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Routine first-trimester combined screening for pre-eclampsia: pregnancy-associated plasma protein-A or placental growth factor?

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Short title: PAPP-A vs PlGF for pre-eclampsia screening
Key words: first trimester, screening, pre-eclampsia, small for gestational age, trisomy 21, PAPP-A, PlGF, blood pressure, uterine Doppler

What are the novel findings of this work?

Maternal serum PAPP-A and PlGF are associated with a similar first-trimester combined screening performance for pre-eclampsia, small-for-gestational age birth or trisomy 21.

What are the clinical implications of this work?

Routine first trimester combined screening for pre-eclampsia can be implemented taking advantage of PAPP-A levels available from screening for aneuploidy.

ABSTRACT

Objective: To compare the screening performance of serum pregnancy-associated plasma protein-A (PAPP-A) versus placental growth factor (PlGF) in routine first trimester combined screening for preeclampsia (PE), small for gestational age (SGA) birth and trisomy 21.

Methods: Retrospective study nested in pregnancy cohorts undergoing first trimester combined screening for PE and trisomy 21 using the Fetal Medicine Foundation (FMF) algorithm based on maternal characteristics, nuchal translucency, PAPP-A, free beta-human chorionic gonadotropin, blood pressure and uterine artery Doppler. Women at high-risk for preterm PE (≥1 in 50) received 150 mg aspirin, serial fetal growth scans at 28 and 36 weeks and were offered elective birth from 40 weeks of gestation. PlGF was retrospectively quantified in stored surplus first-trimester serum samples. The performance of combined first trimester screening for PE, SGA and trisomy 21 using either PAPP-A or PlGF was calculated.

Results: Maternal serum PAPP-A was assayed in 1094 women including 82 with PE, 111 with SGA <10th centile, 53 with both PE and SGA <10th centile, and 94 with trisomy 21. PlGF levels were retrospectively obtained from 1066 out of 1094 women. Median serum PlGF MoM was significantly lower in PE (1.0, IQR=0.8-1.4, *P*<0.01), SGA (1.0, IQR=0.8-1.3, *P*<0.001) and trisomy 21 pregnancies (0.6, IQR=0.5-0.9, *P*<0.0001) compared to controls (1.2, IQR=0.9-1.5). There was no significant difference in the performance of first trimester screening using PAPP-A versus PlGF for either preterm PE (AUC 0.78 vs 0.79, *P*=0.55) or term PE (AUC 0.74 vs 0.74, *P*=0.60). These findings persisted even after correction for the effect of targeted aspirin use on prevalence of PE. Similarly, there were no significant differences in combined screening sensitivity or specificity for SGA or trisomy 21 when using PAPP-A versus PlGF.

Conclusion: Using PlGF or PAPP-A in routine first trimester combined screening with maternal characteristics, blood pressure and uterine artery Doppler does not make an appreciable clinical difference to the detection of PE or SGA. Depending on the setting, biomarkers should be chosen to achieve a good compromise between performance and measurement requirements. This pragmatic effectiveness study suggests that combined screening for PE can be successfully implemented in public healthcare settings without changing current protocols for the assessment of PAPP-A in the first trimester.

INTRODUCTION

The efficacy of a first trimester screening algorithm for pre-eclampsia (PE) combining maternal history, blood pressure, uterine artery Doppler and maternal serum pregnancy-associated plasma protein-A (PAPP-A) and serum placental growth factor (PlGF) was established in the Aspirin for Evidence-Based Preeclampsia Prevention (ASPRE) multi-centre randomised controlled trial [1]. Women who were at high-risk based on the screening algorithm and who received 150 mg of aspirin started before 16 weeks of gestation had a 62% reduction in the incidence of preterm (less than 37 weeks) PE when compared to those receiving placebo [2].

The external validity of this trial was recently established in a clinical effectiveness study, where an 80% decrease in the rate of preterm PE was observed over two years in a public healthcare setting [3]. In that study, the first trimester combined risk for developing preterm PE was assessed in 4841 women using maternal history, blood pressure, uterine artery Doppler and PAPP-A instead of PlGF. This was a decision based on logistical and cost-effectiveness considerations as PAPP-A is already measured routinely as part of combined screening for Down syndrome, according to National Screening Committee guidelines [4]. In this clinical effectiveness study, women at high-risk (≥1 in 50) of preterm PE received 150 mg of aspirin, were scheduled to have serial fetal ultrasound assessments at 28 and 36 weeks, and offered elective birth from 40 weeks of gestation [3].

The decision to use PAPP-A instead of PlGF was partly based on the findings of the first trimester Screening Program for PE (SPREE) study. Here, the primary comparison was of screening performance of standard maternal history-based screening using National Institute for Health and Care Excellence (NICE) guidelines versus an algorithm-based on maternal history, blood pressure and PAPP-A in 16,747 routinely screened women – with secondary analyses including PlGF and uterine artery Doppler indices. In a post-hoc analysis, the authors showed that all factors plus PlGF gave an 82% sensitivity for preterm PE compared to 76% with PAPP-A, at a 10% screen-positive rate [5]. The screening performance was not improved by combining PAPP-A and PlGF. Nevertheless, the SPREE study which undertook a comparison of NICE guidelines versus combined testing for PE involving various combinations of maternal factors, MAP, PAPP-A and PlGF, did not directly compare the clinical value of screening of these factors with PAPP-A versus PlGF in a routine healthcare setting.

The primary aim of this study was to compare the clinical effectiveness of first trimester combined screening for PE by using maternal history, blood pressure, uterine artery Doppler and either PAPP-A or PlGF. Secondary aims were to assess the screening performance for small for gestational age (SGA) birth and trisomy 21.

METHODS

This is a retrospective cohort study in which first trimester routine screening for PE was undertaken. In order to assess the relative value of PAPP-A and PlGF, both were measured in the participating women, and the screening performance based on maternal history, blood pressure, uterine artery Doppler plus PAPP-A or PlGF were assessed. In order to study the impact of using PAPP-A or PlGF in screening for other outcomes we also assessed the screening performance, using the same approach, for small for gestational age (SGA) birth and trisomy 21.

Study cohorts

Two nested cohorts were used in this study. The first included 1000 women from a population of 4841 women who underwent first trimester combined PE screening at St George's University Hospitals NHS Foundation Trust between March 2018 and March 2019. The risk for developing PE was assessed by the Fetal Medicine Foundation (FMF) algorithm combining maternal risk factors, mean arterial blood pressure, PAPP-A and uterine artery pulsatility index for each woman. Women with a risk ≥1 in 50 for developing preterm PE received a 150 mg aspirin prophylaxis from 11-14 to 36 weeks' gestation [3]. 246 women who subsequently developed PE (n=82), an SGA baby <10th centile at birth (n=111) or both (n=53) were compared to consecutively screened women (n=754) who did not develop these pregnancy complications. The second cohort included 94 pregnancies with a fetus affected by trisomy 21 who had undergone first trimester maternal serum biomarker assessment at the Prenatal Screening Unit, King George's Hospital between July 2008 and Mars 2016. Samples were stored at -40°C between collection and analysis and underwent a maximum of 3 freeze-thaw cycles.

Laboratory analysis and assessment of pregnancy risks

In both cohorts, surplus first trimester maternal serum samples that had been assayed for PAPP-A as part of first trimester combined screening for PE and/or trisomy 21 were stored at −20°C. Stored aliquots were retrieved and PlGF levels determined using the Kryptor Compact Plus system analyser (BRAHMS, ThermoFisher Scientific, Hennigsdorf, Germany). Samples were thawed and analysed in batches of 50-80 samples. The BRAHMS PlGF plus is a homogeneous sandwich immunoassay based on Trace technology and designed to measure the free PlGF-1 isoform with a range of 3.6–7000 pg/ml with a limit of quantitation of 6.9 pg/ml.

In the PE-screened cohort, the risks for developing PE or SGA at term or preterm were calculated by using maternal characteristics, mean arterial blood pressure, uterine artery Doppler and PAPP-A or PlGF in the FMF first trimester combined PE screening algorithm, as previously described [3]. The definition for PE was based on the NICE hypertension in pregnancy guidelines [6] and SGA was defined as a birthweight below the 10th centile for gestation using the Intergrowth-21 birthweight reference standards [7]. In both cohorts, the risk for trisomy 21 was calculated by either using PAPP-A or PlGF in addition to maternal age, nuchal translucency and free beta-human chorionic gonadotropin using the first trimester combined aneuploidy FMF algorithm [8].

Statistical analysis

The sensitivity and specificity of screening using PAPP-A or PlGF for the detection of term and preterm PE, as well as SGA and trisomy 21, were calculated following the STARD guidelines for diagnostic accuracy studies [9]. Receiver Operating Characteristic (ROC) curve analysis was used to compare the performance of screening for PE and SGA at term and preterm. A pairwise comparison of ROC curves was performed according to the method described by DeLong *et al.* [10].

The prevalence of PE and SGA is influenced by the targeted use of aspirin, serial growth scans and elective birth from 40 weeks of gestation in the high-risk cohort. In combination, these would act to decrease the subsequent development of preterm PE by 80% [3], of term PE by 20% [3] and of SGA birth at term by 45% [11]. This in turn would result in a decrease in sensitivity and have a negative impact on screening performance. In order to avoid this, we undertook an additional analysis using a correction factor that was applied to calculate the adjusted sensitivity of screening using PAPP-A and PlGF, as previously described by Wright and Nicolaides [12].

A Mann Whitney test was used to compare the median PlGF and PAPP-A values (MoM) and interquartile range (IQR) between control women and women with PE, SGA or trisomy 21 fetuses. The algorithms using PAPP-A versus PlGF for trisomy 21 screening were compared by means of a McNemar test. MedCalc statistical software version 19.6.1 (Ostend, Belgium) was used to perform the statistical analyses.

RESULTS

Serum samples from a total of 1094 pregnant women were assayed for PAPP-A - including 82 with PE (10 at <37 weeks), 111 with SGA (21 at <37 weeks), 53 who developed both PE and SGA (20 at <37 weeks), and 94 with trisomy 21. PlGF levels were retrospectively obtained from 1066 out of these 1094 women. Median serum PlGF MoM was significantly lower in PE (1.0, IQR=0.8-1.4, *P*<0.01), SGA (1.0, IQR=0.8-1.3, *P*<0.001) and trisomy 21 pregnancies (0.6, IQR=0.5-0.9, *P*<0.0001) compared to controls (1.2, IQR=0.9-1.5). Similarly, median serum PAPP-A MoM was significantly lower in PE (0.9, IQR=0.6-1.4, *P*<0.01), SGA (0.9, IQR=0.6-1.3, *P*<0.0001) and trisomy 21 pregnancies (0.4, IQR=0.3-0.7, *P*<0.0001) compared to controls (1.1, IQR=0.8-1.6).

Comparison of PAPP-A and PlGF in screening for PE

There was no significant difference in the performance of first trimester screening using PAPP-A or PlGF for either preterm PE (Table 1, Fig 1a, AUC PAPP-A=0.78, AUC PlGF=0.79, *P*=0.55) or term PE (Table 1, Fig 1b; AUC PAPP-A=0.74, AUC PlGF=0.74, *P*=0.60). At a 10% screen-positive rate, the sensitivity of combined screening for preterm PE was 46.7% for PAPP-A and 51.7% for PlGF, with corresponding values of 26.7% and 27.0% for term PE (Table 2). Sensitivities were not significantly different even after adjusting for the effect of targeted aspirin use in high-risk women (Table S1).

Comparison of PAPP-A and PlGF in screening for SGA and trisomy 21

There were no significant differences in screening sensitivity or specificity for SGA when using PAPP-A compared to PlGF (Table 1). For preterm SGA the combined screening algorithm showed a sensitivity of 34.2% and 37.5% when using PAPP-A or PlGF with corresponding values of 16.3% and 17.8% for term SGA (Table 2). Sensitivities were not significantly different even after adjusting for the effect of targeted aspirin use in high-risk women for SGA at term (Table S1). The sensitivity in screening for trisomy 21 was assessed at a cut-off of a chance of 1:150 at term; using PAPP-A this was 84.0% (95% CI 75.1-90.8) with a specificity of 97.0% (95% CI 95.7-98.0). The corresponding sensitivity for PlGF was 79.8% (95% CI 70.3-87.4) with a specificity of 97.3% (95% CI 96.1-98.2). The McNemar test showed no significant difference in the algorithms using PAPP-A versus PlGF for trisomy 21 screening (*P*=0.24).

DISCUSSION

Main findings

In this study, PAPP-A and PlGF are associated with a similar screening performance for PE when used as part of first-trimester screening that also includes maternal characteristics, blood pressure and uterine artery Doppler. High sensitivities for preterm PE were observed when performed with either PAPP-A or PlGF. Similarly, the use of either of the two markers resulted in very similar sensitivities for term PE, SGA birth and trisomy 21.

Strengths and limitations

This study concomitantly evaluates the performance of screening for PE, SGA and trisomy 21 and helps to evaluate the relative merits of using either PAPP-A or PlGF as a maternal serum biomarker. A strength of this study is the real-world performance of routine first trimester screening for PE in a public healthcare setting, where aspirin prophylaxis was guided by the results of risk assessment. This is also a study limitation as it is - by design - a retrospective nested cohort analysis. Using this design means that the prevalence of PE, SGA and trisomy 21 is higher than expected in a routine population, thereby influencing estimates of positive and negative predictive values. Mitigation of these biases was undertaken by emphasising sensitivities and specificities which are not affected by disease prevalence. Furthermore, ROC curve analysis was only used to compare relative performance of PAPP-A versus PlGF, rather than as a demonstration of their screening performance. The main limitation of this study is that there were only 30 cases of preterm PE out of the total of 135 PE cases, making this study potentially underpowered. This was an unexpected effect of the efficacy of targeted aspirin use in reducing the number of preterm PE cases in our population. We mitigate this issue by reporting and discussing a post hoc analysis of a much larger study containing 142 cases of preterm PE, which showed similar results to the current study.

Comparison to previous studies

The largest study to date retrospectively evaluated first-trimester screening for PE on 57,131 pregnancies in a research setting and concluded that the preferred biomarker is PlGF [13]. At a 10% screen-positive rate, the authors achieved a sensitivity for preterm PE of 74.1% with PlGF, which was significantly higher (7.1%, 95% CI 3.8-10.6%) than using PAPP-A, but non-significant for the prediction of the majority of cases of PE that occurred at term (1.8%, 95% CI 0.0-3.6%). It should be noted that this and other studies by the same research group were conducted only in women who had accepted first trimester trisomy screening; this limits the generalisability of the study findings as in the UK for example a third of women decline such assessment [14,15]. These studies were also labelled as non-interventional because the

researchers did not prescribe aspirin prophylaxis. However, PAPP-A levels were routinely revealed to clinicians at a time when national guidelines recommended the use of aspirin prophylaxis in pregnancies with low PAPP-A levels [16]. Therefore, it is difficult to ascertain whether aspirin was prescribed by clinicians managing the pregnancy, leading to selective suppression of PAPP-A screening efficiency. In a prospective multicentre observational study, the same researchers compared conventional risk-factor based assessment by NICE guidelines to first trimester combined screening for PE in 16,747 pregnancies. They convincingly demonstrated that for the same screen positive rate, the sensitivity for preterm PE by combined first trimester screening with maternal characteristics, blood pressure and PAPP-A was significantly higher than that of current NICE guidelines. Unfortunately, in that study, no direct comparison of screening with PAPP-A versus PlGF was undertaken as it was not part of the pre-specified commissioned analysis [17]. A post hoc analysis of the latter data applying McNemar test for comparison of the screening using maternal characteristics, blood pressure, uterine artery Doppler and either PlGF or PAPP-A for detection of preterm PE at a 10% screen positive rate showed no significant difference (P=0.10). The corresponding 95% confidence interval for the difference in sensitivity for the use of PlGF versus PAPP-A ranges from -0.2% to 12.1%. Furthermore, a more recent analysis of first trimester combined screening for PE from China of 10,899 pregnancies included 312 cases of PE (117 cases of preterm PE) in a clinical setting where aspirin prophylaxis is not routinely prescribed [18]. The authors concluded that the first trimester combined test for PE using maternal characteristics, blood pressure, uterine artery Doppler and PAPP-A achieved a detection rate of 65.0% for preterm PE at a 10% fixed false-positive rate, without any improvement in detection when replacing PAPP-A with PlGF, or when adding PlGF to the screening test. These data do not demonstrate equivalence of PAPP-A with PlGF in first trimester combined screening for preterm PE, but neither do they demonstrate inferiority of PAPP-A compared to PlGF in this context.

Implications of study findings

First-trimester combined screening for PE is useful because treatment of the high-risk group with aspirin reduces the rate of preterm PE by 60 to 80% in both efficacy trials and effectiveness studies [2,3]. Previous studies suggested that the best way of identifying the high-risk group is by a combination of maternal factors, blood pressure, uterine Doppler and PlGF. It was inferred that with the use of PAPP-A in place of PlGF, the same sensitivity may be achieved but at a much higher screen-positive rate [13]. If public healthcare budgets allow measurement of one of these markers, which is preferable? The current study demonstrates that PAPP-A, which is already routinely offered as part of first trimester trisomy screening in most countries, appears to have acceptable clinical effectiveness for detection of PE and SGA. A possible advantage using first trimester PlGF is that this would confer significant economies of scale for PlGF testing in later pregnancy [19,20]. However, substituting PAPP-A with PlGF as a marker for the national screening programme for trisomy would require extensive prior evaluation. Depending on the setting, biomarkers should be chosen to achieve a good compromise between performance and measurement requirements. If first-trimester combined screening for PE is to be undertaken without the use of uterine artery Doppler, current evidence would support the superiority of using PlGF over PAPP-A [17].

Research implications of study findings

There is now robust evidence to demonstrate intervention bias with aspirin prophylaxis (lowers preterm PE by 60%) and elective birth from 40 weeks' gestation (lowers term PE by 36%) [2,21]. Future evaluations of screening performance should account for these factors by statistical adjustment for aspirin prophylaxis and competing risk analysis to account for the effect of scheduled birth [22,23]. Secondly, it is established that population characteristics influence detection and false positive rates when screening for PE [17]. Therefore, screen positive rates will vary according to local population characteristics even when using the same screening threshold – something that is not conventionally expected or observed in other national screening programmes, such as aneuploidy screening.

Conclusion

Early pregnancy combined screening for PE is superior to conventional risk-factor based screening that is typically undertaken in most clinical settings. Although there is evidence of superiority of PLGF over PAPP-A when used as a single marker or in combination with blood pressure, this study shows that PAPP-A is as clinically effective as PlGF in first trimester combined PE screening when used in combination with maternal characteristics, blood pressure and uterine artery Doppler. Consideration should be given to routine implementation of first trimester combined screening for preterm PE without changing current protocols for the assessment of PAPP-A.

Disclosures of interest: None

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Ethics approval: This retrospective study of routinely collected clinical data and surplus serum samples undertaken for ongoing clinical audit and assay validation was deemed not to require ethics approval or signed patient consent as per the HRA decision tool. Patients and the public were not involved in the design of this study.

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TABLE AND FIGURE LEGENDS

Table 1. Comparison of receiver operator characteristic (ROC) analysis for the detection of pre-eclampsia (PE) or small-for-gestational-age (SGA) ≤10th centile with either PAPP-A or PlGF using a first trimester combined screening algorithm [2].

Table 2. Performance of screening for pre-eclampsia (PE) or small-for-gestational-age (SGA) ≤10th centile by combining maternal risk factors, mean arterial blood pressure, uterine artery pulsatility index and PAPP-A or PlGF at a fixed 10% screen-positive rate [2].

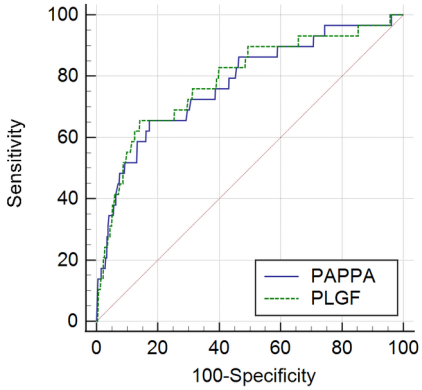
Figure 1. Receiver Operating Characteristic (ROC) curves comparing the performance of first trimester screening for preterm pre-eclampsia (PE) <37 weeks (**a**) and term PE ≥37 weeks (**b**) by combining maternal risk factors, mean arterial blood pressure, uterine artery pulsatility index and PAPP-A (continuous blue line) or PlGF (dotted green line) to assess the patient's individual risk [2].

Table 1 Comparison of receiver operator characteristic (ROC) analysis for the detection of pre-eclampsia (PE) and small-for-gestational-age (SGA) birth ≤10th centile using either PAPP-A or PlGF in the first trimester combined FMF screening algorithm [2].

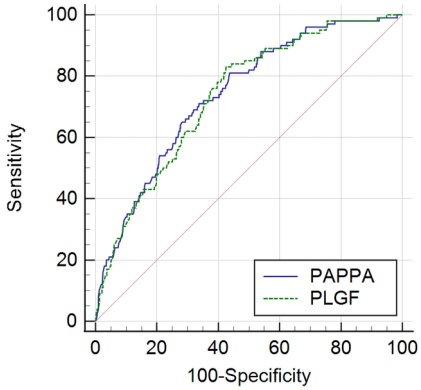
	AUC PAPP-A (95% CI)	**AUC PlGF (95% CI)**	***P*-value**
PE <37 weeks	0.78 (0.75 - 0.80)	0.79 (0.76 - 0.81)	0.55
PE ≥37 weeks	0.74 (0.71 - 0.77)	0.74 (0.71 - 0.76)	0.60
SGA <37 weeks	0.68 (0.65 - 0.71)	0.68 (0.65 - 0.71)	0.85
SGA ≥37 weeks	0.59 (0.56 - 0.62)	0.59 (0.55 - 0.62)	0.69

Table 2 Performance of screening for pre-eclampsia (PE) and small-for-gestational-age (SGA) birth ≤10th centile by using the FMF algorithm combining maternal risk factors, mean arterial blood pressure, uterine artery pulsatility index and PAPP-A or PlGF at a fixed 10% screen-positive rate [2].

	PE <37 weeks		PE ≥37 weeks	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
PAPP-A	46.7 (14/30) (28.3 - 65.7)	91.2 (885/970) (89.3 - 92.9)	26.7 (28/105) (18.5 - 36.2)	92.1 (824/895) (90.1 - 93.8)
PlGF	51.7 (15/29) (32.5 - 70.6)	91.3 (861/943) (89.3 - 93.0)	27.0 (27/100) (18.6 - 36.8)	92.0 (802/872) (90.0 - 93.7)
Difference	5.1 (-20.5 to 30.6)	-	0.3 (-11.8 to 12.5)	-
	SGA <37 weeks		**SGA ≥37 weeks**	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
PAPP-A	34.2 (14/41) (20.1 - 50.6)	91.1 (874/959) (89.2 - 92.9)	16.3 (20/123) (10.2 - 24.0)	91.0 (798/877) (88.9 - 92.8)
PlGF	37.5 (15/40) (22.7 - 54.2)	91.2 (850/932) (89.2 - 92.9)	17.8 (21/118) (11.4 - 25.9)	91.1 (778/854) (89.0 - 92.9)
Difference	3.4 (-17.5 to 24.2)	-	1.5 (-8.0 to 11.0)	-



UOG_23669_Fig 1a.tif



UOG_23669_Fig 1b.tif