Placental growth factor in prediction of stillbirths at 11-13 weeks

Ranjit Akolekar,[1,2] Mirian Machuca,[1,2] Mariane Mendes,[1] Vasileios Paschos,[1] Kypros H. Nicolaides.[1]

Short title: Prediction of stillbirth from biomarkers

Key words: Stillbirth, First trimester screening, Placental growth factor, Pyramid of pregnancy care

1. Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK
2. Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, UK

Acknowledgement: This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116). The reagents and equipment for the measurement of serum placental growth factor were provided by PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland and Roche Diagnostics Limited.

Corresponding author
Professor R Akolekar
Fetal Medicine Research Institute,
King's College Hospital,
16-20 Windsor Walk,
Denmark Hill, London SE58BB
Telephone: +442032998256
Fax: +442077339534

Abstract

Objectives: To investigate whether measurement of maternal serum placental growth factor (PLGF) 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), ductus venosus pulsatility index for veins (DV-PIV) and uterine artery pulsatility index (UT-PI) PI and to 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 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. 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 and PAPP-A, DV-PIV and UT-PI.

Results: Significant contribution to the prediction of stillbirths was provided by maternal factor derived *a priori* risk and MoM values of PLGF, DV-PIV and UT-PI but not serum PAPP-A. A model combining these variables predicted 42% of all stillbirths and 61% 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 (71% vs 46%; p=0.031).

Conclusions: The results of our study demonstrate that a high proportion of stillbirths due to impaired placentation can be effectively identified in the first trimester of pregnancy. The extent to which such stillbirths could be prevented remains to be determined.

Introduction

Antepartum stillbirths can be broadly classified into those thought to be the consequence of impaired placentation and those due to other causes or being unexplained; 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 false positive rate (FPR) of 10%.[4] A first-trimester screening study combining maternal factors with measurements of uterine artery pulsatility index (UT-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 FPR of 10%, the detection rate (DR) of stillbirth due to impaired placentation improved to 55%, whereas the DR of stillbirths due to other cases or those that 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 maternal spiral arteries.[6-8] Maternal serum levels at 11-13 weeks' gestation are decreased in pregnancies with impaired placentation that develop preeclampsia (PE) and those that deliver small for gestational age (SGA) neonates.[9-11]

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 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 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).[12] Transabdominal colour Doppler ultrasound was performed to measure ductus venosus pulsatility index for veins (DV-PIV) and uterine artery pulsatility index (UT-PI).[13,14] Maternal serum concentration of free ß-hCG, PAPP-A and PLGF was measured using automated analysers which provide reproducible results within 10 minutes 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 (CRL).[15] 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 <10th percentile for gestational age [16] or it was due to other reasons or 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 χ2-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 values of PAPP-A, PLGF, UT-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 those characteristics that provide a substantial contribution to the $\log_{10}$ transformed value.[13,17-19] 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.[4] 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 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 UT- 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 characteristic (ROC) curves analysis and the DR and FPR were estimated. The significane of improvement in performance of screening by additional of maternal serum PLGF was assessed by comparison of the areas under the ROC curves (AUROC).[20]

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 45,452 singleton pregnancies fulfilling the 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 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 PLGF MoM (0.78 vs 1.00; p<0.0001) and higher UT-PI MoM (1.23 vs 1.00, p<0.0001), but there was no significant difference in DV-PIV MoM. 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 PLGF MoM (0.63 vs 1.00; p<0.0001) and higher DV-PIV MoM (1.05 vs 1.00; p<0.0001) and UT-PI MoM (1.39 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 (sTable 2, 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 stillbirth due to impaired placentation from maternal factor derived *a priori* risk and MoM values of PLGF, DV-PIV and UT-PI, but not serum PAPP-A (R^2=0.193; p<0.0001). Multivariate regression analysis demonstrated that the odds ratio for maternal factors, DV-PIV, UT-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 of each unit of PAPP-A MoM was associated with an odds ratio for stillbirth of 0.46.

The performance of screening for stillbirth from maternal factors and biomarkers is shown in Table 1. Combinated screening by maternal factors, PAPP-A, DV-PI and UT-PI detected 50% of all stillbirths due to impaired placentation, at a FPR of 10% (AUROC curve 0.809, 95CI% 0.767-0.850). Addition of serum PLGF, which resulted in making the contribution of serum PAPP-A non-significant, improved the DR to 61% (AUROC curve 0.852, 95% CI 0.816-0.888). In the impaired placentation group, screening by maternal factors, PLGF, DV-PI and UT-PI, the DR of stillbirths at < 32 weeks' gestation was higher than those at ≥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, UT-PI and fetal DV-PIV at 11-13 weeks' gestation can potentially predict about 60% of stillbirths due to impaired placentation, at 10% FPR; the performance of screening is better for stillbirths at <32 weeks' gestation (71%) compared to those at term (46%). Although serum PAPP-A on its own or in combination with UT-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 multivariate regression model in this study, the odds ratio 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 DV-PIV and UT-PI, fourth, use of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of 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 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; 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 SGA neonates.[9-11,21] The performance of early screening for stillbirth due to impaired placentation by an algorithm incorporating serum PLGF is superior to that relying on maternal factors alone or a combination of maternal factors with UT-PI, DV-PIV and serum PAPP-A.[4,5] This is not surprising since we have previously reported that in early screening for PE by a combination of maternal factors and biomarkers serum PLGF had a better performance than 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 effectively identified in the first trimester of pregnancy. In the case of PE there is widely accepted process of screening which is based on a combination of maternal factors alone; we have demonstrated that the performance of screening by a combination of maternal factors with biomarkers including UT-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 such drugs as low-dose aspirin starting at <16 weeks' gestation, could potentially improve placentation and reduce the associated risk of PE, SGA and stillbirths by more than 50%.[23]

References

1. Wigglesworth JS. Monitoring perinatal mortality. A pathophysiological approach. *Lancet* 1980; **2**: 684-686.

2. Gardosi J, Kady SM, McGeown P, Francis A and Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. *BMJ* 2005; **331**: 1113-1117.

3. Froen JF, Pinar H, Flenady V, Bahrin S, Charles A, Chauke L, Day K, Duke CW, Facchinetti F, Fretts RC, Gardener G, Gilshenan K, Gordijn SJ, Gordon A, Guyon G, Harrison C, Koshy R, Pattinson RC, Petersson K, Russell L, Saastad E, Smith GC, Torabi R. Causes of death and associated conditions (Codac): a utilitarian approach to the classification of perinatal deaths. *BMC Pregnancy Childbirth* 2009; **9**: 22.

4. Yerlikaya G, Akolekar R, McPherson K, Syngelaki A, Nicolaides KH. Prediction of stillbirth from maternal demographic and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2016; in press.

5. Mastrodima S, Akolekar R, Yerlikaya G, Tzelepis T, Nicolaides KH. Prediction of stillbirth from biochemical and biophysical markers at 11-13 weeks. *Ultrasound Obstet Gynecol* 2016; in press.

6. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci* 1991; **88**: 9267–9271.

7. Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. *Placenta* 1997; **18**: 657–665.

8. Vuorela P, Hatva E, Lymboussaki A, Kaipainen A, Joukov V, Persico MG, Alitalo K, Halmesmaki E. Expression of vascular endothelial growth factor and placenta growth factor in human placenta. *Biol Reprod* 1997; **56**: 489–494.

9. Akolekar R, Zaragoza E, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11+0 to 13+6 weeks of gestation in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2008; **32**: 732–739.

10. Poon LC, Zaragoza E, Akolekar R, Anagnostopoulos E, Nicolaides KH. Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11(+0) to 13(+6) weeks of gestation. *Prenat Diagn* 2008; **28**: 1110–1115.

11. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation. *Am J Obstet Gynecol* 2016; **214**: 103.e1-103.e12.

12. Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta human chorionic gonadotropin, and pregnancy associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008; **31**: 618–624.

13. Maiz N, Wright D, Ferreira AF, Syngelaki A, Nicolaides KH. A mixture model of ductus venosus pulsatility index in screening for aneuploidies at 11-13 weeks' gestation. *Fetal Diagn Ther* 2012; **31**: 221-229.

14. Plasencia W, Maiz N, Poon L, Yu C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks and 21 + 0 to 24 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2008; **32**: 138-146.

15. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **182**: 702–710.

16. Poon LCY, Tan MY, Yerlikaya G, Syngelaki A, Nicolaides KH. Birthweight in live births and stillbirths. Ultrasound Obstet Gynecol 2016; in press.

17. Wright D, Silva M, Papadopoulos S, Wright A, Nicolaides KH. Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **46**: 42-50.

18. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 591-598.

19. Tayyar A, Guerra L, Wright A, Wright D, Nicolaides KH. Uterine artery pulsatility index in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 689-697.

20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993; **39**: 561-577.

21. Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. *Fetal Diagn Ther* 2011; **29**: 148-154.

22. Gallo DM, Wright D, Casanova C, Campanero M, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19–24 weeks' gestation. *Am J Obstet Gynecol* 2016; **214:** 619-e1.

23. Roberge S, Nicolaides K, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 491-499.

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	227			
Maternal factors		0.647 (0.607-0.686)	19.4 (14.3-24.5)	30.4 (24.4-36.4)
+ UT PI + DV PIV + PlGF		0.731 (0.693-0.769)	31.7 (25.7-37.8)	41.9 (35.5-48.3)
Unexplained				
Maternal factors	96	0.625 (0.567-0.683)	14.6 (7.5-21.7)	26.0 (17.2-34.8)
Abnormal placentation				
All stillbirths	131			
Maternal factors		0.663 (0.610-0.716)	22.9 (15.7-30.1)	33.6 (25.5-41.7)
+ 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)
+ UT-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)
+ UT-PI + DV-PIV		0.795 (0.754-0.836)	36.6 (28.1-44.5)	48.1 (39.5-56.7)
+ PlGF + UT-PI		0.848 (0.811-0.884)	47.9 (39.4-56.5)	60.8 (52.4-69.2)
+ UT-PI + DV-PIV + PlGF		0.852 (0.816-0.888)	48.1 (36.2-60.0)	61.1 (49.5-72.7)
< 32 weeks	68			
Maternal factors		0.664 (0.587-0.741)	29.4 (18.6-40.2)	36.8 (25.3-48.3)
+ UT-PI + DV-PIV + PlGF		0.900 (0.859-0.940)	61.8 (50.3-73.4)	70.6 (59.8-81.4)
< 37 weeks	98			
Maternal factors		0.666 (0.604-0.727)	24.5 (16.0-33.1)	32.7 (23.4-42.0)
+ UT-PI + DV-PIV + PlGF		0.875 (0.835-0.915)	54.1 (44.2-64.0)	66.3 (56.9-75.7)
≥ 37 weeks	33			
Maternal factors		0.655 (0.551-0.758)	18.2 (5.1-31.4)	36.4 (20.0-52.8)
+ UT-PI + DV-PIV + PlGF		0.785 (0.710-0.860)	30.3 (14.6-45.9)	45.5 (28.5-62.5)

AUROC=area under receiver operating characteristic curves; CI= confidence interval; FPR = False positive rate; 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=45,225)	All stillbirths (n=227)	Unexplained (n=96)	Impaired placentation (n=131)
Age, median (IQR)	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, median (IQR)	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, median (IQR)	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, n (%)	32,834 (72.6)	122 (53.7)	55 (57.3)	67 (51.1)
Afro-Caribbean, n (%)	8,359 (18.5)	86 (37.9)**	34 (35.4)*	52 (39.7)*
South Asian, n (%)	1,927 (4.3)	8 (3.5)	3 (3.1)	5 (3.8)
East Asian, n (%)	929 (2.1)	4 (1.8)	1 (1.0)	3 (2.3)
Mixed, n (%)	1,176 (2.6)	7 (3.1)	3 (3.1)	4 (3.1)
Method of conception				
Spontaneous, n (%)	43,877 (97.0)	214 (94.3)	91 (94.8)	123 (93.9)
Assisted conception, n (%)	1,348 (3.0)	13 (5.7)	5 (5.2)	8 (6.1)
Cigarette smoking, n (%)	4,335 (9.6)	25 (11.0)	9 (9.4)	16 (12.2)
Chronic hypertension, n (%)	699 (1.5)	15 (6.6)**	1 (1.0)	14 (10.7)*
SLE / APS, n (%)	97 (0.2)	2 (0.9)	0	2 (1.5) †
Diabetes mellitus, n (%)	435 (1.0)	9 (4.0)*	5 (5.2)*	4 (3.1)†
Parity				
Nulliparous, n (%)	21,266 (47.0)	109 (48.0)	46 (47.9)	63 (48.1)
Previous miscarriage, n (%)	574 (1.3)	2 (0.9)	1 (1.0)	1 (0.8)
Previous stillbirth, n (%)	360 (0.8)	10 (4.4)**	3 (3.1)†	7 (5.3)*
Previous SGA, n (%)	1,494 (3.3)	9 (4.0)	2 (2.1)	7 (5.3)
Inter-pregnancy interval, median (IQR)[a]	1.1 (0.0-3.1)	1.0 (0.0-4.4)	1.2 (0.0-4.0)	0.8 (0.0-4.5)

Post hoc Bonferroni correction for multiple comparisons; † = p<0.025; * = 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=45,225)	All stillbirths (n=227)	Unexplained (n=96)	Abnormal placentation (n=131)
Pregnancy associated plasma protein-A (MoM)	1.00 (0.69-1.43)	0.85 (0.53-1.23)***	1.04 (0.70-1.44)	0.69 (0.46-1.10)***
Placental growth factor (MoM)	1.00 (0.78-1.29)	0.78 (0.51-1.09)***	1.07 (0.75-1.30)	0.63 (0.43-0.89)***
Ductus venosus pulsatility index for veins (MoM)	1.00 (0.90-1.09)	1.01 (0.91-1.15)	1.00 (0.88-1.10)	1.04 (0.94-1.17)**
Uterine artery pulsatility index (MoM)	1.00 (0.81-1.22)	1.23 (0.97-1.52)***	1.03 (0.83-1.34)	1.39 (1.14-1.61)***

MoM= multiple of the median; *Post hoc* Bonferroni correction for multiple comparisons; † = p< 0.01; * = p< 0.001

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)	13.98 (8.55-22.88)	<0.0001	8.77 (5.09-15.12)	<0.0001
Log_{10} PAPP-A MoM	0.19 (0.13-0.30)	<0.0001		
Log_{10} PlGF MoM	0.008 (0.004-0.015)	<0.0001	0.016 (0.008-0.030)	<0.0001
Log_{10} Ductus venosus PIV MoM	196.02 (36.86-1042.46)	<0.0001	49.73 (7.86-314.52)	<0.0001
Log_{10} Uterine artery PI MoM	$4.63e^{03}$ ($1.02e^{03}$-$20.91e^{03}$)	<0.0001	713.01 (155.83-3262.38)	<0.0001

PAPP-A = pregnancy associated plasma protein-A; PlGF = placental growth factor; 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 placental growth factor (PlGF) multiple of the median (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.