Association of maternal serum PAPP-A levels, nuchal translucency and crown rump length in first trimester with adverse pregnancy outcomes: Retrospective cohort study

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.5069

B15 2TT

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Running title: First trimester PAPP-A and adverse pregnancy outcome

Word count: 3676

Tables: 3

Figures: 0

References: 29

Funding

There was no specific funding for this study.

Disclosure of interests

Dr Morris is an author of the RCOG Greentop guideline on Investigation and Management of the Small for Gestational Age Fetus.

What's already known on this topic?

Low levels of PAPP-A are associated with small for gestational age and pre-eclampsia.

What does this study add?

NT, CRL and PAPP-A are independent prognostic markers for adverse pregnancy outcome. Further work is required to assess the predictive ability of these factors in prediction models.

Abstract

Objective

Are first trimester serum pregnancy-associated plasma protein-A (PAPP-A), nuchal translucency (NT) and crown rump length (CRL) prognostic factors for adverse pregnancy outcomes?

Method

Retrospective cohort women, singleton pregnancies (UK 2011-2015). Unadjusted and multivariable logistic regression, outcomes: small for gestational age (SGA), pre-eclampsia (PE), pre-term birth (PTB), miscarriage, stillbirth, perinatal mortality and neonatal death (NND).

Results

12,592 pregnancies: 852 (6.8%) PTB, 352 (2.8%) PE, 1824 (14.5%) SGA, 73 (0.6%) miscarriages, 37(0.3%) stillbirths, 73 perinatal deaths (0.6%) and 38 (0.30%) NND. Multivariable analysis: lower odds of SGA [adjusted odds ratio (aOR) 0.88 (95% CI 0.85,0.91)], PTB [0.92 (95%CI 0.88,0.97)], PE [0.91 (95% CI 0.85,0.97)] and stillbirth [0.71 (95% CI 0.52,0.98)] as PAPP-A increases. Lower odds of SGA [aOR 0.79 (95% CI 0.70,0.89)] but higher odds of miscarriage [aOR 1.75 95% CI (1.12,2.72)] as NT increases, and lower odds of stillbirth as CRL increases [aOR 0.94 95% CI (0.89,0.99)]. Multivariable analysis of

three factors together demonstrated strong associations: a) PAPP-A, NT, CRL and SGA, b) PAPP-A and PTB, c) PAPP-A, CRL and PE, d) NT and miscarriage.

Conclusions

PAPP-A, NT and CRL independent prognostic factor for adverse pregnancy outcomes, especially PAPP-A and SGA with lower PAPP-A associated with increased risk.

Keywords: PAPP-A, small for gestational age, stillbirth, pre-eclampsia, pre-term delivery.

Introduction

Adverse pregnancy outcomes have a psychological impact for the family as well as an increased cost of healthcare. Methods of prediction would allow obstetricians to provide increased obstetric surveillance, focusing optimum management and possibly improving the outcome of the pregnancy.

Pregnancy associated plasma protein A (PAPP-A) is a placental glycoprotein produced by syncytial trophoblast of the placenta, which cleaves insulin-like growth factor binding protein 4 (IGFBP4) and is a positive regulator of insulin-like growth factors (IGFs) [1], potentially influencing fetal growth and wellbeing.

Studies have tested the hypothesis that low maternal serum levels of PAPP-A in the first trimester are prognostic factors for adverse pregnancy outcomes associated with poor placental function [2 3, 4 5 6]. International Guidelines on "*The Investigation and Management of the Small for Gestational Fetus*" have recommended that pregnant women with a serum PAPP-A <0.4MoM (5th centile) in the first trimester receive increased ultrasound surveillance for fetal growth disorders[7]. This recommendation was based on a previous

systematic review by our group in 2008 assessing Down's syndrome markers to predict pre-eclampsia and SGA[8]. This review included only 16 studies, did not assess all outcomes and did not distinguish between prognosis and prediction. However, contradictory results have been observed in publications [6, 9] and few studies have investigated the association of first trimester fetal biometry [nuchal translucency (NT) and crown rump length (CRL)] with adverse outcomes and their relationship with PAPP-A [3, 10]. Resolution of this is required, because genuine prognostic factors in this field have many potential uses. As outlined by the PROGRESS series [11-14], they may guide clinical decisions and monitoring strategies, inform the design and analysis of new trials, and improve models for individualised risk prediction. Factors that add additional (independent) prognostic information are difficult to find, but are needed to improve the discrimination performance of a 'prognostic model'[13] that produces absolute risk predictions for women based on a set of individual characteristics[11]. Our objective was to undertake a large cohort study to determine whether serum PAPP-A, NT and CRL in the first trimester are independent prognostic factors for the risk of subsequent adverse pregnancy outcomes.

Methods

Data collection

In a retrospective cohort study, data were collected from patients booked from 1st August 2011 (commencement of electronic maternity record) to 31st March 2015 at the Birmingham Women's Foundation Trust (BWNFT), a secondary and tertiary care NHS hospital in West Midlands, UK.

All pregnant women who accepted first trimester aneuploidy screening and delivered in BWNFT were included in the study. First trimester aneuploidy screening is offered to all pregnant women between 11+2 to 14+1 weeks of gestation (crown–rump length (CRL) measures from 45 mm to 84 mm) as part of the National Downs Syndrome Screening Programme[15, 16]. This involves measuring maternal serum levels of PAPP-A and free beta human chorionic gonadotrophin (fβHCG), along with the NT, and the pregnancy is dated on the basis of CRL. All first trimester scans and measurements performed at BWNFT are performed by accredited sonographers according to National NEQAS guidelines[17]. First day of the last menstrual period was obtained from the referring letter from the community midwife or hospital. This date was confirmed with the mother at the ultrasound visit and additional information on the regularity and cycle duration was obtained.

Analysis for PAPP-A was performed on the Auto-Delfia immunoassay platform (Perkin Elmer Ltd, Seer Green, UK). For the purpose of Down's syndrome screening, PAPP-A values are converted to multiples of the median for gestation. In this study, to prevent any loss of data[18] and to remove the need for considering an absolute threshold, PAPP-A values were considered as a continuous variable using the absolute value (mU/L). To aid the presentation and interpretation of results, PAPP-A values were rescaled by dividing the values by 1000, therefore the results relate to PAPP-A values in U/L.

Multiple hospital-based, secure and confidential computerized databases were used to extract the information for the study and governance from the hospital-based IT and Caldecott Guardian were prospectively obtained. Maternal and pregnancy outcome data were collected from K2 database, Neonatal data were collected from Badgernet database, and biochemistry data were collected from a biochemistry database. The collected data

consisted of maternal characteristics and demographics; maternal medical, antepartum, peripartum and pregnancy outcome data along with first trimester serum PAPP-A levels, NT and CRL. Multiple gestations, pregnancies with donor eggs, missing outcome and known fetal aneuploidy were excluded. Cases of aneuploidy were cross referenced with the West Midlands Regional Genetics Laboratory database.

Definitions of maternal and obstetric characteristics

Preterm birth (PTB) was defined as live delivery prior to 37 weeks, both spontaneous and iatrogenic. Pre-eclampsia (PE) was defined according to the International Society for the study of Hypertension in Pregnancy (ISSHP) definition as *de-novo* hypertension at or after 20 weeks gestation (at least 2 readings of Blood Pressure >140 mmHg systolic or >90 mmHg diastolic) with proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L ['2 + '] on dipstick testing)[19]. Small for gestational age (SGA) was defined as birthweight below the 10th percentile of the customised growth chart[20]. Miscarriage was defined as fetal demise before 24 weeks of gestation. Stillbirth was defined as intrauterine death after 24 completed weeks of pregnancy. Perinatal death was defined as fetal or neonatal death between 24 weeks of gestation and 7 days after birth. Neonatal death was defined as death between birth and 28 days.

Data Analysis

Mother and fetus demographics and clinical features

Distributions of demographic characteristics and known prognostic factors were summarised. The existing prognostic factors were those deemed to be important by the clinical team a prior and defined as: maternal age at test, gestational age at test, parity,

body mass index (BMI), deprivation category (Index of Multiple Deprivation 2010 – IMD [16], calculated using National Perinatal Epidemiology Unit calculator), ethnicity, assisted conception (IVF), smoking status (non-smoker, stopped at booking or on-going smoker), pre-pregnancy insulin-dependent diabetes mellitus, and gender of the baby. Mean and standard deviation (SD), or median and interquartile range (IQR), is reported for continuous variables, according to whether the variables were normally distributed. The number and percentage are reported for categorical variables.

Analysis of prognostic association with outcomes

Univariable logistic regression analysis was used to estimate the unadjusted odds ratio (OR) for each potential prognostic factor (PAPP-A/NT/CRL) separately. The odds ratios indicate how much the odds of the outcome are increased for each 1-unit increase in the factor.

Again for each of the three factors separately, multivariable logistic regression analyses were fitted to examine the odds ratio adjusted for the known (or likely) existing prognostic factors of maternal age (years), parity, BMI, smoking status, IVF, ethnicity, deprivation category and gestational diabetes. This provided the adjusted odds ratio for a 1-unit increase in each factor, to reveal their independent prognostic value over and above other factors.

Then, for each outcome, the three factors were analysed in combination in one multivariable logistic regression model, whilst adjusting for the other factors detailed above, to explore whether the prognostic value of each factor is the same after adjusting for the

other two potential prognostic factors. The linearity assumption of all continuous variables was assumed for these multivariable models.

Therefore, finally, the fully adjusted models with all three potential factors in combination were fitted again; however, additionally, the linearity assumption of the prognostic effects for the three factors of interest was assessed, and alternative functional forms were considered if the assumption was violated. Else, a linear relationship was specified for all three prognostic factors of interest. The functional form was chosen using fractional polynomials, where all possible fractional polynomials up to the second degree were considered based on their statistical significance[21]. A linear relationship was specified for the other continuous covariates (maternal age and BMI).

In all multivariable models described above, no model selection process was used to determine which factors were included in each model, since all variables were pre-specified.

Handling of missing data

The percentages of missing values for each covariate and outcome were calculated. Missing data was imputed with multiple imputation with chained equations with 35 imputed datasets equal to the percentage of patients with missing data [22]. For non-normally distributed variables, predictive mean matching was used to impute the missing data [23]. The imputation model contained all complete outcomes and covariates that were included in the multivariable analyses. Rubin's rules were used to combine the parameter estimates and standard errors into a single inference [24]. A complete-case analysis was also conducted as a sensitivity analysis.

Estimates of prognostic effects are reported as odds ratios (OR) with 95% confidence intervals (CI) and p-values. Analyses were performed using Stata version 14 [25] and all regression models were fitted using maximum likelihood estimation.

Results

Between August 2011 and March 2015, first-trimester combined screening was performed in 12,837 pregnant women. Ten pregnancies conceived with donor eggs were excluded. Three pregnancies with an unclear pregnancy outcome showing neonatal death post 28 days were excluded since this outcome would not be routinely recorded in the databases. After excluding 232 multiple pregnancies, the final study cohort was of 12,592 singleton pregnancies.

As depicted in Table 1, the mean maternal age and median BMI were 30.6 (SD 5.6) and 25.1 (IQR 22.4 to 28.8), respectively. The majority of the women were White British European (62.6%) followed by South Asian (19.9%). Mean gestational age at first trimester ultrasound was 88.2 days (SD 4.3). About 2% (n=250) of pregnancies were the result of assisted conception. Smoking status was known for all women and 12.4% were on-going smokers in the pregnancy, 84.3% non-smokers and 3.3% had given up at the time of first trimester booking appointment. Nearly half (43%, n=5495) patients lived in the most deprived areas (deprivation score ≥ 34.18) and 3.6% (n=455) patients lived in the least deprived areas (deprivation score ≤ 8.49). Gender distribution was almost equal in the fetuses (48.6% male and 51.1% female).

BMI was missing in 4466 (35.5%) records, parity was missing in 567 (4.5%) and deprivation score was missing in 175 (1.4%) records. There were missing data for 152 (1.2%) fetal weight and four (0.03 %) neonatal outcomes. Multiple imputation was performed for missing BMI (height, weight), parity and deprivation score values. Although some variables were normally distributed, predictive mean matching was used for all variables because imputation with chained regression analysis imputed unrealistic values for weight and height.

Table 2 displays the number of events for each outcome for the cohort, and the prognostic association of PAPP-A, NT and CRL with these outcomes in the unadjusted analyses. Of 12,592 women, 852 had pre term birth (6.8%), 352 patients had pre-eclampsia (2.8%) and 1824 babies were SGA (14.5%). There were 73 pregnancies that ended in miscarriage (0.6%) and 37 stillbirths (0.3%). There were 38 neonatal deaths (0.31%) of which 36 were early and thus giving a total of 73 perinatal deaths (0.6%).

PAPP-A Results

In the unadjusted analysis (Table 2), a one unit increase in the concentration of PAPP-A (U/L) was estimated to lower the odds of an SGA neonate by 13%, which was highly significant [OR 0.87 (95% CI 0.85, 0.90), p<0.0001]. Similar conclusions can be drawn for the association between PAPP-A and pre-term birth [OR 0.93 (95%CI 0.90, 0.97), p<0.0001] In addition, PAPP-A and pre-eclampsia demonstrated a similar relationship [OR 0.92 (95% CI 0.86, 0.97), p=0.004]. The results for stillbirth were in the same direction and quantitatively similar, although the CI was slightly wider and the p-value was just above the 5% [OR 0.81

(95% CI 0.66, 1.0), p=0.052]. There was no evidence of a strong association between PAPP-A and miscarriage, perinatal death or neonatal death.

After adjusting for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes in the multivariable analysis there was evidence of strong independent prognostic associations between: PAPP-A and SGA [OR 0.88 (95%CI 0.85, 0.91), p<0.0001] (Table 3; full model parameter estimates for all adjusted models are shown in the Supporting Information Tables S1-S7); PAPP-A and PTB [OR 0.92 (95% CI 0.88, 0.97), p=<0.0001]; and PAPP-A and PE [OR 0.91 (95% CI 0.85, 0.97), p=0.003]. There was also evidence of a lower odds of a stillbirth as PAPP-A increases [OR 0.71 (95% CI 0.52, 0.98), p=0.038]. The association of PAPP-A with the outcomes of miscarriage (Table S4), perinatal death (Table S6) and neonatal death (Table S7) remained non-significant in the multivariable analyses.

NT Results

In the unadjusted analyses (Table 2), for higher values of NT there was a strong association with an increased odds of miscarriage [OR 1.94 (95% CI 1.54, 2.45), p<0.0001], and a significant decreased odds of SGA [OR 0.81 (95% CI 0.72, 0.91), p<0.0001]. There was also some evidence that higher values are associated with an increased risk of PTB [OR 1.15 (95% CI 1.00, 1.32), p=0.053] though the CI overlapped one.

After multivariable analysis, there was independent prognostic value of NT for SGA [OR 0.79 (95% CI 0.70, 0.89), p<0.0001] (Table 3 and Table S8), and for miscarriage [OR 1.75 (95% CI 1.12, 2.72), p=0.013] (Table S11). There was no significant relationship between NT and PTB, PE, stillbirth, perinatal or neonatal death in the unadjusted or adjusted analyses (Table S9, S10, S12, S13, S14).

CRL Results

For CRL in the unadjusted analysis there was no significant association with any of the outcomes (Table 2). There was a borderline statistical significance for SGA which remained after adjustment [OR 0.99 (95% CI 0.99, 1.00, p=0.057)] (Table 3), but the magnitude of the OR was close to one. After adjustment for other known predictors and potential confounders, there was evidence of a strong association between CRL and stillbirth [OR 0.94 (95% CI 0.89, 0.99), p=0.027], thus between 1% and 11% lower odds of stillbirth for a one unit increase in CRL. The adjusted analyses for PTB, PE, miscarriage, perinatal death and neonatal death demonstrated no significant association with CRL (Table S15 - S21).

PAPP-A, NT and CRL in combination

Assuming linear functions for all continuous variables, the three potential prognostic factors were then considered in combination with adjustment for confounders and known prognostic factors as discussed (Tables S22-28 in the Supporting Information). For SGA, this analysis demonstrated strong associations with PAPP-A [OR 0.87 (95% CI 0.84, 0.90), p<0.0001); NT [OR 0.80 (95% CI 0.70, 0.91); p=0.001] and CRL [OR 1.01 (95% CI 1.00, 1.03); p=0.004).

For preterm birth (Table S23), only PAPP-A was significantly associated with reduced odds [OR 0.92 (95% CI 0.87, 0.96), p<0.0001], as seen when the factors were considered individually. For pre-eclampsia, PAPP-A was still significantly associated [OR 0.88 (95% CI 0.82, 0.94); p=<0.0001] and now there was evidence of increased odds of pre-eclampsia as CRL increases [OR 1.02 (95% CI 1.01, 1.04); p=0.004] (Table S24).

There remained a statistically significantly increased odds of miscarriage as NT increases [OR 1.67 (95% CI 1.01, 2.76), p=0.047], and no evidence of associations between miscarriage and PAPP-A or CRL (Table S25). There was not any strong evidence (e.g. based on statistical significance at the 5% level) of any associations between stillbirth and any of PAPP-A, NT or CRL, unlike the individual models (Table S26). There was no evidence of associations between any of the three factors of interest and perinatal or neonatal death (Table S27-28).

After checking the linearity assumption of the three prognostic factors, all the previously identified prognostic associations remained the same. However, the log transformation was statistically the best fitting functional form of PAPP-A for SGA, and $1/\sqrt{\text{PAPP-A}}$ was statistically the best fitting functional form of PAPP-A for PTB. For PAPP-A for all other outcomes and for CRL and NT for all outcomes, the best fitting functional form was the linear function (See Tables S29-S31).

All the findings remained the same in the complete case analysis.

Discussion

Main Findings

This large cohort study provides strong evidence that lower values of PAPP-A are associated with an increased odds of SGA, stillbirth, PE and PTB. As NT increases there is evidence of a lower odds of SGA but higher odds of miscarriage. As CRL decreases there is evidence of higher odds of stillbirth. Neonatal and perinatal deaths were not associated with any of the prognostic factors measured in the first trimester. When considered in combination there is a statistically significant association of PAPP-A, NT and CRL with SGA; preterm birth with PAPP-A, pre-eclampsia with PAPP-A and CRL, and miscarriage with NT. In the combined model stillbirth is no longer associated with any of the factors.

Strengths and Limitations:

Our study has several strengths. This is a large cohort study looking at multiple pregnancy outcomes providing reproducible statistical results. The UK is a country where high quality and homogenous universal health care is provided to its residents free of charge irrespective of socioeconomic and other statuses. This made our cohort a true representative of the general population avoiding bias due to skewed demographics. Certain factors that are known to affect pregnancy outcome, such as ethnicity, parity, maternal age and BMI, socio economic deprivation, smoking status and pre-pregnancy insulin-dependent diabetes mellitus, have been adjusted for in our analysis. We have made an effort to look for lesser researched possible associations such as miscarriage and neonatal/perinatal death. Despite these strengths, our study is not without limitations. The data for potential confounding factors (existing prognostic factors) was limited to that which is routinely

collected in our electronic maternity record as this was a retrospective study. Similarly, the outcomes are limited to those routinely recorded and thus it was not possible to look at different thresholds that might confer a more severe outcome e.g. birth weight <5th or 3rd customised centile or severe pre-eclampsia. However, as our study was designed to investigate the independent prognostic ability of first trimester factors with adverse outcome it can be argued that when a factor is prognostic this relationship will be stronger for higher thresholds for the same outcome. Although the databases used were not designed for this particular study they are populated by qualified health professionals and data was obtained from multiple sources to allow cross-referencing and checking of outcomes. The biochemistry data is part of the National Screening Programme and thus subject to the relevant quality assurance (UK National external quality assurance scheme (UKNEQAS), Edinburgh Royal Infirmary, UK and Downs syndrome screening quality assurance and support service (DQASS), University of Plymouth UK). Our sample was determined by the number of patients available with an electronic record and outcome data and thus not determined by a sample size calculation. Most confidence intervals were quite narrow, but we recognise that non-significant findings do not necessarily mean that no prognostic association exists, and may simply reflect a low power to detect genuine associations. Nevertheless, many confidence intervals were relatively narrow and the prognostic associations identified were often strongly significant [26].

The clinical utilisation of CRL as an individual prognostic factor (i.e. outside of a model using it as a continuous factor) is less clear as standard care in the UK is for a single first trimester ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk. Previous studies have assessed the prognostic value of difference in expected to observed CRL based on the

last menstrual period[10], observed versus expected change in CRL in the first trimester[27] and CRL as a continuous factor in multivariable analysis[28]. The use of CRL to date a pregnancy assumes that there is no growth variation within the first trimester nor association with factors such as fetal sex, maternal age or ethnicity[29]. A study from the Netherlands demonstrated that CRL in the first trimester was associated with an increased risk of adverse birth outcomes and postnatal growth acceleration[28]. As standard care in the UK is only to offer one first trimester ultrasound, thus it is not possible to assess CRL change and the use of the CRL to date the pregnancy does not allow the assessment of observed to expected CRL. We thus wished to assess whether CRL, measured between the 11+2-14+1 week window, assessed as a continuous variable had a relationship with adverse pregnancy outcome i.e. in particular assessing extremes of the continuum.

Interpretation

The aim was to provide more evidence toward establishing if the prognostic factors of interest can be used to further inform the management of potential adverse outcomes, for example by increased surveillance for pregnant women at greater risk. The results showed evidence of associations between the potential prognostic factors and several outcomes, and the associations remained largely the same when the factors were considered in combination. Future work is now important to establish whether the findings from all prognostic factor studies are consistent by synthesising the evidence.

The evidence from this study supports the need for women with pregnancies with a low PAPP-A and increased NT being under Consultant led care and the recommendation within

the RCOG guidelines[7] for increased surveillance for SGA in pregnancies with a low PAPP-A and supports this being extended to pregnancies in the first trimester with an increased NT. At present until a model is developed that can incorporate these factors as continuous variables it would be appropriate to use accepted thresholds of <5th centile for PAPP-A and >99th centile for NT. Due to the association with low PAPP-A and PTB and PE these pregnancies should be assessed comprehensively for other risk factors for PTB and consideration given to the commencement of aspirin prior to 16 weeks. Independent prognostic factors have a broad array of potential uses in both clinical practice and health research[12]. For instance, they help to define disease at diagnosis; they may be modifiable in order for interventions to improve outcomes; they aid the design and analysis of trials; they are confounders to consider in observational studies and unbalanced trials; and they are the building blocks of prognostic models [12]. Prognostic factor research is therefore important to discover and evaluate such factors.

We emphasise that our multivariable models were fitted to examine if there is evidence of an independent association between the potential prognostic factors of interest and the maternal and fetal outcomes after adjustment for known prognostic factors. Our objective was to assess the prognostic factors themselves and not on an overall prognostic model for individual risk prediction. This is especially important since there was no external data to validate such a model [13]. Future work could use new datasets to develop individual risk prediction models in order to tailor treatment choices to the individual and to look at different thresholds for the outcomes e.g. severe PET. Such models should build on the findings of this study, in terms of the prognostic factors that were identified as important.

Conclusion

When three first trimester potential prognostic factors are considered in combination there remains strong evidence of associations between: a) PAPP-A, NT, CRL and SGA, b) PAPP-A only with PTB, c) PAPP-A and CRL for PE, d) NT and miscarriage. Further work is required to assess the predictive ability of these factors in prediction models for adverse pregnancy outcome.

Acknowledgements

We acknowledge the help of the IT department at Birmingham Women's Hospital NHS Foundation Trust with obtaining the maternity outcome data.

Contribution to authorship

RKM, MDK, AB designed the study, collected, analysed and interpreted the data. IM was responsible for collecting the biochemistry data and in the interpretation of other results. RDR and DB designed the analysis, performed the analysis and interpreted the data. All authors were involved in the writing of the manuscript.

Ethics approval

The study had ethical approval from the research ethics committee (REC reference 14/NW/1394) and Confidentiality Advisory Group (CAG reference 14/CAG/1033)

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Table 1: Mother and fetus demographics and clinical features at the test.

		Summary (N=12,592)
Mother's age (years)		30.6 (5.6)
Gestational age at test (days)		88.2 (4.3)
Parity (number)		1 [0,1]
BMI		25.1 [22.4, 28.8]
Deprivation score*, n (%)	≤8.49	455 (3.6)
	8.5 – 13.79	846 (6.7)
	13.8 – 21.35	2629 (20.9)
	21.36 – 34.17	2992 (23.8)
	≥34.18	5495 (43.6)
	Missing	175 (1.4)
Ethnicity, n (%)	African-Caribbean	944 (7.5)
	South-Asian	2502 (19.9)
	Oriental	358 (2.8)
	Other mixed	898 (7.1)
	White	7879 (62.6)
	Not stated	11 (0.1)
Assisted conception, n (%)		250 (2.0)
Smoking status, n (%)	Smoker	1569 (12.4)
	Non-smoker	10611 (84.3)
	Stopped during pregnancy	412 (3.3)
Pre-pregnancy insulin-dependent Diabetes mellitus, n (%)		36 (0.3)
Gender of baby, n (%)	Male	6118 (48.6)
	Female	6435 (51.1)
	Missing	39 (0.3)

Mean (standard deviation) or median [interquartile range] for continuous variables and n (%) for categorical variables; * Deprivation score calculated using the National Perinatal Epidemiology Unit Index of Multiple Deprivation (NPEU IMD) calculator. BMI: body mass index

Table 2: Number of events for each outcome in singleton pregnancies (N=12,592) and unadjusted association with PAPP-A, nuchal translucency and crown rump length and each outcome (univariable logistic regression).

Outcome	Number of events (%)	PAPP-A (U/L)	Nuchal translucency (mm)	Crown rump length (mm)
		Odds ratio (95% CI), p-value	Odds ratio (95% CI), p-value	Odds ratio (95% CI), p-value
SGA (<10th customised centile)	1824 (14.5)	0.87 (0.85 to 0.90), <0.0001	0.81 (0.72 to 0.91), <0.0001	0.99 (0.99 to 1.00), 0.065
Pre-term birth (<37 weeks)	852 (6.77)	0.93 (0.90 to 0.97), <0.0001	1.15 (1.00 to 1.32), 0.053	1.00 (0.99 to 1.01), 0.730
Pre-eclampsia	352 (2.80)	0.92 (0.86 to 0.97), 0.004	0.90 (0.70 to 1.14), 0.378	1.01 (1.00 to 1.02), 0.123
Miscarriage (death prior to birth <24 weeks gestation)	73 (0.58)	0.97 (0.86 to 1.09), 0.598	1.94 (1.54 to 2.45), <0.0001	1.02 (0.99 to 1.05), 0.147
Stillbirth (death prior to birth >24 weeks gestation)	37 (0.29)	0.81 (0.66 to 1.00), 0.052	0.77 (0.35 to 1.68), 0.503	0.97 (0.93 to 1.01), 0.150
Perinatal death* (Death between 24 weeks gestation and 7 days after birth)	73 (0.58)	0.93 (0.82 to 1.05), 0.245	0.70 (0.39 to 1.23), 0.213	0.98 (0.95 to 1.01), 0.160
Neonatal death$^{\$}$ (Death between birth and 28 days)	38 (0.31)	1.03 (0.89 to 1.20), 0.686	0.57 (0.25 to 1.29), 0.176	0.98 (0.95 to 1.02), 0.432

* Perinatal death includes stillbirths; $^{\$}$ neonatal death includes babies that die between birth and 7 days after birth that are captured within the perinatal death category. SGA small for gestational age (birth weight<10th customised centile. Odds ratios indicate the effect of 1 –unit increase in the factor on the odds of the outcome.

Table 3: Adjusted odds ratio estimates for the association between each adverse outcome and PAPP-A, nuchal translucency and crown rump length.

Outcome	PAPP-A (U/L) OR (95% CI), p-value	NT (mm) OR (95% CI), p-value	CRL (mm) OR (95% CI), p-value
SGA (<10th customised centile)	0.88 (0.85 to 0.91), <0.0001	0.79 (0.70 to 0.89), <0.0001	0.99 (0.99 to 1.00), 0.057
Preterm birth (<37 weeks)	0.92 (0.88 to 0.97), <0.0001	1.09 (0.93 to 1.27), 0.313	1.00 (0.99 to 1.01), 0.624
Pre-eclampsia toxaemia	0.91 (0.85 to 0.97), 0.003	0.87 (0.67 to 1.12), 0.287	1.01 (0.99 to 1.02), 0.325
Miscarriage (death <24 weeks gestation)	1.01 (0.84 to 1.21), 0.929	1.75 (1.12 to 2.72), 0.013	1.03 (0.99 to 1.07), 0.129
Stillbirth (death >24 weeks gestation)	0.71 (0.52 to 0.98), 0.038	0.69 (0.25 to 1.93), 0.485	0.94 (0.89 to 0.99), 0.027
Perinatal death (death between 24 weeks gestation and 7 days after birth)	0.88 (0.73 to 1.06), 0.173	0.89 (0.43 to 1.83), 0.746	0.97 (0.93 to 1.01), 0.110
Neonatal death (death between birth and 28 days)	1.04 (0.85 to 1.29), 0.687	1.07 (0.39 to 2.93), 0.896	1.00 (0.95 to 1.05), 0.919

OR odds ratio; CI confidence interval; NT nuchal translucency; CRL crown rump length; SGA small for gestational age; all odds ratio estimates for PAPP-A, NT and CRL from separate multivariable models, adjusted for maternal age, BMI, parity, ethnicity, deprivation score, smoking status, IVF, and gestational diabetes.