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The association between first trimester AFP to PAPP-A ratio and placentally-related adverse pregnancy outcome

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ABSTRACT

Introduction: Low maternal serum levels of pregnancy-associated plasma protein A (PAPP-A) measured in the first trimester and high levels of alpha fetoprotein (AFP) measured in the second trimester have been associated with adverse pregnancy outcomes reflective of placental insufficiency, and there is a synergistic relationship between the two. We investigated the utility as a screening test of a simple ratio of maternal serum AFP to PAPP-A (AFP:PAPP-A) measured in the first trimester.

Methods: We studied 4057 nulliparous women with a singleton pregnancy from the Pregnancy Outcome Prediction (POP) study. We studied the predictive ability for adverse outcome of the AFP:PAPP-A ratio measured in the first trimester with and without correction for maternal weight and gestational age at measurement. We compared the AFP:PAPP-A ratio with corrected AFP and PAPP-A on their own and in combination.

Results: An AFP:PAPP-A ratio > 10 was associated with placentally-related adverse outcomes, including fetal growth restriction (risk ratio (RR) 3.74, 95% confidence interval (CI) 2.30–6.09), severe preeclampsia (RR 2.12, 95% CI 1.39–3.25) and stillbirth (RR 5.05, 95% CI 1.48–17.18). The ratio performed favorably in predicting adverse pregnancy outcomes when compared with corrected measurements of either AFP or PAPP-A, and was equivalent to a model combining the two. Its predictive ability was not affected by correction for maternal weight or gestational age at measurement.

Discussion: An elevated maternal AFP:PAPP-A ratio in the first trimester is associated with placentally-related adverse outcomes in a cohort of unselected nulliparous women.

1. Introduction

Maternal serum biomarkers measured as part of aneuploidy screening have been shown to be associated with adverse pregnancy outcomes reflective of placental insufficiency [1–4]. Pregnancy-associated plasma protein A (PAPP-A) is a protease which targets insulin-like growth factor binding proteins (IGFPBs) and low first trimester levels have been robustly associated with small for gestational age (SGA), preeclampsia (PE) and spontaneous preterm birth (PTB) [2,5–10]. Alpha fetoprotein (AFP) is a protein of fetal origin, and raised levels measured in the second trimester have been similarly linked to adverse outcomes [2,5,11–13]. Unlike PAPP-A, which influences availability of important placental insulin-like growth factors (IGFs) and directly impacts on fetal growth [14], high maternal serum AFP is thought to reflect excessive placental permeability [15]. Analyses of data where PAPP-A was measured in the first trimester and AFP in the second trimester have shown a synergistic relationship between the two markers, with a combination of low PAPP-A and high AFP being strongly associated with SGA and spontaneous PTB [5].

Whilst these two markers are traditionally measured at different time points in pregnancy, as maternal serum AFP levels begin to rise from the first trimester [16], it is possible that AFP measured at this

Abbreviations: AFP, alpha fetoprotein; FGR, fetal growth restriction; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A; PE, preeclampsia; PlGF, placental growth factor; POP, Pregnancy Outcome Prediction study; PTB, preterm birth; sFLT-1, soluble fms-like tyrosine kinase-1; SGA, small for gestational age; wkGA, weeks of gestation

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time could still be informative, providing earlier, additional information and preventing the need for two blood tests. Furthermore, AFP and PAPP-A require correction for maternal weight and gestational age at the time of measurement in screening for aneuploidy [17,18]. Previous work has demonstrated a simple ratio of sFlt-1 (soluble fms-like tyrosine kinase 1) to PlGF (placental growth factor) to be a clinically useful predictor of preeclampsia [19,20]. This led us to consider whether a ratio of AFP to PAPP-A as uncorrected measurements may be as, or more, informative than the corrected measurements in screening for adverse pregnancy outcome. Therefore, in this study, we aimed to analyze and compare the associations and predictive ability for adverse pregnancy outcomes of a simple AFP:PAPP-A ratio with corrected AFP or PAPP-A as individual measurements and in combination.

2. Materials and methods

2.1. Study design

We studied unselected, nulliparous women from the Pregnancy Outcome Prediction (POP) study [21,22]. Details of the study protocol and power calculations for detection of adverse outcome are described in detail elsewhere [21,22]. Briefly, women with a viable, singleton pregnancy who attended for their dating scan at the Rosie Hospital (Cambridge, United Kingdom) between January 2008 and July 2012 were invited to take part in the study. Blood tests and ultrasound scans were performed at time of recruitment (approximately 12 weeks of gestation) and at approximately 20, 28 and 36 weeks of gestational age (wkGA). Maternal age, body mass index (BMI), smoking status and alcohol intake were recorded at approximately 20 wkGA and other characteristics, pregnancy and birth outcome data were collected by review of paper case records and relevant electronic databases. Socioeconomic deprivation was measured using the Index of Multiple Deprivation 2007 score and calculated from the woman's postcode [23]. Exact gestational age was estimated from the crown-rump length measurement or head circumference, according to national guidance [24].

2.2. Exposures and outcomes

Our primary exposure was the simple (uncorrected) AFP:PAPP-A ratio measured at approximately 12 wkGA as a continuous variable and categorized as > 10, for simplicity (representing approximately the top decile), or ≤ 10. The results for the AFP:PAPP-A ratio were compared with the corresponding AFP or PAPP-A values corrected for maternal weight and exact gestation of measurement on their own and in combination. We also compared the uncorrected ratio with the ratio corrected for maternal weight and gestation of measurement.

Outcomes studied were FGR (birth weight less than the 10th percentile, based on a United Kingdom population based reference [25]) with one of the following complications: (i) non-anomalous perinatal death, (ii) any neonatal morbidity (as previously described in Ref. [22]), or (iii) maternal PE; severe SGA (birth weight less than the 3rd population percentile adjusted for gestational age and fetal sex); severe PE (defined according to the 2013 American College of Obstetricians and Gynecologists' (ACOG) guidelines [26]); PE with PTB (PTB defined as delivery ≥ 24 and < 37 wkGA); PE with SGA (SGA defined as birth weight less than the 10th population percentile); spontaneous PTB (sPTB); iatrogenic (medically-indicated) PTB (iPTB), pre-viable loss (defined as delivery of an infant with no signs of life at < 24 wkGA) and stillbirth (defined as delivery of an infant with no signs of life ≥ 24 wkGA).

2.3. Samples and measurement of AFP and PAPP-A

Maternal blood samples were obtained at time of recruitment (median gestational age 12.7 wkGA, inter-quartile range (IQR) 12.1–13.1) and used for analysis in this study. AFP and PAPP-A were measured as previously described [27] using the Roche Elecsys immunoassay (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany). Researchers performing the assays were blinded to the study participants' clinical information and outcome data. AFP has been reported in international units (IU) per L and PAPP-A in IU per mL. These raw values were used to calculate the AFP:PAPP-A ratio.

2.4. Exclusion criteria

Women were excluded from the analyses if they had no AFP or PAPP-A measurement at approximately 12 wkGA, had a therapeutic termination of pregnancy, withdrew from study, or had no outcome data.

2.5. Statistical analyses

Standard screening summary statistics and relative risks from two by two tables were calculated from the analyses of elevated (> 10) uncorrected AFP:PAPP-A ratio, the top decile of corrected AFP, bottom decile of corrected PAPP-A and women in the top 10% of risk from a predictive model including both corrected AFP and PAPP-A. For the corrected values, we log-transformed the raw values, calculated maternal weight and gestation of measurement-adjusted multiples of the median (MoM), and converted them into standardized Z scores. The discrimination between cases and non-cases was also analyzed using the uncorrected AFP:PAPP-A ratio as a continuous variable by plotting the receiver operating characteristic (ROC) curve and estimating the C statistic, i.e. the area under the ROC curve (AUC). We compared the predictive performance of the uncorrected AFP:PAPP-A ratio against corrected AFP and PAPP-A alone and in combination, using the method of DeLong [28]. Using the same methods, the performance of the AFP:PAPP-A ratio was compared with and without correction for gestational age and maternal weight. For this purpose, the AFP:PAPP-A ratio values were log-transformed to improve normality and turned into a Z score. The corrected AFP:PAPP-A ratios were obtained as standardized residuals from the linear regression analysis between the AFP:-PAPP-A Z score (dependent variable) and gestational age and maternal weight (independent variables). All analyses were performed in STATA version 15.1 (StataCorp, College Station, TX).

2.6. Ethics approval and study reporting

Ethics approval for the POP study was obtained from the Cambridgeshire 2 Research Ethics Committee (reference number 07/H0308/163) and written consent was obtained from all study participants. The reporting of this study conforms to the STARD (Standards for Reporting Diagnostic Accuracy) guidelines [29].

3. Results

3.1. Description of study participants

Out of the 4512 women recruited to the POP study, 67 women (1.5%) withdrew and 233 (5.7%) delivered elsewhere, leaving 4212 eligible women. There were 4057 women who had biochemical and outcome data and they were included in the analysis. Of these, 416 women (10.3%) had one or more of the outcomes described above. A table showing pairwise overlaps of the outcomes is provided in Supplemental Table 1. The distributions of the individual biomarker values and the AFP:PAPP-A ratio in the study population are given in Supplemental Table 2. The clinical characteristics of all women analyzed in this study are tabulated by adverse outcome status in Table 1.

Table 1
Characteristics of the study participants (n = 4057) according to presence or absence of adverse pregnancy outcome.

Characteristic[a]	Absence of adverse outcome	Presence of adverse outcome[b]
n (%)	3641 (89.8%)	416 (10.3%)
Maternal characteristics		
Median maternal age in years (IQR)	30 (27–33)	30 (27–34)
Median height in cm (IQR)	165 (161–170)	164 (159–168)
Missing (%)	0 (0.0%)	3 (0.7%)
Median BMI (IQR)	24.0 (21.8–27.2)	24.7 (22.5–28.1)
Missing (%)	1 (0.03%)	3 (0.7%)
Smoker (%)	161 (4.4%)	38 (9.1%)
Alcohol use (%)	169 (4.6%)	13
Missing (%)	2 (0.1%)	(3.1%)
Ethnicity (%)		
Non-white	206 (5.7%)	27 (6.5%)
White	3372 (92.6%)	382 (91.8%)
Missing (%)	63 (1.7%)	7 (1.7%)
Socioeconomic deprivation quartile (%)		
1 (Least deprived)	873 (24.0%)	105 (25.2%)
2	874 (24.0%)	91 (21.9%)
3	861 (23.7%)	119 (28.6%)
4 (Most deprived)	884 (24.3%)	87 (20.9%)
Missing	149 (4.1%)	14 (3.4%)
Median age stopped FTE in years (IQR)	21 (18–23)	21 (18–23)
Missing (%)	109 (3.0%)	12 (2.9%)
Married (%)	2473 (67.9%)	286 (68.8%)
Birth outcomes		
Median birth weight in grams (IQR)	3460 (3170–3760)	2670 (2290–3110)
Missing (%)	0 (0.0%)	10 (2.1%)
Median birth weight Z score[c] (IQR)	−0.13 (−0.64-0.44)	−0.52 (−1.79-0.27)
Missing (%)	0 (0.0%)	13 (3.1%)
Median birth weight percentile[c] (IQR)	44.7 (26.0–67.0)	30.3 (3.70–60.6)
Missing (%)	0 (0.0%)	13 (3.1%)
Median gestational age at delivery in weeks (IQR)	40 (39–41)	38 (36–40)
Female fetal sex (%)	1826 (50.2%)	188 (45.2%)
Missing (%)	0 (0.0%)	6 (1.4%)
Induced (%)	1158 (31.8%)	143 (34.4%)
Mode of delivery (%)		
Vaginal	1798 (49.4%)	171 (41.1%)
Assisted vaginal	877 (24.1%)	84 (20.2%)
Intrapartum cesarean	622 (17.1%)	75 (18.0%)
Pre-labour cesarean	335 (9.2%)	80 (19.2%)
Missing (%)	0 (0.3%)	6 (1.4%)

BMI, body mass index; FTE, full time education; IQR, inter-quartile range.

[a] For characteristics where there is no missing category, data was 100% complete.

[b] Presence of adverse outcome included any one of the adverse outcomes studied (fetal growth restriction, severe small for gestational age, preeclampsia and preterm birth, severe preeclampsia, preeclampsia and small for gestational age, spontaneous preterm birth, iatrogenic preterm birth, pre-viable loss and stillbirth).

[c] Birth weight percentiles and Z scores were derived from a United Kingdom population-based reference [25].

3.2. Associations with adverse pregnancy outcomes

An elevated uncorrected AFP:PAPP-A ratio > 10 was associated with several placentally-related adverse outcomes (Fig. 1A). The estimated relative risks of FGR, severe SGA, PE with SGA, iPTB, pre-viable loss and stillbirth were about three to fivefold higher in women with an AFP:PAPP-A ratio > 10 compared to women with a ratio ≤10. The relative risks for PE with preterm delivery, severe PE, and sPTB were slightly lower at two to threefold.

The uncorrected AFP:PAPP-A ratio had comparable risk ratios for adverse outcome with either the top decile of corrected AFP MoM or bottom decile of corrected PAPP-A MoM on their own or in combination (Fig. 1). For PE with PTB and stillbirth, the risk ratio was statistically significantly associated (P value < 0.05) with the AFP:PAPP-A ratio (Fig. 1A), whereas there was no statistically significant association with the corrected AFP MoM or PAPP-A MoM (Fig. 1B and C, respectively). Additionally, a PAPP-A MoM in the bottom decile was not associated with severe PE or pre-viable loss at P value < 0.05 (Fig. 1C). The associations between an AFP:PAPP-A ratio > 10 and adverse outcome were similar to the top 10% of predicted risk using a combination of corrected AFP MoM and PAPP-A MoM (Fig. 1D).

3.3. Screening test performance

Standard screening statistics for the AFP:PAPP-A ratio > 10 are provided in Table 2. For the outcomes studied, the AFP:PAPP-A ratio had high specificity (range 89.8–90.5%), but relatively low sensitivity (range 19.4–36.4%). The highest positive likelihood ratio of 3.6 was observed for stillbirth (Table 2). The highest AUC of 0.716 was observed for preeclampsia with SGA (Tables 2 and 3). Correction of the ratio for gestational age and maternal weight did not make a substantial difference to the discrimination between cases and non-cases (Supplemental Table 3, P value > 0.05 for all comparisons). Moreover, the uncorrected AFP:PAPP-A ratio had similar screening statistics for adverse outcome compared to the top decile of corrected AFP MoM or bottom decile of corrected PAPP-A MoM alone or in combination (Supplemental Tables 4 to 6).

When the AUCs were compared, the uncorrected ratio generally had higher AUCs than either corrected AFP MoM or PAPP-A MoM on their own for screening of adverse pregnancy outcomes (Table 3). In particular, the AUC for sPTB using the uncorrected AFP:PAPP-A ratio was higher than using corrected AFP MoM on its own and for iPTB, using the uncorrected AFP:PAPP-A ratio resulted in a higher AUC than corrected PAPP-A MoM on its own. When corrected AFP MoM and PAPP-A MoM were included in the same predictive model the AUCs were comparable to that of the uncorrected AFP:PAPP-A ratio except for sPTB, where the AUC was higher using the combined corrected measurements.

3.4. Addition of AFP:PAPP-A to ultrasound findings associated with adverse outcome

Our previous work has shown that an estimated fetal weight (EFW) < 10th percentile with a reduction in growth velocity of the abdominal circumference (ACGV) on ultrasound is associated with most neonatal morbidity [22]. An AFP:PAPP-A > 10 in the first trimester or an EFW < 10th percentile with reduced ACGV in the third trimester provided greater sensitivity for adverse outcome than the ultrasound finding on its own, but specificity was lower (Supplemental Table 7).

4. Discussion

The major finding of this study is that a simple ratio of AFP to PAPP-A > 10 in the first trimester is associated with several important placentally-related adverse pregnancy outcomes, including FGR, PE, PTB and stillbirth. In addition, the ratio does not require correction for maternal weight and gestational age at measurement, simplifying its use as potential screening tool, and may provide more information than either AFP or PAPP-A on their own.

Whilst AFP and PAPP-A are normally corrected for maternal weight and gestational age to improve their predictive ability for aneuploidy, correcting the ratio for these parameters did not substantially influence predictive ability for the adverse outcomes analyzed in this study cohort. The maximum improvement in AUC was 0.026 for stillbirth and for five of the nine studied outcomes the AUC was less after correction, hence these corrections are unnecessary for the ratio.

Moreover, there was a trend for the uncorrected ratio to be more

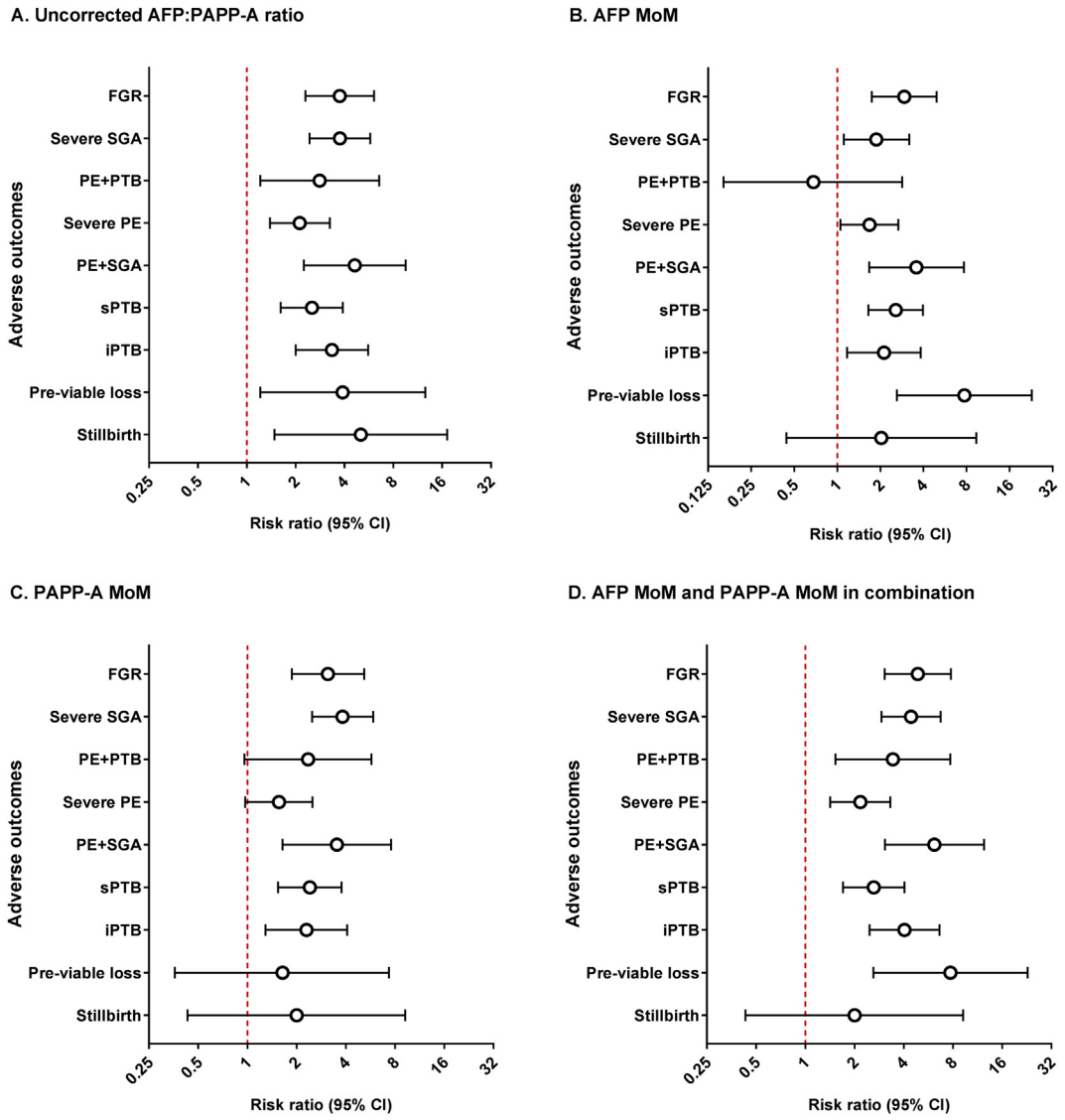

Fig. 1. Risk ratios with 95% CIs for adverse outcomes according to an uncorrected AFP:PAPP-A ratio > 10 (A), top decile AFP MoM (B), bottom decile PAPP-A MoM (C) and top 10% of predicted risk for a model combining AFP MoM and PAPP-A MoM (D). All measurements were performed at approximately 12 wkGA. CI, confidence interval; iPTB, iatrogenic preterm birth; FGR, fetal growth restriction; MoM, multiples of the median; PE, preeclampsia; SGA, small for gestational age; sPTB, spontaneous preterm birth; wkGA, weeks of gestational age.

Table 2
Screening statistics of an uncorrected AFP:PAPP-A ratio > 10 at approximately 12 wkGA.

Outcome	TP/FP	TN/FN	Positive LR	Negative LR	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
FGR	22/389	3581/52	3.0	0.78	29.7	90.2	5.4	98.6	0.674
Severe SGA	28/383	3567/66	3.1	0.78	29.8	90.3	6.8	98.2	0.651
PE + PTB	7/404	3611/22	2.4	0.84	24.1	89.9	1.7	99.4	0.596
Severe PE	25/386	3529/104	2.0	0.89	19.4	90.1	6.1	97.1	0.597
PE + SGA	11/400	3612/21	3.4	0.73	34.4	90.0	2.7	99.4	0.716
sPTB	24/367	3485/87	2.3	0.87	21.6	90.5	6.1	97.6	0.675
iPTB	19/367	3485/52	2.8	0.81	26.8	90.5	4.9	98.5	0.637
Pre-viable loss	4/411	3633/9	3.0	0.77	30.8	89.8	1.0	99.8	0.605
Stillbirth	4/407	3626/7	3.6	0.71	36.4	89.9	1.0	99.8	0.611

AFP, alpha fetoprotein; AUC, area under the curve; FGR, fetal growth restriction; FN, false negative; FP, false positive; iPTB, iatrogenic preterm birth; LR, likelihood ratio; NPV, negative predictive value; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; PTB, preterm birth; SGA, small for gestational age; sPTB, spontaneous preterm birth; TN, true negative; TP, true positive; wkGA, weeks of gestational age.
All screening statistics were calculated comparing the uncorrected AFP:PAPP-A ratio > 10 to an uncorrected AFP:PAPP-A ratio ≤10 whilst the AUC was calculated using the AFP:PAPP-A ratio as a continuous variable.

Table 3
Comparison of uncorrected AFP:PAPP-A ratio *vs.* AFP MoM and PAPP-A MoM corrected for maternal weight and gestation on their own or in combination.

Outcome	AFP:PAPP-A[a] AUC (95% CI)	AFP MoM[b] AUC (95% CI) *P* value[d]	PAPP-A MoM[b] AUC (95% CI) *P* value[d]	Combination[c] AUC (95% CI) *P* value[d]
FGR	0.674 (0.607–0.740)	0.619 (0.556–0.683) 0.09	0.638 (0.567–0.710) 0.16	0.684 (0.619–0.750) 0.65
Severe SGA	0.651 (0.589–0.712)	0.599 (0.541–0.657) 0.08	0.614 (0.545–0.683) 0.11	0.651 (0.589–0.715) 0.92
PE + PTB	0.596 (0.484–0.708)	0.511 (0.408–0.613) 0.14	0.586 (0.463–0.710) 0.78	0.595 (0.474–0.716) 0.95
Severe PE	0.597 (0.546–0.648)	0.564 (0.513–0.615) 0.12	0.559 (0.509–0.608) 0.08	0.590 (0.540–0.640) 0.36
PE + SGA	0.716 (0.614–0.818)	0.633 (0.538–0.729) 0.09	0.675 (0.572–0.777) 0.15	0.721 (0.619–0.824) 0.69
sPTB	0.675 (0.624–0.726)	0.605 (0.549–0.661) 0.002	0.643 (0.594–0.691) 0.15	0.689 (0.640–0.738) 0.04
iPTB	0.637 (0.563–0.710)	0.599 (0.523–0.668) 0.22	0.572 (0.497–0.646) 0.009	0.623 (0.550–0.696) 0.09
Pre-viable loss	0.605 (0.400–0.810)	0.660 (0.470–0.851) 0.50	0.557 (0.358–0.757) 0.54	0.644 (0.446–0.843) 0.40
Stillbirth	0.611 (0.413–0.809)	0.623 (0.467–0.779) 0.83	0.563 (0.381–0.745) 0.39	0.631 (0.467–0.796) 0.64

CI, confidence interval; iPTB, iatrogenic preterm birth; FGR, fetal growth restriction; MoM, multiples of the median; PE, preeclampsia; SGA, small for gestational age; sPTB, spontaneous preterm birth; wkGA, weeks of gestational age.

[a] The uncorrected AFP:PAPP-A ratio was calculated by dividing AFP in IU/L by PAPP-A in IU/mL (measured at approximately 12 wkGA) and women with a ratio > 10 were compared with women with a ratio ≤10.

[b] Both AFP and PAPP-A (measured at approximately 12 wkGA) were log transformed, converted into MoM and corrected for maternal weight and gestation of measurement before categorizing into deciles. Women with an AFP MoM in the top decile were compared with women with an AFP MoM in deciles 1–9 and women with a PAPP-A MoM in bottom decile were compared with women with a PAPP-A MoM in deciles 2–10.

[c] Both AFP and PAPP-A (measured at approximately 12 wkGA) were log transformed, converted into MoM and corrected for maternal weight and gestation of measurement before being included as predictors in a logistic regression model with the adverse outcome as the dependent variable. The women in the top 10% (10th decile) of predicted risk were derived from this model and compared with women who had a predicted risk in deciles 1–9.

[d] *P* value is given for the comparison against the uncorrected AFP:PAPP-A ratio.

strongly associated with adverse outcome than either corrected AFP or PAPP-A on their own. The comparison of AUCs between the uncorrected ratio and the individual corrected measurements was not statistically significant at the 5% level, but the DeLong method is conservative [30] and the greater AUCs could be clinically important. Furthermore, sensitivity using the uncorrected AFP:PAPP-A ratio was between 0.9% and 18.4% higher than the commonly used corrected PAPP-A on its own. A corrected AFP in the top decile had the greatest sensitivity for pre-viable loss (42.6%), but otherwise sensitivity was the same (for sPTB) or better using the uncorrected ratio (between 3.9% and 17.2%). In addition, the positive and negative likelihood ratios for PE or SGA using AFP measured in the first trimester were similar to previous studies where it had been measured in the second trimester [31], supporting the case for its utility when measured at an earlier stage of pregnancy.

The model combining AFP and PAPP-A as corrected measurements resulted in AUCs which were largely comparable to the uncorrected AFP:PAPP-A ratio. Moreover, the lower limit of sensitivity for the model was the same as the simple ratio (19.4%) and there did appear to be a stronger association with stillbirth using the uncorrected ratio, with a relative risk of more than fivefold. But, due to the rarity of the outcome of stillbirth (n = 11) the confidence intervals were wide. However, the model was fitted to the specific dataset studied, meaning its performance may not be similar in other populations, whereas the ratio is easier to calculate and likely to be reproducible in other settings.

A strength of our study was that we were able to assess the utility of a screening test in a low-risk, nulliparous population. This is an advantage as inclusion of multiparous women who have not had the outcome in a previous pregnancy would alter the interpretation of the predictive ability of the ratio, where the positive predictive value would necessarily be lower due to a lower prior odds for the outcome. Furthermore, our definitions of adverse outcomes (e.g. FGR and severe SGA) were likely to represent situations where there is a substantial risk of morbidity; FGR was defined as a birth weight < 10th percentile with a complication and severe SGA infants had a birth weight < 3rd percentile. Inclusion of all SGA babies is likely to capture a substantial proportion of constitutionally small babies where there is a low risk of pathology [2], and where a biomarker such as AFP:PAPP-A is unlikely to be altered.

There are weaknesses and limitations to our work. Among the women recruited to the study, 455 (10.1%) did not have complete outcome and biochemical data and could not be analyzed. We also studied women predominantly of Northern European ancestry, so our conclusions may be limited to this population. Additional analyses of AFP and PAPP-A should be performed in women of non-Northern European ancestry, as levels have been shown to differ amongst ancestral groups [32]. AFP and PAPP-A levels are also affected by other factors, such as maternal smoking [33,34] and assisted conception [35,36], which could affect the interpretation of the ratio. In addition, the utility of the ratio with both markers measured in the second trimester, which may be relevant to women who book late in pregnancy, was not specifically studied. We have found that the ratio remains positively associated with adverse outcome when measured at 20 wkGA (unpublished data), therefore it is likely that the ratio can be interpreted similarly in women who have it measured up to 20 wkGA.

The combination of low maternal PAPP-A in the first trimester and high AFP levels in the second trimester has previously been shown to be associated with preterm delivery or delivery of an infant with SGA [5]. PAPP-A is commonly measured in the first trimester as part of aneuploidy screening, and is typically corrected for maternal weight and gestation of measurement [17,18]. Previous studies have shown a low PAPP-A in the first trimester to be associated with SGA, FGR, PE, PTB and stillbirth [7,37,38] and currently, the Royal College of Obstetricians and Gynaecologists (RCOG) recommend serial ultrasound scans for growth and umbilical artery Dopplers in women who screen positive for low PAPP-A (< 0.4 corrected MoMs) [39]. However, there is no consensus for monitoring based on low PAPP-A in the United States as there is no direct evidence it improves outcome. A systematic

review of studies investigating the associations between PAPP-A and adverse pregnancy outcomes found that a PAPP-A < ~0.4 MoMs had a sensitivity up to 25.7% for all PE and 16.0% for severe SGA (birth weight < 3rd percentile) [7], and the highest sensitivity for stillbirth has been reported as 15.0% [40]. Therefore, given that PAPP-A is measured almost routinely, an improvement in sensitivity would be welcome. The key advantage of the ratio is its simplicity, and an uncorrected AFP:PAPP-A ratio greater than 10 in the first trimester may alert clinicians to women at increased risk of developing pregnancy complications and who may benefit from closer monitoring. However, the ratio did not perform well as a screening test as a single parameter. For every correctly identified case of any adverse outcome there would be about three false positive results, which may prompt unnecessary additional monitoring and maternal anxiety. It may, however, enhance clinical prediction of adverse outcome when added to other measurements and maternal characteristics, as has been shown for PAPP-A and the prediction of early onset preeclampsia [41]. Addition of biomarkers to ultrasound data may provide better diagnostic accuracy than either on their own [42], and we found that an AFP:PAPP-A ratio > 10 in the first trimester or an EFW < 10th percentile with reduced growth velocity of the abdominal circumference in the third trimester (which we have previously shown to be the only ultrasonic marker of FGR to be associated with neonatal morbidity [22]) may improve sensitivity for detection of adverse outcome, compared with ultrasound on its own. However, to confirm its suitability as a screening test would require further investigation and analysis, taking into account the challenges associated with developing a multivariable predictive model in Obstetrics [43–45].

In summary, an elevated simple AFP:PAPP-A ratio > 10 measured in the first trimester is associated with a higher risk for severe adverse pregnancy outcomes and provides a simple alternative to correcting AFP and PAPP-A for maternal weight and exact gestational age of measurement.

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Author contributions

GCSS and DSC-J designed the study and managed the teams who collected the data. FG and EC collected the data and supervised the technicians who assisted in performing the biochemical assays. US and AEH performed statistical analysis of the data and AEH, US, FC and GCSS contributed to the interpretation of the data. AEH, US and FC drafted the manuscript and all authors critically revised the work. All authors approved the final version of the manuscript. We confirm that all persons designated as authors qualify for authorship, that all persons who qualify for authorship are listed as authors and we approved the order of authors.

Declaration of interest statement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.placenta.2019.04.005.

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