 (6.3)*	2 (1.8)	15 (9.4)*
SLE / APS, n (%)	132 (0.2)	4 (1.5)*	0	4 (2.5)*
Diabetes mellitus, n (%)	638 (0.9)	7 (2.6) †	3 (2.8)	4 (2.5)
Parity				
Nulliparous, n (%)	34,279 (49.2)	132 (49.3)	56 (51.4)	76 (47.8)
Previous miscarriage, n (%)	883 (1.3)	4 (1.5)	2 (1.8)	2 (1.3)
Previous stillbirth, n (%)	604 (0.9)	15 (5.6)*	3 (2.8)	12 (7.5)*
Previous SGA, n (%)	2,315 (3.3)	12 (4.5)	2 (1.8)	10 (6.3)
Inter-pregnancy interval, median (IQR)[a]	3.0 (2.0-5.1)	4.2 (2.2-7.1)*	3.9 (2.2-7.0)	4.3 (2.2-8.0)†

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

Maternal characteristics	Live births (n=9,870)	Stillbirths		
		All cases (n=86)	Unexplained (n=41)	Impaired placentation (n=45)
Age, median (IQR)	31.1 (26.6-34.8)	32.0 (26.6-35.7)	31.0 (25.1-36.6)	33.4 (26.6-35.5)
Weight, median (IQR)	67.0 (59.0-78.0)	75.4 (63.5-87.3)*	75.0 (62.9-85.6)	76.0 (63.8-88.8)*
Height, median (IQR)	1.65 (1.60-1.69)	1.64 (1.60-1.67)	1.64 (1.61-1.67)	1.65 (1.60-1.69)
Racial origin				
Caucasian, n (%)	7,234 (73.3)	52 (60.5)	25 (61.0)	27 (60.0)
Afro-Caribbean, n (%)	1,812 (18.4)	28 (32.6)*	14 (34.1)*	14 (31.1)
South Asian, n (%)	412 (4.2)	1 (1.2)	0	1 (2.2)
East Asian, n (%)	194 (2.0)	2 (2.3)	0	2 (4.4)
Mixed, n (%)	218 (2.2)	3 (3.5)	2 (4.9)	1 (2.2)
Method of conception				
Spontaneous, n (%)	9,523 (96.5)	84 (97.7)	40 (97.6)	44 (97.8)
Assisted conception, n (%)	347 (3.5)	2 (2.3)	1 (2.4)	1 (2.2)
Cigarette smoking, n (%)	999 (10.1)	14 (16.3)	8 (19.5)	6 (13.3)
Chronic hypertension, n (%)	139 (1.4)	3 (3.5)	1 (2.4)	2 (4.4)
SLE / APS, n (%)	18 (0.2)	1 (1.2)	0	1 (2.2)
Diabetes mellitus, n (%)	100 (1.0)	4 (4.7)*	2 (4.9)	2 (4.4)
Parity				
Nulliparous, n (%)	4,751 (48.1)	42 (48.8)	20 (48.8)	22 (48.9)
Previous miscarriage, n (%)	118 (1.2)	1 (1.2)	1 (2.4)	0
Previous stillbirth, n (%)	71 (0.7)	5 (5.8)*	1 (2.4)	4 (8.9)*
Previous SGA, n (%)	288 (2.9)	5 (5.8)	2 (4.9)	3 (6.7)
Inter-pregnancy interval, median (IQR)[a]	3.0 (1.9-5.0)	4.0 (2.7-6.1)	3.9 (2.6-5.9)	4.0 (2.7-6.3)

Post hoc Bonferroni correction for multiple comparisons; * = p< 0.01
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 3. Median and interquartile range of placental growth factor, uterine artery pulsatility index and fetal biometry at 19-24 week's gestation in pregnancies with livebirths compared to those that had a stillbirth

Biomarker	Live births (n=9,870)	Stillbirths		
		All cases (n=86)	Unexplained (n=41)	Impaired placentation (n=45)
Placental growth factor (MoM)	1.00 (0.74-1.36)	0.65 (0.34-1.15)**	1.13 (0.64-1.52)	0.42 (0.16-0.68)**
Uterine artery pulsatility index (MoM)	1.00 (0.84-1.21)	1.37 (1.01-1.71)**	1.17 (0.80-1.38)	1.68 (1.23-2.06)**
Head circumference z-score	-0.01 (-0.33-0.29)	-0.20 (-0.57-0.17)**	-0.08 (-0.29-0.42)	-0.38 (-0.81 - -0.17)**
Abdominal circumference z-score	0.00 (-0.40-0.38)	-0.29 (-0.71-0.11)**	0.06 (-0.27-0.47)	-0.66 (-1.16 - -0.28)**
Femur length z-score	-0.01 (-0.35-0.30)	-0.12 (-0.56-0.23)*	0.11 (-0.23-0.38)	-0.46 (-0.86 - -0.21)**

MoM= multiple of the median; Significance value (p): *Post hoc* Bonferroni correction for multiple comparisons; * = p< 0.01; ** = p< 0.001

Supplementary Table 4. Univariate and multivariate logistic regression analysis for the prediction of stillbirths due to impaired placentation by maternal factors and combination of placental growth factor, uterine artery pulsatility index and fetal biometry at 19-24 week's gestation

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Maternal factor derived logit (*a priori* risk)	14.52 (9.29-22.69)	<0.0001	4.84 (2.53-9.28)	<0.0001
Log_{10} uterine artery pulsatility index MoM	$22.75e^4$ ($73.20e^3$-$70.72e^4$)	<0.0001	$4.20e^3$ ($1.06e^3$-$16.69e^3$)	<0.0001
Head circumference z-score	0.07 (0.05-0.09)	<0.0001	0.50 (0.32-0.77)	0.002
Abdominal circumference z-score	0.09 (0.07-0.12)	<0.0001	0.31 (0.22-0.43)	<0.0001
Log_{10} placental growth factor MoM	0.001 (0.001-0.002)	<0.0001	0.007 (0.004-0.012)	<0.0001

MoM= multiple of the median; OR = odds ratio; CI = confidence interval

Figure 1. Box and whiskers plot of placental growth factor 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 combined screening with maternal factors, fetal biometry and uterine artery pulsatility index (blue curve) and with addition of maternal serum placental growth factor (red curve).