Ultrasound Obstet Gynecol 2015; **46**: 42–50
Published online 3 June 2015 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.14870

Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history

D. WRIGHT*, M. SILVA†, S. PAPADOPOULOS†, A. WRIGHT* and K. H. NICOLAIDES†

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

KEYWORDS: first-trimester screening; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; second-trimester screening; third-trimester screening; trisomy 18; trisomy 21

ABSTRACT

Objective *To define the contribution of maternal variables which influence the measured level of maternal serum pregnancy-associated plasma protein-A (PAPP-A) in screening for pregnancy complications.*

Methods *Maternal characteristics and medical history were recorded and serum PAPP-A was measured in women with a singleton pregnancy attending for three routine hospital visits at 11 + 0 to 13 + 6, 19 + 0 to 24 + 6 and 30 + 0 to 34 + 6 weeks' gestation. For pregnancies delivering phenotypically normal live births or stillbirths ≥ 24 weeks' gestation, variables from maternal demographic characteristics and medical history that are important in the prediction of PAPP-A were determined from a linear mixed-effects multiple regression.*

Results *Serum PAPP-A was measured in 94 966 cases in the first trimester, 7785 in the second trimester and 8286 in the third trimester. Significant independent contributions to serum PAPP-A were provided by gestational age, maternal weight, height, racial origin, cigarette smoking, diabetes mellitus, method of conception, previous pregnancy with or without pre-eclampsia (PE) and birth-weight Z-score of the neonate in the previous pregnancy. The effects of some variables were similar and those for others differed in the three different trimesters. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured level of serum PAPP-A and express the values as multiples of the median (MoMs). The model was shown to provide an adequate fit of MoM values for all covariates, both in pregnancies that developed PE and in those without this pregnancy complication.*

Conclusions *A model was fitted to express the measured serum PAPP-A across the three trimesters of pregnancy as MoMs, after adjusting for variables from maternal characteristics and medical history that affect this measurement.* *Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Maternal serum levels of pregnancy-associated plasma protein-A in the first trimester of pregnancy are decreased in pregnancies with fetal trisomies 21, 18 or 13, digynic triploidy, monosomy X[1–6] and those with impaired placentation resulting in pre-eclampsia (PE) and delivery of small-for-gestational-age (SGA) neonates[7–10]. There is also some evidence that serum PAPP-A is reduced in the second trimester in pregnancies that develop PE[11], but the levels are increased in cases with established disease[12–14].

Our approach to risk assessment and screening for aneuploidies and pregnancy complications is to apply Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy[10,15,16]. In normal pregnancy, serum PAPP-A concentration is affected by gestational age and maternal characteristics, including weight, racial origin, cigarette smoking, diabetes mellitus and method of conception[1,2,17]. Therefore, for the effective use of serum PAPP-A measurements in risk assessment, these variables need to be taken into account which can be achieved by standardizing the measured levels into multiples of the normal median (MoM) values.

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: 25 March 2015

The objectives of this study were to first, identify and quantify the effects of variables from maternal characteristics and medical history on serum PAPP-A levels, second, present a model for standardizing serum PAPP-A measurements obtained in all three trimesters of pregnancy into MoM values and third, summarize the distribution of MoM values in pregnancies with normal outcome and those that subsequently develop PE. The main focus of this paper is on the pregnancies with a normal outcome. Further details of the distribution of PAPP-A MoM values in pregnancies with PE, SGA and fetal aneuploidies are the subject of other publications.

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 January 2006 and March 2014. In the first visit, at 11 + 0 to 13 + 6 weeks' gestation, maternal characteristics and medical history were recorded and combined screening for aneuploidies was performed[6]. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and third visit, at 30 + 0 to 34 + 6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size from measurement of fetal head circumference, abdominal circumference and femur length and maternal blood sampling for biochemical testing. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks or the fetal head circumference at 19–24 weeks[18,19].

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 delivering a phenotypically normal live birth or stillbirth ≥ 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/assisted conception requiring the use of ovulation drugs/*in-vitro* fertilization (IVF)), cigarette smoking during pregnancy (yes/no), medical history of chronic hypertension (yes/no), diabetes mellitus (yes/no), systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), family history of PE in the mother of the patient (yes/no) and obstetric history including parity (parous/nulliparous if no previous pregnancies ≥ 24 weeks), previous pregnancy with PE (yes/no), 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 pregnancy-associated plasma protein-A

Of the patients included in the study, maternal serum PAPP-A was measured at each visit by automated biochemical analyzers within 10 min of blood sampling. Samples obtained in the first trimester were analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) and those obtained in the second and third trimester were analyzed by the Cobas e411 system (Roche Diagnostics, 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 chronic hypertension, PE or non-proteinuric gestational hypertension (GH).

The definitions of GH and PE were those of the International Society for the Study of Hypertension in Pregnancy[20]. GH was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women. PE was defined as GH with proteinuria of ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above) developing after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease). The birth-weight *Z*-score for the neonate in the last pregnancy was derived from our reference range of birth weight for gestational age at delivery[21].

Statistical analysis

The effect on serum PAPP-A levels of the following variables from maternal characteristics and medical history were examined: age, weight, height, racial origin, history of chronic hypertension, diabetes mellitus Type 1 or 2, SLE or APS, family history of PE, parous or nulliparous, previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interpregnancy interval, method of

conception, smoking during pregnancy and gestational age at assessment.

The modelled relationship with gestational age in the first trimester from our earlier work[2] has proved to provide a good fit across a range of settings and analyzers and provides a model for standardization of PAPP-A in the first trimester, from 8 weeks' gestation. This is important in some settings for aneuploidy screening. The current dataset is restricted to pregnancies of a gestational age of 11 weeks or more. We therefore applied a penalized regression so that the relationship with gestational age between 8 and 11 weeks was consistent with our previously published model[2]. This enabled us to produce a model that captures the relationship between PAPP-A and gestational age across all three trimesters, from as early as 8 weeks' gestation.

Multiple linear regression models were fitted to $\log_{10}$ values of PAPP-A within each trimester. Continuous variables were coded initially into groups and represented as factors to identify suitable parametric forms. Backward elimination was used to identify potentially important terms in the model by sequentially removing non-significant ($P > 0.05$) variables. Effect sizes were assessed relative to the error standard deviation (SD) and a criterion of 0.1 SD was used to identify terms that had little substantive impact in model predictions. Residual analyses were used to assess the adequacy of the model.

Graphical displays of the relationship between gestational age and PAPP-A levels and the effects of variables from maternal characteristics including maternal age, weight, height and other characteristics on PAPP-A MoM values were produced for the final model. Having identified potential models for each trimester, a parsimonious model was selected to cover the data for the three trimesters combined. This model was fitted using a linear mixed model with random effects to represent between-women random effects. A full analysis of residuals, including an investigation of interactions, was used to check the model fit and, on the basis of this model, refinements were made.

The statistical software package R was used for data analyses[22].

RESULTS

Characteristics of the study population

The maternal characteristics and medical history of women that fulfilled the entry criteria are presented in Table 1. Serum PAPP-A was measured in 94 966 cases in the first trimester, 7785 in the second trimester and 8286 in the third trimester. In the first phase of the study, serum PAPP-A was measured only in the first-trimester visit but this was subsequently extended to the second- and then the third-trimester visits. There were 4092 measurements taken in all three trimesters, 2725 in the first and second trimesters, 449 in the second and third trimesters, 2966 in the first and third trimesters, 85 183 in the first trimester only, 519 in the second trimester only and 779 in the third trimester only.

Variables affecting serum PAPP-A

The variables with substantial effect on serum PAPP-A were gestational age, maternal weight, height, racial origin, cigarette smoking, diabetes mellitus, method of conception, previous pregnancy with or without PE and birth-weight *Z*-score of the neonate in the previous pregnancy. Median levels of serum PAPP-A showed a curvilinear relationship with gestational age; the increase in the first and second trimester reaching a maximum at around 30 weeks (Figure 1a). Serum PAPP-A decreased with maternal weight (Figure 1b) and increased with height (Figure 1c), it was higher in women of Afro-Caribbean, South Asian and East Asian racial origin, than in Caucasian women, and it was decreased in cigarette smokers in comparison to non-smokers (Figure 2). In women who conceived after the use of ovulation drugs, serum PAPP-A in the first trimester was decreased and in the third trimester was increased (Figure 2). In women who conceived by IVF, serum PAPP-A was decreased in the first trimester and was increased in the second and third trimesters (Figure 2). In women with diabetes mellitus, serum PAPP-A was decreased and the greatest decrease was observed in those with Type 2 disease treated by insulin (Figure 3). In parous women with and without previous PE, serum PAPP-A was lower than in nulliparous women and the levels increased with a greater birth-weight *Z*-score of the neonate in the previous pregnancy (Figure 4).

Final model on serum PAPP-A

A linear mixed model, with random effects to represent between-women random effects, was fitted to the subset of variables that contributed substantively to the linear regression models (Table 2). Trimester effects were included, with the first trimester being used as the reference. Effects of maternal weight, racial origin, smoking and diabetes mellitus on the median level of serum PAPP-A were considered constant across the three trimesters. In contrast, the effects of method of conception, birth-weight *Z*-score of the neonate in the last pregnancy and previous pregnancy with or without PE were trimester dependent. The relationship between gestational age and median level of serum PAPP-A was curvilinear with a maximum at around 30 weeks. The regression coefficient of 0.077634 for the effect of the second and third trimesters means that, in these trimesters, levels of PAPP-A, are increased by about 20% after adjusting for all the variables in the model. Such difference could be the consequence of the machine or reagents used for the measurements which were different in the first than in the second and third trimesters and/or other trimester-related effects.

Figure 5 shows MoM diagnostics for racial origin, chronic hypertension, diabetes mellitus, method of

Table 1 Maternal and pregnancy characteristics of women with singleton pregnancy attending for routine visits between January 2006 and March 2014, according to trimester of pregnancy

Characteristic	*11 + 0 to 13 + 6 weeks (n = 94 966)*	*19 + 0 to 24 + 6 weeks (n = 7785)*	*30 + 0 to 34 + 6 weeks (n = 8286)*
Maternal age (years)	31.7 (27.4–35.4)	30.9 (26.4–34.7)	31.0 (26.6–34.7)
Maternal weight (kg)	66.0 (59.0–75.8)	71.2 (63.4–82.0)	77.0 (68.8–87.9)
Maternal height (cm)	164.5 (160.0–169.0)	165.0 (160.0–169.0)	165.0 (160.0–169.0)
GA at examination (weeks)	12.7 (12.3–13.1)	21.9 (21.2–22.1)	32.1 (32.0–32.5)
Racial origin			
Caucasian	69 145 (72.8)	5910 (75.9)	6198 (74.8)
Afro-Caribbean	15 753 (16.6)	1257 (16.1)	1450 (17.5)
South Asian	5046 (5.3)	329 (4.2)	310 (3.7)
East Asian	2575 (2.7)	139 (1.8)	148 (1.8)
Mixed	2447 (2.7)	150 (1.9)	180 (2.2)
Medical history			
Chronic hypertension	1189 (1.3)	105 (1.3)	121 (1.5)
Diabetes mellitus	766 (0.8)	82 (1.1)	82 (1.0)
SLE/APS	195 (0.2)	11 (0.1)	15 (0.2)
Cigarette smoker	8177 (8.6)	799 (10.3)	833 (10.1)
Family history of PE	3901 (4.1)	239 (3.1)	245 (3.0)
Obstetric history			
Nulliparous	46 691 (49.2)	3733 (48.0)	4081 (49.3)
Parous with no previous PE	45 261 (47.7)	3770 (48.4)	3901 (47.1)
Parous with previous PE	3014 (3.2)	282 (3.6)	304 (3.7)
Interpregnancy interval (years)	2.9 (1.9–4.9)	3.1 (2.0–5.0)	3.2 (2.1–5.1)
GA at delivery of previous pregnancy (weeks)	40.0 (39.0–40.0)	40.0 (39.0–40.0)	40.0 (39.0–40.0)
Birth weight of previous pregnancy (g)	3350 (3008–3700)	3398 (3030–3717)	3377 (3008–3700)
Mode of conception			
Spontaneous	91 376 (96.2)	7523 (96.6)	8016 (96.7)
Ovulation induction	1269 (1.3)	79 (1.0)	78 (0.9)
In-vitro fertilization	2321 (2.4)	183 (2.4)	192 (2.3)
Pregnancy outcome			
PE	2149 (2.3)	201 (2.6)	193 (2.3)
No PE	92 817 (97.7)	7584 (97.4)	8093 (97.7)

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; GA, gestational age; PE, pre-eclampsia; SLE, systemic lupus erythematosus.

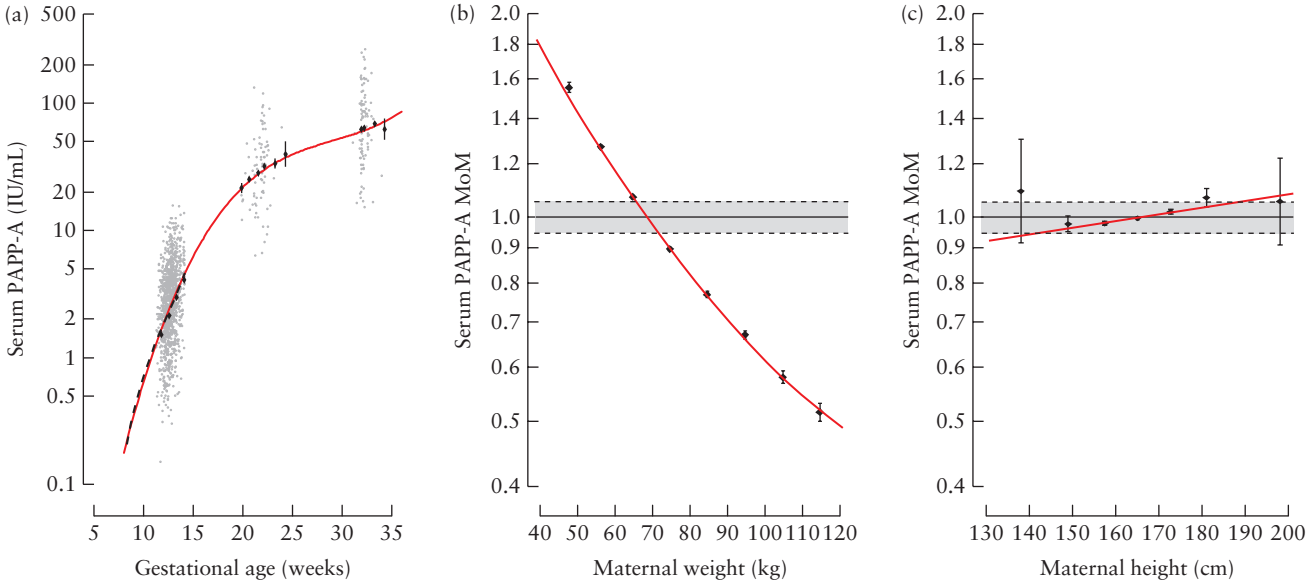

Figure 1 Relationship between median (95% CI) serum pregnancy-associated plasma protein-A (PAPP-A) and gestational age across three trimesters of pregnancy (a) and maternal weight (b) and height (c) plotted on the multiples of the median (MoM) scale after correcting for other factors. The red curve in (a) is the penalized weighted regression model, the black dashed curve represents the relationship between serum PAPP-A and gestational age in our previous model[2], the gray points are a random sample of 5000 points and the black points are the estimated weekly medians with 95% CI. Fitted effects (——), median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) are indicated in (b) and (c).

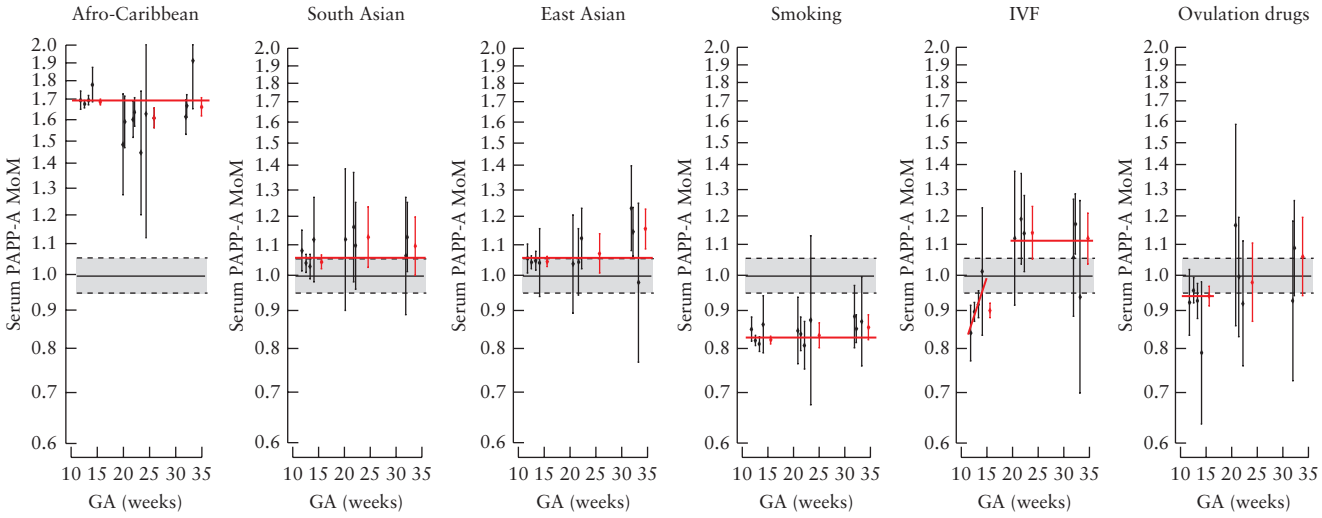

Figure 2 Effect of maternal racial origin, smoking and method of conception on median (95% CI) serum pregnancy-associated plasma protein-A (PAPP-A), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) are indicated. Black vertical lines represent values for individual gestational weeks and red vertical lines represent pooled estimates for each trimester. GA, gestational age; IVF, *in-vitro* fertilization.

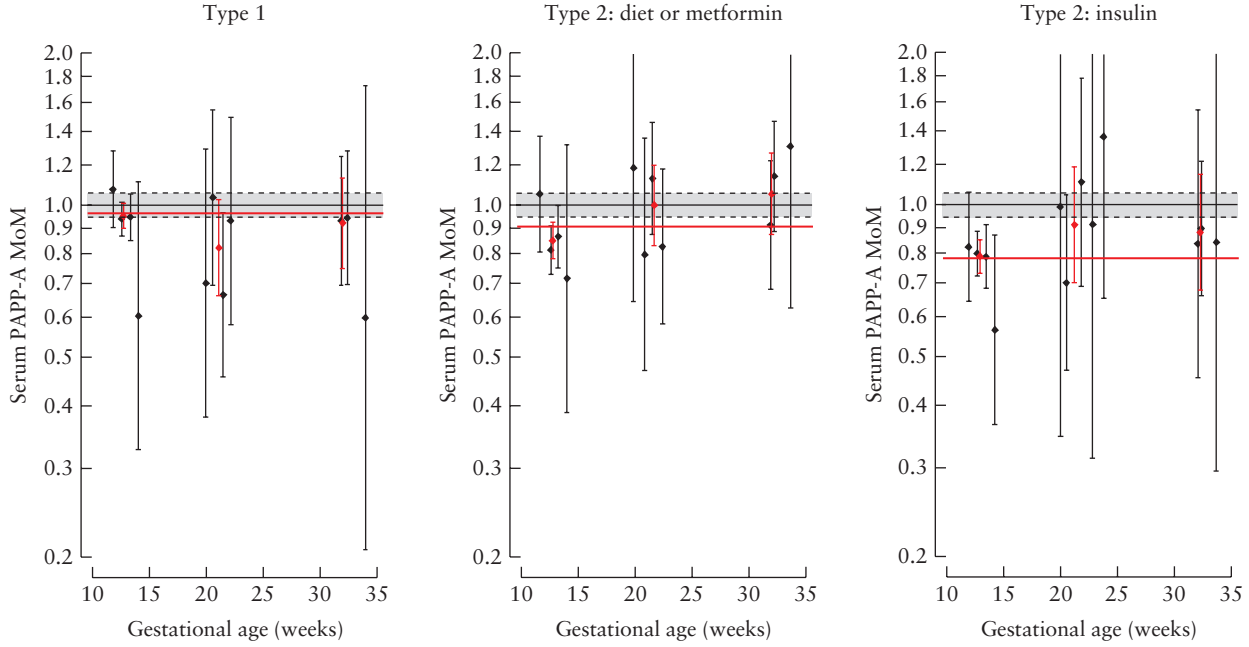

Figure 3 Effect of maternal diabetes mellitus Type 1 and Type 2 according to treatment on median (95% CI) serum pregnancy-associated plasma protein-A (PAPP-A), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) are indicated. Black vertical lines represent values for individual gestational weeks and red vertical lines represent pooled estimates for each trimester.

conception and smoking in pregnancies unaffected by PE and those that developed PE. In unaffected pregnancies, the model provided an adequate fit with median MoM values falling well within 0.1 SDs of 1 MoM. In the PE group, the overall median MoM was 0.8496 (95% CI, 0.8299–0.8698) in the first trimester, 1.0185 (95% CI, 0.997–1.0404) in the second trimester and 1.2363 (95% CI, 1.2053–1.2681) in the third trimester; in the first trimester, the levels were decreased and in the third trimester they were increased. These changes were consistent across the range of variables.

Distributional properties of serum PAPP-A MoM values

Figure 6 shows a Gaussian distribution of serum PAPP-A MoM values. The median and 5th, 10th, 90th and 95th percentiles were 1.00000 (95% CI, 0.99577–1.00423) and 0.38025 (95% CI, 0.37713–0.3834), 0.47799 (95% CI, 0.47468–0.48091), 1.92919 (95% CI, 1.91986–1.93969) and 2.31549 (95% CI, 2.30075–2.33083), respectively. Estimated SD and correlations with 95% CI are given in Tables 3 and 4, respectively. The SDs decreased slightly from first to

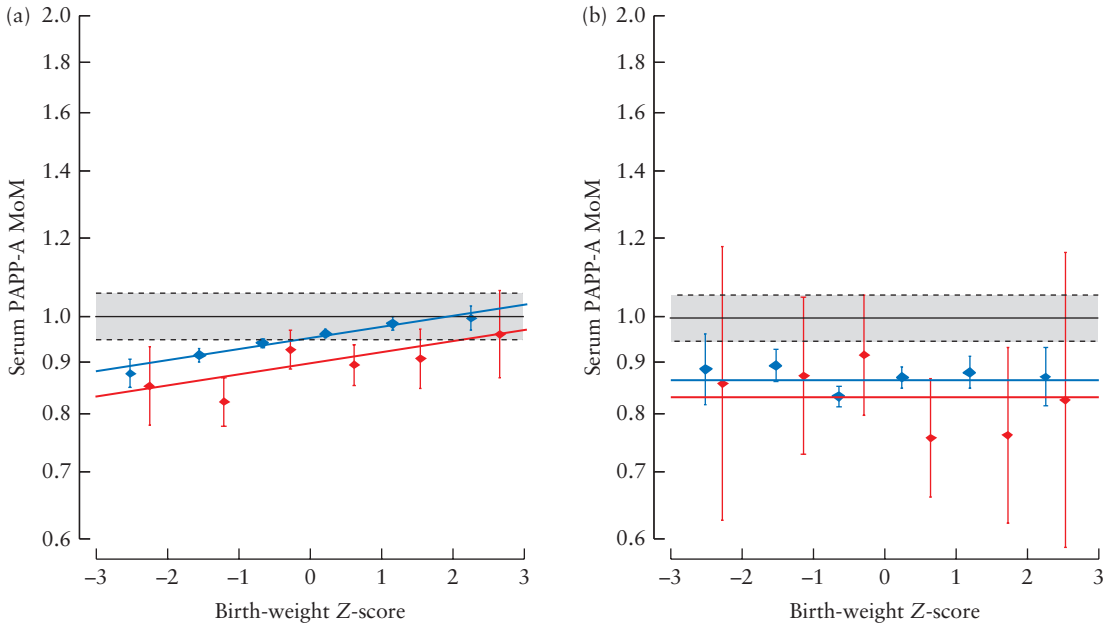

Figure 4 Effect of birth-weight *Z*-score of the neonate in the last pregnancy in parous women with (red vertical lines) and without (blue vertical lines) history of pre-eclampsia (PE) on median (95% CI) serum pregnancy-associated plasma protein-A (PAPP-A) in the first (a) and second and third (b) trimesters, plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects for those with previous PE (——) and for those without previous PE (——), median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) are indicated.

second trimester and increased in the third trimester. The correlations between $\log_{10}$ serum PAPP-A MoM across trimesters were slightly stronger between first and second trimesters and second and third trimesters than between first and third trimesters.

DISCUSSION

Main findings of the study

The findings of this study demonstrate that, in pregnancy, significant independent contributions to the measured maternal serum PAPP-A concentration are provided by maternal characteristics and variables from medical history. Serum PAPP-A has a curvilinear relationship with gestational age, decreases with maternal weight and increases with height, is increased in women of Afro-Caribbean, South Asian and East Asian racial origin and decreased in cigarette smokers and parous women with or without previous PE. In parous women, serum PAPP-A is related to the birth-weight *Z*-score of the neonate in the last pregnancy. In women conceiving after use of ovulation induction drugs or by IVF, serum PAPP-A is reduced in the first trimester but levels increased in the third trimester of IVF pregnancies. In women with diabetes mellitus, serum PAPP-A was decreased, with the greatest decrease observed in Type 2 disease treated by insulin.

Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum PAPP-A concentration and express the values as MoMs. The model was shown to provide an adequate fit of MoM values for all covariates, both in pregnancies that developed PE and in those without this pregnancy complication. In pregnancies affected by PE, serum PAPP-A was reduced in the first trimester but increased in the third trimester.

Strengths and limitations of the study

The strengths of this study are first, prospective examination of a large population of pregnant women attending for routine care in three well-defined gestational-age ranges which are widely used for first-trimester screening for chromosomal defects and second- and third-trimester assessment of fetal anatomy, growth and wellbeing, second, measurement of serum PAPP-A by automated machines that provide reproducible results within 40 min of sampling so that complete assessment and counseling can potentially be undertaken in the same hospital visit and third, application of multiple regression analysis to define the contribution and interrelations of maternal variables that influence the measured serum PAPP-A across the three trimesters of pregnancy.

An alternative to the use of data from three gestational-age ranges would have been a cross-sectional study with inclusion of each gestational week, from the beginning to the end of pregnancy. However, we adopted the pragmatic approach of collecting data from the gestational-age ranges used in routine clinical practice.

Comparison with findings of previous studies

Previous studies, mainly undertaken in the first trimester, have also reported that serum PAPP-A concentration is affected by gestational age and maternal characteristics, including maternal weight, racial origin, cigarette smoking, method of conception and diabetes mellitus[1,2,17].

Table 2 Linear mixed model with random effects for variables from maternal characteristics and history that contribute substantively to the measurement of serum pregnancy-associated plasma protein-A

Term	*Estimate*	*95% CI*	*SE*	P
Intercept	0.0454785310	0.042755 to 0.048202	0.001389376	< 0.0001
Trimester-dependent effects				
First trimester				
Mode of conception				
IVF	−0.0783723230	−0.11153 to −0.045217	0.0169157300	< 0.0001
IVF × (GA (−77))*	0.0027462050	0.00018744 to 0.005305	0.0013054940	0.0177
Ovulation drugs	−0.0264109990	−0.039743 to −0.013079	0.0068021850	< 0.0001
Obstetric history				
Parous: no PE	−0.0219839800	−0.025224 to −0.018744	0.001653096	< 0.0001
Parous: PE	−0.0469429490	−0.056231 to −0.037655	0.004738944	< 0.0001
Parous: birth-weight *Z*-score of last pregnancy	0.0102555100	0.0082999 to 0.012211	0.000997774	< 0.0001
Second and third trimesters				
Constant	0.0776339530	0.071968 to 0.0833	0.0028907500	< 0.0001
IVF conception	0.0457197440	0.02153 to 0.06991	0.0123419080	< 0.0001
Parous: no PE	−0.0588522160	−0.066383 to −0.051322	0.003842188	< 0.0001
Parous: PE	−0.0714002590	−0.091649 to −0.051151	0.010331057	< 0.0001
Trimester-independent effects				
Gestational age				
GA (−77)*	0.0326879700	0.032288 to 0.033088	0.000204098	< 0.0001
$(GA (-77))^2$*	−0.0002324214	−0.00024905 to −0.00021579	0.000008484	< 0.0001
$(GA (-77))^3$*	0.00000061284	0.00000052464 to 0.00000070104	0.000000045	< 0.0001
Maternal weight (−69)†	−0.0078350880	−0.0079782 to −0.007692	0.0000730000	< 0.0001
$(Maternal weight (-69))^2$†	0.0000354000	0.000031343 to 0.000039457	0.0000020700	< 0.0001
Maternal height (−164)‡	0.0010400690	0.00078965 to 0.0012905	0.0001277630	< 0.0001
Racial origin				
Afro-Caribbean	0.2309686060	0.2267 to 0.23524	0.0021769840	< 0.0001
East Asian	0.0231496220	0.013474 to 0.032825	0.0049365610	< 0.0001
South Asian	0.0201601760	0.013051 to 0.02727	0.0036273050	< 0.0001
Mixed	0.0754382560	0.065784 to 0.085092	0.0049254720	< 0.0001
Smoker	−0.0822814970	−0.08772 to −0.076843	0.0027748110	< 0.0001
Medical history				
Type 1 DM	−0.0169311260	−0.04138 to 0.0075177	0.0124738960	0.0873
Type 2 DM on insulin	−0.1079358750	−0.14108 to −0.074794	0.0169090680	< 0.0001
Type 2 DM on diet or metformin	−0.0432446890	−0.076978 to −0.0095118	0.0172106370	0.006

Continuous variables were centered by subtracting the mean from each measured value: *77 from gestational age in days; †69 from maternal weight in kg; ‡164 from maternal height in cm. DM, diabetes mellitus; GA, gestational age; IVF, *in-vitro* fertilization; PE, pre-eclampsia; SE, standard error.

In this series of pregnancies in all three trimesters, we developed a model that incorporates variables with common effects across the trimesters and those with trimester-specific effects. In the context of diabetes mellitus, we found that the levels are reduced and this decrease is most marked in Type 2 disease requiring treatment with insulin. In the model, we included variables such as outcome of the previous pregnancy because standardizing the measured values of biomarkers for any variables included in the prior model is essential for the application of Bayes' theorem in combined screening for pregnancy complications by maternal characteristics and biomarkers. The distribution of serum PAPP-A should be specified conditionally on any terms included in the prior model[23]. It is also important for the interpretation of PAPP-A that these effects are accounted for.

Implications for clinical practice

Measurement of serum PAPP-A may be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome. Effective use of serum PAPP-A in risk assessment and screening necessitates that variables from maternal characteristics and medical history which affect this measurement in normal pregnancy are taken into account. In the clinical implementation of the presented model, it is important that adjustments are made to the various coefficients for the machines or reagents used and other possible local effects.

To show the need for standardizing into MoM values, consider two women with a spontaneous pregnancy at 11 weeks' gestation, a Caucasian and an Afro-Caribbean, both nulliparous, non-smokers, non-diabetics, both of age 35 years, weight 69 kg and height 160 cm and both with serum PAPP-A measurements of 0.9 IU/L. The corresponding MoM values would be 0.81 in the Caucasian woman and 0.48 in the Afro-Caribbean woman, which are on the 33rd and 9th percentiles, respectively. Consequently, for the same measurement of PAPP-A, the risks for both PE and trisomy 21 are higher in the Afro-Caribbean woman than in the Caucasian woman.

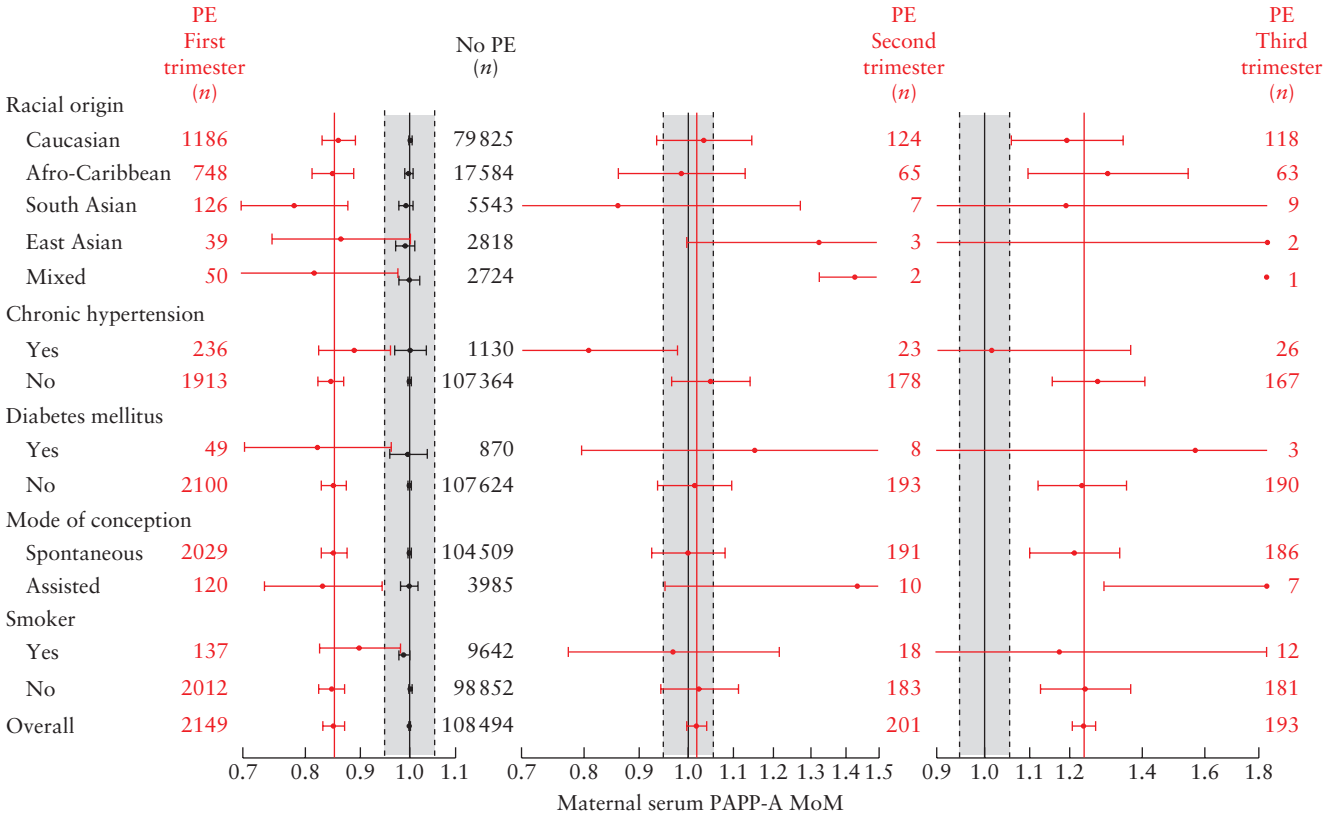

Figure 5 Median serum pregnancy-associated plasma protein-A (PAPP-A) multiples of the median (MoM) (with 95% CI) derived from the model according to racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women who developed pre-eclampsia (PE) (red values) and in those unaffected by PE (black values). Data for PE are presented separately for each trimester. Median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) of women unaffected by PE and median MoM (——) of women with PE for each trimester are indicated.

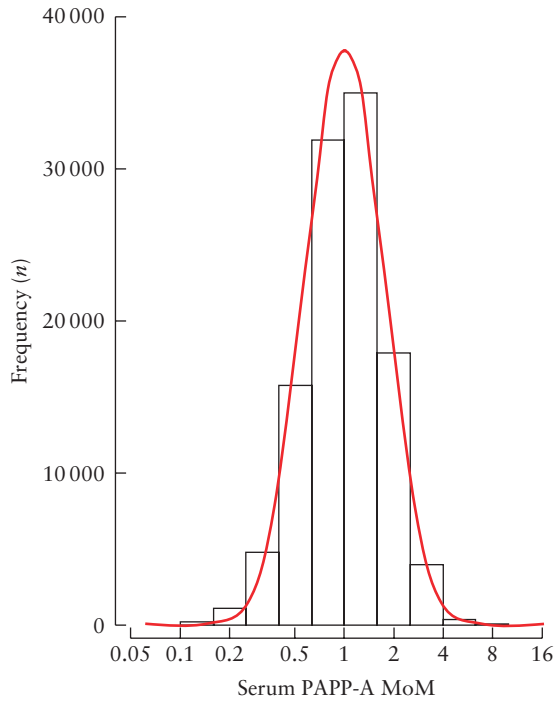

Figure 6 Gaussian distribution of serum pregnancy-associated plasma protein-A (PAPP-A) multiples of the median (MoM) values.

Table 3 Standard deviations (SD) for $\log_{10}$ serum pregnancy-associated plasma protein-A multiples of the median values for each trimester

Trimester	*SD Estimate (95% CI)*
First	0.23740 (0.23644–0.23836)
Second	0.22579 (0.22488–0.22670)
Third	0.27131 (0.27022–0.27241)

Table 4 Correlation of $\log_{10}$ serum pregnancy-associated plasma protein-A multiples of the median (MoM) values in each trimester of pregnancy

Trimester	*Second*	*Third*
First	0.58023 (0.55917–0.60054)	0.38813 (0.36133–0.41429)
Second	1	0.81997 (0.80948–0.82993)
Third	—	1

Values in parentheses are 95% CI.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116) and by the European Union 7th Framework Programme -FP7-HEALTH-2013-INNOVATION-2 (ASPRE Project # 601852). The reagents and equipment for the measurement of serum PAPP-A in the second and third trimesters were provided by Roche Diagnostics Limited.

REFERENCES

1. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2008; **31**: 493–502.
2. Wright D, Spencer K, Kagan K, Tørring N, Petersen OB, Christou A, Kallikas J, Nicolaides KH. First-trimester combined screening for trisomy 21 at 7–14 weeks' gestation. *Ultrasound Obstet Gynecol* 2010; **36**: 404–411.
3. Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free ß-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008; **23**: 1968–1975.
4. Kagan KO, Anderson JM, Anwandter G, Neksasova K, Nicolaides KH. Screening for triploidy by the risk algorithms for trisomies 21, 18 and 13 at 11 weeks to 13 weeks and 6 days of gestation. *Prenat Diagn* 2008; **28**: 1209–1213.
5. Spencer K, Tul N, Nicolaides KH. Maternal serum free beta-hCG and PAPP-A in fetal sex chromosome defects in the first trimester. *Prenat Diagn* 2000; **20**: 390–394.
6. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011; **31**: 7–15.
7. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* 2009; **33**: 23–33.
8. 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.
9. Poon LC, Syngelaki A, Akolekar R, Lai J, Nicolaides KH. Combined screening for pre-eclampsia and small for gestational age at 11–13 weeks. *Fetal Diagn Ther* 2013; **33**: 16–27.
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. Bersinger NA, Ødegård RA. Second- and third-trimester serum levels of placental proteins in pre-eclampsia and small-for-gestational-age pregnancies. *Acta Obstet Gynecol Scand* 2004; **83**: 37–45.
12. Bersinger NA, Smárason AK, Muttukrishna S, Groome NP, Redman CW. Women with pre-eclampsia have increased serum levels of pregnancy-associated plasma protein-A (PAPP-A), inhibin A, activin A and soluble E-selectin. *Hypertens Pregnancy* 2003; **22**: 45–55.
13. Deveci K, Sogut E, Evliyaoglu O, Duras N. Pregnancy-associated plasma protein-A and C-reactive protein levels in preeclamptic and normotensive pregnant women at third trimester. *J Obstet Gynaecol Res* 2009; **35**: 94–98.
14. Atis A, Aydin Y, Basol E, Kaleli S, Turgay F, Goker N. PAPP-A levels of late pregnancy in pre-eclampsia and HELLP syndrome. *Arch Gynecol Obstet* 2012; **285**: 45–49.
15. 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.
16. Nicolaides KH. Turning the pyramid of prenatal care. *Fetal Diagn Ther* 2011; **29**: 183–196.
17. Savvidou MD, Syngelaki A, Muhaisen M, Emelyanenko E, Nicolaides KH. First trimester maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein A in pregnancies complicated by diabetes mellitus. *BJOG* 2012; **119**: 410–416.
18. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *BJOG* 1975; **82**: 702–710.
19. Snijders RJ, Nicolaides KH. Fetal biometry at 14–40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34–48.
20. 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**: 19–24.
21. Poon LCY, Volpe N, Muto B, Syngelaki A, Nicolaides KH. Birthweight with gestation and maternal characteristics in live births and stillbirths. *Fetal Diagn Ther* 2012; **32**: 156–165.
22. 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/.
23. 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; in press

