Ultrasound Obstet Gynecol 2016; **47**: 466–471
Published online 8 March 2016 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15818

Maternal serum alpha-fetoprotein at 12, 22 and 32 weeks' gestation in screening for pre-eclampsia

F. E. BREDAKI*, M. MATALIOTAKIS*, A. WRIGHT†, D. WRIGHT† and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Institute of Health Research, University of Exeter, Exeter, UK*

KEYWORDS: alpha fetoprotein; Bayes' theorem; impaired placentation; pre-eclampsia; pyramid of pregnancy care; screening

ABSTRACT

Objective *To examine the distribution of maternal serum alpha-fetoprotein (AFP) at 12, 22 and 32 weeks' gestation in singleton pregnancies which develop pre-eclampsia (PE) and examine the performance of this biomarker in screening for PE.*

Methods *Serum AFP was measured in 17 071 cases at 11–13 weeks, in 8583 cases at 19–24 weeks and 8609 cases at 30–34 weeks' gestation. Bayes' theorem was used to combine the* a-priori *risk from maternal characteristics and medical history with AFP. The performance of screening for PE requiring delivery < 32, at 32 + 0 to 36 + 6, < 37 and ≥ 37 weeks' gestation was estimated.*

Results *In pregnancies that developed PE, serum AFP multiples of the median (MoM) was increased at 11–13 and 19–24 weeks' gestation, but not at 30–34 weeks, and the values were inversely related to gestational age at delivery. Combined screening with maternal factors and serum AFP improved the prediction provided by maternal factors alone for PE delivering < 37 weeks, but not for PE delivering ≥ 37 weeks. The performance of screening for preterm PE was better at 19–24 weeks than at 11–13 weeks and the detection rate (DR) for a given false-positive rate (FPR) was higher for PE delivering < 32 weeks than for PE delivering at 32 + 0 to 36 + 6 weeks. The DRs, at 10% FPR, of combined screening at 11–13 weeks for PE delivering < 32 and at 32 + 0 to 36 + 6 weeks were 54% and 45%, respectively, and these improved to 72% and 53% with screening at 19–24 weeks.*

Conclusions *Measurement of serum AFP at 11–13 and 19–24 weeks' gestation improves the prediction of preterm PE provided by maternal factors alone.* *Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Maternal serum alpha-fetoprotein (AFP) levels during the first and or second trimester of pregnancy are altered in pregnancies with aneuploidy, neural tube defects and adverse pregnancy outcome, including fetal death, pre-eclampsia (PE), fetal growth restriction and preterm birth[1–7].

We have proposed that the best approach to screening for PE is to use Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the measurement of biomarkers[8–10]. Our approach assumes that, if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. The effect of maternal factors and biomarkers is to modify the mean of the distribution of gestational age at delivery with PE so that, in pregnancies at low-risk of PE, the gestational age distribution is shifted to the right with the implication that in most pregnancies delivery will actually occur before development of PE. In high-risk pregnancies the distribution is shifted to the left and the smaller the mean gestational age the higher is the risk of PE.

The objectives of this study were to present the distribution of serum AFP values at 11–13, 19–24 and 30–34 weeks' gestation in pregnancies that develop PE and examine the performance of screening for PE by serum AFP at these stages in pregnancy.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending three routine hospital visits at King's College Hospital, University College London Hospital and Medway Maritime Hospital, UK between March 2006

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: kypros@fetalmedicine.com)

Accepted: 13 November 2015

and April 2014. Samples were collected routinely during two time periods within this 8-year interval. In the first hospital visit, at 11 + 0 to 13 + 6 weeks' gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies[11]. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and the third, at 30 + 0 to 34 + 6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size. Gestational age was determined by the measurement of fetal crown–rump length (CRL) at 11–13 weeks or fetal head circumference at 19–24 weeks[12,13].

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 pregnancy delivering phenotypically normal live birth or stillbirth at or after 24 weeks' gestation. Pregnancies with aneuploidies or major fetal abnormalities, and those ending in termination, miscarriage or fetal death before 24 weeks 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 or assisted conception requiring the use of ovulation drugs or *in-vitro* fertilization), cigarette smoking during pregnancy, medical history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), family history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancy at or after 24 weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal height was measured at the first visit and weight at each visit.

Measurement of maternal serum alpha-fetoprotein

Serum AFP was measured by automated biochemical analyzers within 10 min of blood sampling. In 17 638 cases, the sample was analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, USA) and, in 16 625 cases, analysis was by the Cobas e411 system (Roche Diagnostics Ltd., Penzberg, Germany).

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[14]. The outcome measures for this study were PE delivering < 32, at 32 + 0 to 36 + 6, < 37 and ≥ 37 weeks' gestation.

Statistical analysis

Competing-risks model

The distribution of gestational age at delivery with PE was defined by two components: first, the prior distribution based on maternal characteristics[8] and second, the distribution of serum AFP multiples of the median (MoM) values with gestational age at delivery in pregnancies affected by PE. The values of AFP were $\log_{10}$ transformed to achieve homogeneity of variance and approximate Gaussian distributional form. Each measured value in the unaffected and PE pregnancies was expressed as a MoM, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$ transformed value; these include gestational age, maternal weight, racial origin, cigarette smoking, machine used to measure serum AFP and gestational age at delivery, birth-weight *Z*-score of the neonate and interval from the previous pregnancy[15]. In the PE group, regression analysis demonstrated that the $\log_{10}$ MoM AFP changed linearly with gestational age at delivery and this linear relationship was assumed to continue until the mean $\log_{10}$ MoM reached zero, beyond which the mean was taken as zero. The point at which the mean $\log_{10}$ MoM reached zero was determined using the method of least squares. Standard errors were obtained using bootstrapping. Risks of PE were obtained by applying Bayes' theorem to derive the posterior distribution of gestational age at delivery with PE from the maternal factors specific prior distribution[8] and the likelihood function of AFP. The likelihood function comprises the regression of $\log_{10}$ MoM AFP on gestational age at delivery with PE.

Model-based estimates of screening performance using Bayes' theorem

To provide model-based estimates of screening performance, the following procedure was adopted. First, we obtained the dataset of 123 406 singleton pregnancies, including 2748 (2.2%) with PE, that was previously used to develop a model for PE based on maternal demographic characteristics and medical history[8,16]. Second, for each of the records, AFP MoM values were simulated from the fitted multivariate Gaussian distribution for log-transformed MoM values. Third, risks were obtained using the competing-risks model from the simulated MoM values and the pregnancy characteristics. These three steps were applied to the pregnancies within the normal group with no restriction on the time of delivery. Fourth, for a given false-positive rate (FPR), risks from the normal group were used to define a risk cut-off. The proportion of PE risks was then used to obtain an estimate of the associated detection rate (DR). The area under the receiver–operating characteristics curve (AUC)

Table 1 Maternal and pregnancy characteristics in women with singleton pregnancy screened at different weeks for prediction of pre-eclampsia (PE)

	11–13 weeks		*19–24 weeks*		*30–34 weeks*	
Characteristic	*Normal* (n = 16 583)	*PE* (n = 488)	*Normal* (n = 8366)	*PE* (n = 217)	*Normal* (n = 8401)	*PE* (n = 208)
Maternal age (years)	31.1 (26.6–34.8)	31.4 (27.0–35.1)	31.1 (26.5–34.8)	31.5 (26.9–35.6)	31.2 (26.8–34.8)	31.4 (27.0–34.8)
Maternal weight (kg)	66.8 (59.0–77.9)	72.0 (63.0–87.5)	71.0 (63.1–81.5)	78.0 (69.0–94.0)	76.6 (68.5–86.9)	81.2 (71.8–95.2)
Maternal height (cm)	164 (160–169)	163 (159–168)	165 (160–169)	164 (160–168)	165 (160–169)	163 (159–168)
BMI (kg/m^2)	24.6 (22.0–28.6)	27.2 (23.8–32.1)	26.0 (23.5–29.8)	29.0 (25.8–33.7)	28.1 (25.4–31.9)	30.9 (27.4–35.1)
GA at screening (weeks)	12.7 (12.3–13.1)	12.6 (12.3–13.0)*	21.9 (21.1–22.1)	21.9 (21.0–22.1)	32.1 (32.0–32.4)	32.1 (32.0–32.4)
Racial origin		*		*		*
Caucasian	11 373 (68.6)	253 (51.8)	6090 (72.8)	117 (53.9)	6097 (72.6)	116 (55.8)
Afro-Caribbean	3214 (19.4)	190 (38.9)	1575 (18.8)	88 (40.6)	1640 (19.5)	78 (37.5)
South Asian	985 (5.9)	33 (6.8)	347 (4.2)	7 (3.2)	311 (3.7)	7 (3.4)
East Asian	635 (3.8)	4 (0.8)	169 (2.0)	3 (1.4)	151 (1.8)	3 (1.4)
Mixed	376 (2.3)	8 (1.6)	185 (2.2)	2 (0.9)	202 (2.4)	4 (1.9)
Medical history						
Chronic hypertension	190 (1.2)	62 (12.7)*	85 (1.0)	30 (13.8)*	92 (1.1)	29 (13.9)*
Diabetes mellitus	207 (1.3)	12 (2.5)*	80 (1.0)	8 (3.7)*	84 (1.0)	4 (1.9)
SLE/APS	30 (0.2)	1 (0.2)	14 (0.2)	0 (0.0)	13 (0.2)	0 (0.0)
Cigarette smoker	1678 (10.1)	33 (6.8)*	833 (10.0)	21 (9.7)	826 (9.8)	14 (6.7)
Family history of PE	574 (3.5)	34 (7.0)*	275 (3.3)	12 (5.5)	264 (3.1)	11 (5.3)
Mode of conception						
In-vitro fertilization	464 (2.8)	16 (3.3)	199 (2.4)	8 (3.7)	182 (2.2)	5 (2.4)
Ovulation drugs	261 (1.6)	9 (1.8)	82 (1.0)	4 (1.8)	80 (1.0)	3 (1.4)
Spontaneous	15 858 (95.6)	463 (94.9)	8085 (96.6)	205 (94.5)	8139 (96.9)	200 (96.2)
Obstetric history		*		*		*
Parous						
No previous PE	8444 (50.9)	149 (30.5)	4130 (49.4)	57 (26.3)	3992 (47.5)	59 (28.4)
Previous PE	543 (3.3)	67 (13.7)	276 (3.3)	30 (13.8)	301 (3.6)	25 (12.0)
Nulliparous	7596 (45.8)	272 (55.7)	3960 (47.3)	130 (59.9)	4108 (48.9)	124 (59.6)
Interpregnancy interval (years)	3.1 (2.0–5.1)	4.1 (2.6–6.6)*	2.9 (1.8–4.8)	4.5 (2.7–6.6)*	3.1 (2.0–5.2)	3.5 (2.4–6.2)*

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; BMI; body mass index; GA, gestational age; SLE, systemic lupus erythematosus. Comparisons with normal group: chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test for continuous variables: *$P < 0.05$.

was also calculated. The simulations were repeated 10 times to reduce variability due to the simulation process and provide suitably precise model-based estimates of performance.

Empirical performance of screening

Five-fold cross validation was used to assess the performance of screening for subgroups of PE according to gestational age at delivery, by models combining maternal factors with serum AFP. The data were divided into five equal subgroups, the model was then fitted five times to different combinations of four of the five subgroups and used to predict risk of PE in the remaining fifth of the data. In each case, the maternal-factor model and the regression models were fitted to the training dataset comprising four-fifths of the data and used to produce risks for the hold-out sample comprising the remaining fifth of the data.

The statistical software package R was used for data analyses[17] and the survival package[18] was used for fitting the maternal-factors model.

RESULTS

The characteristics of the study population of singleton pregnancies with measurements of serum AFP are summarized in Table 1. Serum AFP was measured in 17 071 cases at 11–13 weeks, in 8583 cases at 19–24 weeks and 8609 at 30–34 weeks.

In pregnancies that developed PE, AFP MoM at 11–13 and 19–24 weeks' gestation was inversely related to gestational age at delivery (Figure 1). The regression equations are given in Table S1. At 30–34 weeks, the median AFP MoM in pregnancies that developed PE was not significantly different from that in normal pregnancies. The standard deviation for $\log_{10}$ AFP MoM in unaffected pregnancies and in those that developed PE are given in Table S2.

Empirical and model-based performance of screening for PE by maternal factors and serum AFP at 11–13 and 19–24 weeks' gestation are shown in Tables 2 and S3 and Figure 2. All model-based results were within the 95% CI of the empirical data. Combined screening with maternal factors and serum AFP improved the prediction provided by maternal factors alone for PE delivering < 37 weeks,

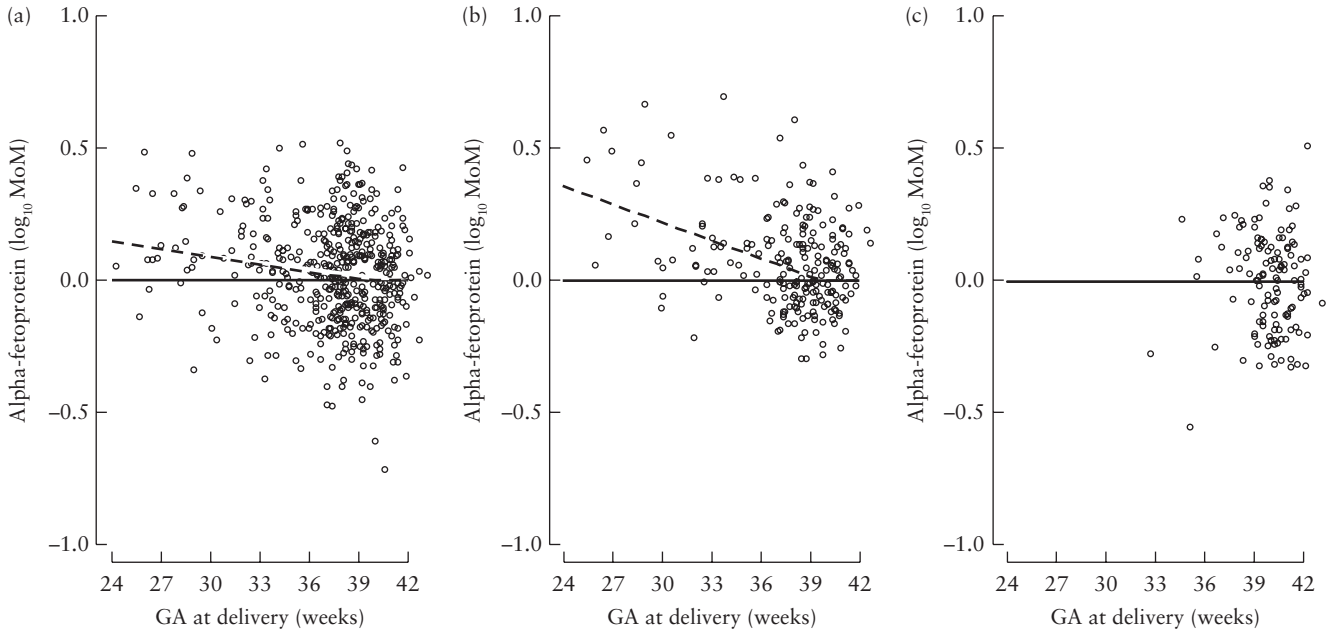

Figure 1 Relationship between maternal serum alpha-fetoprotein multiples of the median (MoM) and gestational age (GA) at delivery in pregnancies with pre-eclampsia, with screening at: (a) 11–13, (b) 19–24 and (c) 30–34 weeks' gestation. Regression lines (– – –) are shown.

Table 2 Empirical and model-based detection rates of pre-eclampsia (PE) by screening with maternal factors and a combination of maternal factors and serum alpha-fetoprotein at 11–13 and 19–24 weeks' gestation

	Detection rate of PE delivering:							
	< 32 weeks		*32 + 0 to 36 + 6 weeks*		*< 37 weeks*		*≥ 37 weeks*	
Screening	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*
Maternal factors								
FPR = 5%								
11–13 weeks	44 (28–62) 16/36	41	26 (19–35) 31/117	31	31 (24–39) 47/153	34	24 (20–29) 82/335	27
19–24 weeks	47 (23–72) 8/17	41	37 (22–54) 14/38	31	40 (27–54) 22/55	34	29 (22–37) 47/162	27
FPR = 10%								
11–13 weeks	64 (46–79) 23/36	52	40 (31–50) 47/117	44	46 (38–54) 70/153	46	36 (30–41) 119/335	37
19–24 weeks	65 (38–86) 11/17	52	50 (33–67) 19/38	44	55 (41–68) 30/55	46	41 (33–49) 66/162	37
Combined								
FPR = 5%								
11–13 weeks	53 (35–70) 19/36	43	26 (18–35) 30/117	32	32 (25–40) 49/153	35	24 (20–29) 81/335	27
19–24 weeks	53 (28–77) 9/17	60	45 (29–62) 17/38	39	47 (34–61) 26/55	44	29 (22–37) 47/162	27
FPR = 10%								
11–13 weeks	67 (49–81) 24/36	54	40 (31–50) 47/117	45	46 (38–55) 71/153	47	36 (30–41) 119/335	37
19–24 weeks	71 (44–90) 12/17	72	58 (41–74) 22/38	53	62 (48–75) 34/55	58	40 (32–47) 64/162	38

FPR, false-positive rate.

but not for PE delivering ≥ 37 weeks. The performance of screening for preterm PE was better at 19–24 weeks than at 11–13 weeks and the DR for a given FPR was higher for PE delivering < 32 weeks than for PE delivering at 32 + 0 to 36 + 6 weeks.

DISCUSSION

Principal findings of the study

The data of this study demonstrate that in pregnancies that develop PE there are increased levels of maternal

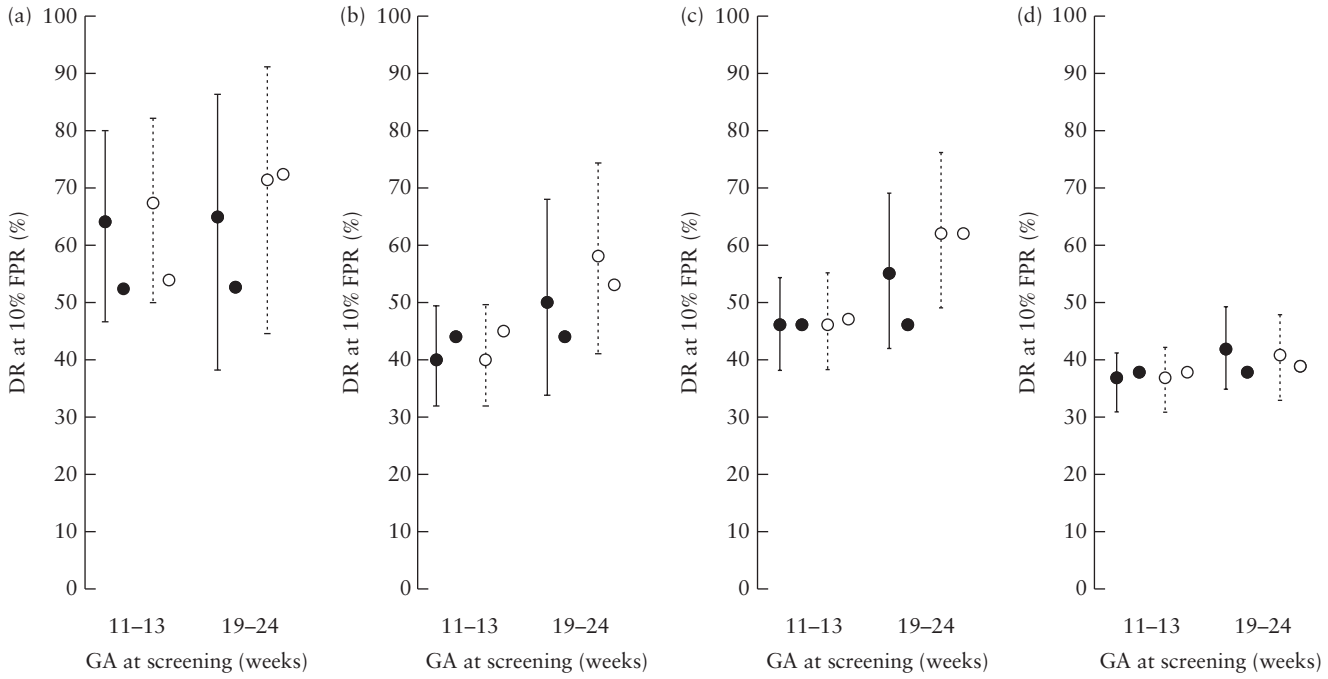

Figure 2 Empirical detection rate (DR) of pre-eclampsia delivering: (a) < 32; (b) at 32 + 0 to 36 + 6; (c) < 37; and (d) ≥ 37 weeks' gestation, when screening by maternal factors (●) and by a combination of maternal factors with serum alpha-fetoprotein (○) at 11–13 and 19–24 weeks' gestation. Vertical lines represent 95% CIs. Adjacent circles with no 95% CI represent model-based DR. FPR, false-positive rate; GA, gestational age.

serum AFP during the first and second trimesters, but not in the third trimester. The separation in MoM values from normal is greater with earlier, compared to later, gestational age at which delivery for PE becomes necessary; consequently, the performance of screening is superior for PE delivering < 32 than PE delivering at 32 + 0 to 36 + 6 weeks. The regression lines of serum AFP MoM with gestational age at delivery in pregnancies that develop PE intersect 1 MoM at about 40 weeks and this marker shows no discriminatory power for term-PE. The slope of the regression lines increases with gestational age at screening; consequently, the performance of screening for PE is superior with screening at 19–24 than at 11–13 weeks.

Strengths and limitations

The strengths of this screening study for PE in the three trimesters of pregnancy are first, examination of a large population of pregnant women attending for routine care, second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE, third, measurement of serum AFP by automated machines that provide reproducible results within 40 min of sampling so that complete assessment and counseling can potentially be undertaken at the same hospital visit, fourth, expression of the values of serum AFP as MoMs after adjustment for factors that affect the measurements, and fifth, use of Bayes' theorem to combine the prior risk from maternal factors with AFP to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

In some of the predictions there were large differences between modeled and empirical results of performance of screening, albeit within the 95% CI of the empirical data. This is likely to be the consequence of the relatively small number of affected cases in the many subgroups of PE. Another reason is the strong contribution of the prior risk derived from maternal characteristics and medical history; these characteristics were not identical in the dataset used for deriving the modeled results and the two datasets for the empirical results of screening at 11–13 and 19–24 weeks.

Comparison with previous studies

A systematic review and meta-analysis of serum analytes used in screening for fetal aneuploidies, including AFP, has reported that these analytes have low predictive accuracy for PE, but may be useful if they are combined with other tests[6]. This study combined serum AFP with maternal factors and demonstrated a modest performance in screening for preterm PE.

Clinical implications of the study

In a proposed new pyramid of pregnancy care[19], assessment at 12 weeks aims to identify the group at high risk for development of preterm PE and, through pharmacological intervention, reduce the prevalence of the disease[20,21]. Assessment at 22 and/or 32 weeks aims to estimate the patient-specific risk of developing PE and on the basis of such risk define the timing and content of subsequent visits to help improve perinatal outcome.

Measurement of serum AFP at 11–13 weeks is not in itself a good predictive marker of PE and is unlikely to improve the performance of screening provided by a combination of maternal factors, uterine artery pulsatility index, mean arterial pressure and serum placental growth factor[22]. At 19–24 weeks, serum AFP may prove to be useful in combination with other biomarkers in identifying pregnancies at high risk of early PE requiring delivery < 32 weeks.

ACKNOWLEDGMENTS

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

REFERENCES

1. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol* 1984; **148**: 886–894.
2. Bredaki FE, Wright D, Matos P, Syngelaki A, Nicolaides KH. First-trimester screening for trisomy 21 using alpha-fetoprotein. *Fetal Diagn Ther* 2011; **30**: 215–218.
3. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM. First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. *Ultrasound Obstet Gynecol* 2013; **42**: 41–50.
4. Wald NJ, Cuckle H, Brock JH, Peto R, Polani PE, Woodford FP. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet* 1977; **1**: 1323–1332.
5. Dugoff L, Hobbins JC, Malone FD, Vidaver J, Sullivan L, Canick JA, Lambert-Messerlian GM, Porter TF, Luthy DA, Comstock CH, Saade G, Eddleman K, Merkatz IR, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME; FASTER Trial Research Consortium. Quad screen as a predictor of adverse pregnancy outcome. *Obstet Gynecol* 2005; **106**: 260–267.
6. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, Mol BW, van der Post JA, Khan KS. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. *BMC Pregnancy Childbirth* 2008; **8**: 33.
7. Beta J, Bredaki FE, Calvo JR, Akolekar R, Nicolaides KH. Maternal serum α-fetoprotein at 11–13 weeks' gestation in spontaneous early preterm delivery. *Fetal Diagn Ther* 2011; **30**: 88–93.
8. Wright D, Syngelaki A, Akolekar R, Poon LC, Nicolaides KH. Competing risks model in screening for pre-eclampsia by maternal characteristics and medical history. *Am J Obstet Gynecol* 2015; **213**: 62.e1–10.
9. Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for pre-eclampsia. *Fetal Diagn Ther* 2012; **32**: 171–178.
10. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for pre-eclampsia by biophysical and biochemical markers. *Fetal Diagn Ther* 2013; **33**: 8–15.
11. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011; **31**: 7–15.
12. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
13. Snijders RJ, Nicolaides KH. Fetal biometry at 14–40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34–48.
14. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX–XIV.
15. Bredaki FE, Sciorio C, Wright A, Wright D, Nicolaides KH. Serum alpha-fetoprotein in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **46**: 34–41.
16. Andrietti S, Silva M, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for pre-eclampsia by maternal factors and biomarkers at 35–37 weeks' gestation. *Ultrasound Obstet Gynecol* 2015. DOI: 10.1002/uog.15812
17. R Development Core Team. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2011; ISBN 3-900051-07-0; http://www.R-project.org/.
18. Therneau T. A package for survival analysis in S. R package version 2.37-7, 2014; https://cran.r-project.org/web/packages/survival/index.html
19. Nicolaides KH. Turning the pyramid of prenatal care. *Fetal Diagn Ther* 2011; **29**: 183–196.
20. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, Forest JC, Giguere Y. Prevention of pre-eclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet Gynecol* 2010; **116**: 402–414.
21. 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.
22. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. Competing risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation. *Am J Obstet Gynecol* 2015; **213**: 62.e1–10.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Regression equations of alpha-fetoprotein multiples of the median in singleton pregnancies that developed pre-eclampsia

Table S2 Standard deviation for $\log_{10}$ serum alpha-fetoprotein multiples of the median in unaffected pregnancies and those that developed pre-eclampsia

Table S3 Modelled and empirical areas under the receiver–operating characteristics curve in screening for pre-eclampsia delivering < 32, < 37 and ≥ 37 weeks' gestation by maternal factors and a combination of maternal factors and serum alpha-fetoprotein at 11–13 and 19–24 weeks' gestation

