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First trimester screening of maternal placental protein 13 for predicting preeclampsia and small for gestational age: in-house study and systematic review

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ABSTRACT

Objective: To describe normative levels of PP13 in first-trimester of pregnancy and determine the accuracy of PP13 in predicting preeclampsia and small for gestational age (SGA) infants.

Methods: We measured PP13 in archived first trimester serum samples from an unselected maternal cohort of 2,989 women. Associations of PP13 levels and diagnostic accuracy in predicting adverse pregnancy outcomes were assessed using multivariate logistic regression models. Due to inadequate number of cases we then conducted a systematic review involving structured search of electronic databases and subsequent meta-analysis of predictive accuracy using data from similar studies.

Results: Overall, 2,678 women were included in the in-house study with 71 (2.7%) preeclampsia cases, 5 (0.2%) early-onset preeclampsia (≤34 weeks) cases; and 191 (7.1%) and 41 (1.5%) infants SGA<10^{th} and <3^{rd} centile. Median (IQR) normative level of PP13 in unaffected pregnancies was 53.5 (37.7-71.8) pg/ml. The area under the receiver operating characteristic curve (AUC) for multivariate models was 0.72 (95%CI 0.66-0.78) for preeclampsia; 0.82 (95%CI 0.63-0.99) for early-onset preeclampsia; 0.73 (95%CI 0.69-0.77) for SGA<10^{th} centile; and 0.83 (95%CI 0.78-0.88) for SGA<3^{rd} centile. Eight studies were included in the systematic review, normative levels of PP13 was assessed in four studies but these were variable; and meta-analysis was performed on seven studies. Sensitivity rates of PP13 based on 5% fixed false positive rates were 24%, 45% and 26% for preeclampsia, for early-onset preeclampsia and SGA, respectively.

Conclusions: First-trimester PP13, in combination with maternal characteristics and other serum biomarkers was inadequate for screening purposes and predicting women at risk.

Keywords: placental protein 13, pregnancy, first trimester, biological markers, prenatal diagnosis

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INTRODUCTION

Placental protein 13 (PP13) is a 32-kd dimer protein which was first isolated and characterized in 1983. [1] PP13 is one of 56 known placental proteins, is exclusively produced by the placenta and facilitates trophoblast invasion and maternal artery remodelling. [2, 3] Moreover, expression is induced by syncytialisation of trophoblast cell lines *in vitro* suggesting that the expression is regulated by terminal differentiation. [2, 4-6] The presence of PP13 on the surface of the placenta indicates that PP13 may have haemostatic and immunobiological functions in the placenta. In support of this, aggregates rich in PP13 are associated with zones of T-cell, neutrophil and macrophage accumulation. [2] Moreover, a lysophospholipase function of PP13 may have a possible protective function during implantation and in the maintenance of pregnancy. [4] Although these properties have not been explored there is potential for dysregulation of PP13 to contribute to multiple complications of pregnancy, such as preeclampsia, where aberrant placentation and immunological responses are central to disease pathology.

The strong association of PP13 with the placenta and potential to impact trophoblast function have generated much interest in PP13 as a biomarker of diagnostic merit. [7-15] However, less remarkable predictive properties have been reported in recent studies, with some describing a potential role of PP13 levels for predicting preeclampsia or small for gestational age (SGA) in combination with other biomarkers. [9, 12, 15] Variation in study design, population characteristics, numbers of cases and reporting of the diagnostic accuracy of PP13, have made it difficult to interpret the clinical significance of these results. [9, 12, 15-19] The original objective of this study was to assess normative levels of PP13 in the first trimester of pregnancy and to examine the diagnostic accuracy for predicting adverse pregnancy outcomes in an unselected maternity

population. However, due to the discontinuation in the production of the PP13 assay after March 2011, the analysis was only possible for a small number of cases. Therefore, to increase the robustness of our findings we incorporated the results from our in-house study (Schneuer et al, 2012 [Sch]) and conducted a systematic review and meta-analysis of all relevant studies to: i) assess normative levels of PP13 in the first-trimester of pregnancy; and ii) examine the association and screening performance of PP13 for preeclampsia and SGA.

METHODS

In-house study

A prospective cohort study was conducted of women with a singleton pregnancy attending first trimester Down syndrome screening between July and October 2006 in New South Wales (NSW), Australia. Serum samples were collected by the Pacific Laboratory Medicine Services (PaLMs), an aneuploidy screening service in NSW and then archived and stored at -80°C. Serum samples for this study were thawed and PP13 levels were measured by an automated immunoassay system (AutoDELFIA® PerkinElmer Inc. Turku, Finland). Laboratory technicians were blinded to pregnancy outcomes. Intra-assay and inter-assay coefficients of variation were <11% and the reported analytic sensitivity of the immunoassay was 0.11 pg/ml.

Maternal information and first trimester screening results derived from the laboratory database were combined via record linkage with women's corresponding health records from routinely collected birth and hospital databases to obtain information on the pregnancy and infant outcomes. Birth information was obtained from the Perinatal Data Collection (PDC), a statutory surveillance system of all births in NSW of at least 400 grams birth weight, or at least 20 weeks' gestation. The PDC includes demographic, medical and obstetric information on the mother, labour, delivery and birth outcome. Hospital data were obtained from The Admitted Patient Data Collection (APDC), a census of all patient hospital admissions from NSW, with records for both mothers and liveborn infants. The APDC includes demographic, clinical and health services information for each admission. Probabilistic record linkage of data was conducted independently by the NSW Centre for Health Record Linkage. [20] The study was approved by the NSW Population and Health Services Research Ethics Committee.

The primary outcomes for this study were preeclampsia, early-onset preeclampsia and small for gestational age (SGA). Information on preeclampsia was obtained from the hospital data (ICD10-

AM O11, O14, O15), based on a diagnosis by the attending clinician. Preeclampsia was defined as the onset of hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg) from 20 weeks' gestation onwards accompanied by proteinuria. [21] Early onset preeclampsia was defined as women with preeclampsia requiring delivery at ≤34 weeks gestation. SGA was defined as birthweight less than the 10^{th} and 3^{rd} centiles of the distribution for gestational age by sex based on Australian birthweight charts. [22] Key explanatory variables included maternal age, parity (nulliparous/ multiparous), smoking during pregnancy, maternal weight (kilograms) and previous history of preeclampsia or gestational hypertension. Information on Down syndrome screening biomarkers: free beta human chorionic gonadotropin (free β-hCG) and Pregnancy Associated Plasma Protein A (PAPP-A) were also available for analysis. Gestational age was reported in completed weeks of gestation as determined by the best clinical estimate, including early ultrasound (>97%) and date of last menstrual period. [23]

Comparison of maternal characteristics and raw concentrations of PP13 between women with and without each clinical outcome was performed using contingency tables, t-test analysis or Wilcoxon-rank sum test for categorical, normal or non-normally distributed variables, respectively. PP13 levels were standardized by gestational age, weight and smoking status, using multiple of the median (MoM) as described by Cuckle and Wald. [24] A logarithmic transformation was used to normalize the distribution of PP13 MoM and multivariate logistic regression analysis was conducted to assess the association between log PP13 MoM with each pregnancy outcome adjusting for significant explanatory variables and other relevant biomarkers.

The area under the receiver operating characteristic curve (AUC) was assessed by a traditional academic point system with an AUC result of 1 representing a perfect test, 0.9-<1 an excellent test, 0.8-<0.9 a good test, 0.7-<0.8 a moderate test, 0.6-<0.7 a poor test and 0.5-<0.6 a test with no discriminatory value. [25] For each outcome, we dichotomized women's specific risk using

probability cut-points corresponding to 5% and 10% fixed false positive rates. We then compared the predicted outcome with the observed outcome for all women in the study and calculated estimates of predictive accuracy including sensitivity, specificity, positive (PPV) and negative predictive value (NPV), likelihood ratios (LR) and diagnostic odds ratios with exact binominal confidence intervals. [26] Statistical analyses were performed using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA) and $P<0.05$ values were considered to be statistically significant.

Systematic review and meta-analysis

Structured searches including all languages were completed in PubMed (1990-April 2012), MEDLINE (1950-April 2012), and EMBASE (1966-April 2012) and supplemented by cross-checking reference lists of relevant publications. Key search terms used alone or in combination included: pregnancy, first trimester, gestational age, placenta, blood supply, physiopathology, pregnancy proteins, biological markers, prenatal diagnosis, early diagnosis, galectins/blood, LGALS13 protein, and placental protein 13. Searches were limited to humans and females.

The systematic review focused on observational studies (cohort, cross-sectional, and case-control) that evaluated the accuracy of PP13 to predict preeclampsia (all or early) or SGA. The inclusion criteria for the review were as follows: i) study population included women with singleton pregnancies; ii) PP13 was measured in serum or plasma in the first trimester of pregnancy, defined as ≤14 weeks gestation or mean specimen collection <14 weeks gestation; and iii) PP13 levels measured using the Auto DELFIA assay. Exclusion criteria included case series or reports; guidelines, editorials, abstracts, comments or reviews without original data; animal or in vitro studies, or studies examining cell lines or genetic components.

The abstracts of articles identified from the electronic search were screened and full-text of eligible articles reviewed for inclusion by two independent assessors. Characteristics, data extraction and quality assessment of studies was conducted by each assessor who completed a standard abstraction

sheet. These were then compared and any discrepancies were resolved through discussion consensus. The diagnostic performance of PP13 for study outcomes was examined by extrapolating extracted data at relevant PP13 cut-points to create 2x2 contingency tables and calculating sensitivity, specificity, PPV, NPV and LR. A bivariate, random effects, meta-regression model was used to calculate pooled estimates of sensitivity with 95% confidence intervals (95% CIs) at the 5% and 10% fixed false positives rate. Chi-square test was used to asses heterogeneity between studies ($P<0.05$). These analyses were conducted using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

In-house study

A total of 2,989 samples were tested before the assay became unavailable. Linked health information was available for 2,784 (93.1%) women and we then excluded 106 women whose blood sample was taken before 10 or after 14 weeks gestation, had a medical abortion, twin pregnancy or an infant with major congenital anomalies. Demographic data and PP13 serum levels are described in Table 1. Maternal age was 32.8 (SD 4.6) years, 1216 (45.4%) where nulliparous and 159 (6.1%) smoked during pregnancy. There were 71 (2.7%) women diagnosed with preeclampsia, including 5 (0.2%) with early-onset; and 191 (7.1%) SGA<10^{th} centile and 41 (1.5%) SGA<3^{rd} centile infants. Median PP13 levels for the total population was 52.7 pg/ml (IQR: 37.0-71.0) and median PP13 MoM was 0.98 (IQR: 0.73-1.32). Compared with unaffected pregnancies, median levels of PP13 were significantly lower for women subsequently diagnosed with preeclampsia or a SGA infant (Table 1).

Based on univariate AUC results, the predictive performance of PP13 MoM in early pregnancy for determining adverse pregnancy outcomes was poor for preeclampsia and fair for SGA (Table 2). Performance increased when maternal factors and other serum biomarkers were included; with good detection of more severe outcomes, early-onset preeclampsia and SGA<3^{rd} centile (Table 2). Table 2 also presents diagnostic accuracy of PP13 and highlights that a positive test result from the adjusted models using a 5% false positive rate cut-point was associated with 3.5 to 9-fold increased risk of adverse pregnancy outcomes. Using a 5% false positive rate cut-point, sensitivity of PP13 in predicting preeclampsia and early-onset preeclampsia was 15.5% and 20.4%, respectively. Both PPVs were poor, with only 7.7% and 0.7% women with a positive test developing preeclampsia and early-onset preeclampsia, respectively. Also, positive LRs highlight that women with low PP13 levels would be more than three times more likely to be seen in women with, as opposed to women without, preeclampsia. Sensitivity of PP13 in predicting SGA<10^{th} and SGA<3^{rd} centile was up to

20.4% and 31.7%, respectively, and PPV and LR for SGA infants were slightly better than for preeclampsia (Table 2). The NPV was high for all pregnancy outcomes, particularly for severe cases, indicating that women that do not have low concentrations of PP13 in early pregnancy were unlikely to have subsequent diagnosis of early-onset preeclampsia or SGA<3rd centile infants (Table 2).

Systematic review and meta-analysis

Electronic searches yielded 198 citations, of which eight were considered to be relevant in addition to our in-house study (Figure 1). One article was omitted as two studies were based on the same population and presented similar data, [16, 27] with the article reporting more complete information included. [16] Characteristics of the eight included studies are presented in Table 3. Seven studies assessed predictive accuracy of PP13 with conflicting results. [Sch][9, 12, 15-18]

Of the 8 studies, only 4 reported raw PP13 concentrations in controls for assessment of normative levels in first trimester of pregnancy, [Sch][16, 17, 19] and these were presented in various formats. One study reported a PP13 median of 66.6 pg/ml for controls, [16] and another presented medians by week of gestation for weeks 11-13; with values of 53.5, 63.1, 65.7 pg/ml, respectively; [19] while a study of nulliparous women reported slightly higher levels of PP-13 (77.3 pg/ml). [17] In comparison, median level of PP13 among controls in the in-house study was 53.5 (IQR; 37.7-71.8) pg/ml for all woman, 54.7 (IQR; 39.8-72.1) pg/ml for nulliparous women and 53.9, 54.0, 52.5 pg/ml for gestational week 11-13, respectively.[Sch]

Although, a number of the studies found a significant difference in PP13 MoM levels between cases and controls for preeclampsia, [Sch][12] early-onset preeclampsia [12, 15, 16] and SGA; [Sch][9] three studies found no association between PP13 levels and adverse pregnancy outcomes. [9, 17, 18] In most studies, diagnostic accuracy of each outcome was determined at fixed cut-points of

false positive rates of 5%, 10% and 20%. The corresponding PP13 MoM values at these cut-points were not reported in any of the included studies, but were determined by Schneuer et al, 2011 as 0.46, 0.55 and 0.68; respectively.

Table 4 provides a summary of the PP13 MoM levels, AUC results and overall diagnostic results from the meta-analysis. From the studies that reported AUC results, two reported fair results for preeclampsia, three reported good results for early-onset preeclampsia and two studies reported no discriminative value for predicting these outcomes. The AUC for SGA was good but was only reported by the in-house study. Combined results for sensitivity at 5% (10%) fixed false positive rates highlight that PP13 in combination with maternal characteristics and other biomarkers identify 24% (40%), 45% (56%) and 26% (39%) of pregnancies that will develop preeclampsia, early-onset preeclampsia and SGA, respectively. There was no evidence of between-study heterogeneity for sensitivity results (Table 4).

DISCUSSION

Our in-house study highlights that lower serum levels of PP13 in early pregnancy are associated with increased risks of women developing preeclampsia and having a SGA infant. However, the diagnostic performance of PP13 for preeclampsia and SGA>10th centile, including information on maternal factors and other serum biomarkers was only fair, based on AUC analysis. Results improved for the more severe adverse pregnancy outcomes of early-onset preeclampsia and SGA<3rd centile. The meta-analysis found that of the studies reporting diagnostic performance of PP13, results were comparable with the in-house study. Overall results revealed that for a given 5% false positive rate, a test including PP13, maternal characteristics and other biomarkers would identify around 25% of pregnancies that will develop preeclampsia and SGA and 45% of early-onset preeclampsia cases.

Our in-house study was a large population-based cohort that had corresponding health information available for 93% of samples collected using record linkage of birth outcome data with laboratory results. Strengths of our in-house study were the assessment of a large sample and unselected consecutive cohort of women attending first trimester screening. Record linkage of laboratory to birth and hospital data ensured follow up and ascertainment of pregnancy outcomes with only minimal missing information. Women with missing health information had similar characteristics compared to those included in the study. However, due to the unavailability of the assay it was limited by small numbers of severe cases for assessment.

The advantage of the systematic review and meta-analysis was the ability to undertake a combined analysis of all relevant studies. Thus, that provided with larger numbers of cases and comprehensive assessment of predictive accuracy of PP13. Strengths of the review were that all included studies used a similar gestational age window for testing and the same assay (AutoDELFIA) to analyse the serum samples. We limited our analysis to this assay as it is reported to be a more robust and reproducible, compared with the previous ELISA format, and has greater sensitivity and dynamic range. [28] Additionally, the studies include women of varying characteristics and from diverse geographical locations. However, results from the review and subsequent meta-analysis may be limited as models from each study were adjusted for different maternal characteristics or biomarkers and may not be comparable. One of the main issues of the systematic review was the variation in findings between the studies. Although, no between-study heterogeneity was found in the meta-analysis for each outcome, this is only relevant for the limited studies that reported complete accuracy results. Differences in results may also be explained by study sample size, study design or representativeness of the clinical population. [29] These studies also had small numbers of cases and prevalence of preeclampsia was overestimated, ranging from 4.5 to 18.3%. [9, 15, 16, 19] Most of the studies included in the review were nested case-control and hospital-based studies, therefore, their population may not be representative for screening purposes. Moreover, all studies

used only uncomplicated pregnancies as controls which may be misleading and bias the discriminative ability of PP13 in detecting cases versus controls.[29] There were four prospective cohort studies included in the review (including our own), [12, 17, 18][Sch], although one included only nulliparous women. [17] The other two studies by Odibo et al 2010 and Di Lorenzo et al 2012 had more comparable results to the in-house study; [Sch] although, the latter reported no association between PP13 and preeclampsia with their results based on a model which includes a range of other variables and appears not to reflect the predictive value of PP13. [18]

Variation in normative PP13 levels among controls across different studies also suggests that there may be differences in population characteristics. Studies did not report wether women were taking preventive treatment for preeclampsia or dietary supplements that may mask the defects in endogenous PP13 production. The manipulation of PP13 levels by anti-oxidants (such as Vitamin C) and Non-steroidal Anti-inflammatory Drugs (such as aspirin) would increase the variability of the PP13 measurements in this population and limit diagnostic value. However, it is uncertain whether this may have altered the results of the studies. [30]

Differences between studies and only fair diagnostic accuracy may also be explained by differences in the underlying aetiology of pregnancy outcomes and the biology of PP13. The causes of preeclampsia and SGA are heterogeneous and multifactorial and PP13 levels may be affected in some phenotypes and not in others. [30] The frequent variation in PP13 levels between studies emphasizes the need for a gold standard or established normative levels for comparison, so that concentrations from heterogeneous populations can be tested by an independent marker body or be compared to a reference range. [30] Detection of PP13 may also be variable based on amounts bound to carbohydrates and/or Immunoglobulin E (IgE) bound which could limit access of the antibodies in the assay platform to the antigenic site. Similarly, if the PP13 was shed in

syncytiotrophoblast membranes or placental exosomes [31] it may not be as readily measurable as a unbound dimeric protein.

In conclusion, although studies of predictive accuracy of PP13 were promising, they were limited due to insufficient numbers of cases and accessibility to do further testing. Meta-analysis of studies highlight that accuracy of PP13 combined with other maternal information and serum biomarkers is inadequate for screening purposes and predicting women at risk. Additional biomarkers such as specific maternal blood mRNA of high placental expression [32] or uterine artery Doppler flow [9, 12, 16, 17] have been identified as having potential value and may improve accuracy, but it is unclear whether this would be significant and further studies exploring these are required.

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Table 1: Demographic characteristics of in-house study population, NSW Australia[Sch]

Maternal characteristics	***Unaffected N= 2423 n (%)***	***Early PE N=5 n (%)***	***All PE N=71 n (%)***	***SGA (<3rd) N=41 n (%)***	***SGA(<10th) N=191 n (%)***
Maternal age (years) Mean (SD)	32.8 (4.6)	32.0 (3.4)	32.0 (4.2)	32.7 (5.7)	32.8 (5.3)
Nulliparous	1057 (43.6)	1 (20.0)	46 (64.8)	27 (65.0)	118 (61.8)
Smoking	134 (5.5)	0	1 (1.5)	9 (22.0)	24 (12.6)
Maternal weight (kg) Mean (SD)	67.4 (14.5)	68.4 (6.8)	73.5 (16.6)	59.9 (11.1)	61.1 (12.0)
Preterm birth (<37 weeks)	110 (4.5)	5 (100)	16 (22.9)	2 (4.9)	12 (6.3)
Previous hypertension	160 (6.6)	1 (20)	10 (14.1)	4 (9.8)	8 (4.2)
Infant birthweight(g) Mean (SD)	3582 (492)	1264 (482)	3100 (779)	2487 (269)	2686 (365)
PP13 (pg/ml) Median (IQR)	53.5 (37.7 - 71.8)	44.0 (20.8 - 52.2)	44.0 (32.4 - 65.8)*	40.5 (30.0 - 58.1)*	42.8 (32.9 - 58.2)*
PP13 MoM Median (IQR)	1.00 (0.74 - 1.33)	0.87 (0.42 - 1.03)	0.87 (0.70 - 1.27)	0.80 (0.54 - 0.93)*	0.77 (0.59 – 1.06)*

PE: preeclampsia; SGA: Small for gestational age; SD: Standard deviation; IQR: inter-quartile range; *P-value<0.05 compared with unaffected pregnancies

Table 2: Accuracy of models using PP-13 concentrations in early pregnancy to predict subsequent adverse pregnancy outcomes based on a 5% false positive rate for in-house study [Sch]

Diagnostic accuracy results	SGA<10th centile (n=191)		SGA<3rd centile (n=41)		Preeclampsia (n=71)		Early-onset preeclampsia (n=5)	
	Univariate	Adjusted*	Univariate	Adjusted^	Univariate	Adjusted#	Univariate	Adjusted#
AUC (95% CI)	0.64 (0.60 – 0.69)	0.73 (0.69 – 0.77)	0.65 (0.57 – 0.74)	0.83 (0.78 – 0.88)	0.55 (0.48 – 0.62)	0.72 (0.66 – 0.78)	0.61 (0.26 – 0.96)	0.82 (0.63 – 0.99)
Detected cases	19	39	4	13	4	11	2	1
Sensitivity (95% CI)	9.9 (6.1 – 15.1)	20.4 (14.9 – 26.8)	9.8 (2.7 – 23.1)	31.7 (18.1 – 48.1)	5.6 (1.6 – 13.8)	15.5 (8 – 26)	40.0 (5.3 – 85.3)	20.0 (0.5 – 71.6)
Positive predictive value (95% CI)	13.5 (8.3 – 13.2)	24.2 (17.8 – 31.6)	3.0 (0.8 – 7.5)	9.2 (5 – 15.1)	3.0 (0.8 – 7.4)	7.7 (3.9 – 13.4)	1.5 (0.2 – 5.3)	0.7 (0 – 3.6)
Negative predictive value (95% CI)	93.1 (92 – 94)	93.8 (92.8 – 94.7)	98.5 (97.9 – 98.9)	98.9 (98.4 – 99.2)	97.4 (96.7 – 98.0)	97.6 (97.0 – 98.2)	99.9 (99.6 – 100)	99.8 (99.6 – 100)
Positive likelihood ratio	2.0	4.1	1.9	6.3	1.1	3.1	8.0	3.4
Negative likelihood ratio	0.9	0.8	1.0	0.7	1.0	0.9	0.6	0.8
Diagnostic odds ratio (95% CI)	2.1 (1.3 – 3.5)	4.8 (3.3 – 7.2)	2.0 (0.7 – 5.8)	8.8 (4.4 – 17.3)	1.1 (0.4 – 3.1)	3.5 (1.8 – 6.7)	12.6 (2.1 – 76.1)	4.0 (0.4 – 36.3)

*Adjusted for parity, smoking, weight, age and β-hCG; ^Adjusted for parity, smoking, weight, age, previous hypertension and PAPP-A; # Adjusted for parity, weight, age, previous hypertension and β-hCG; SGA: small for gestational age

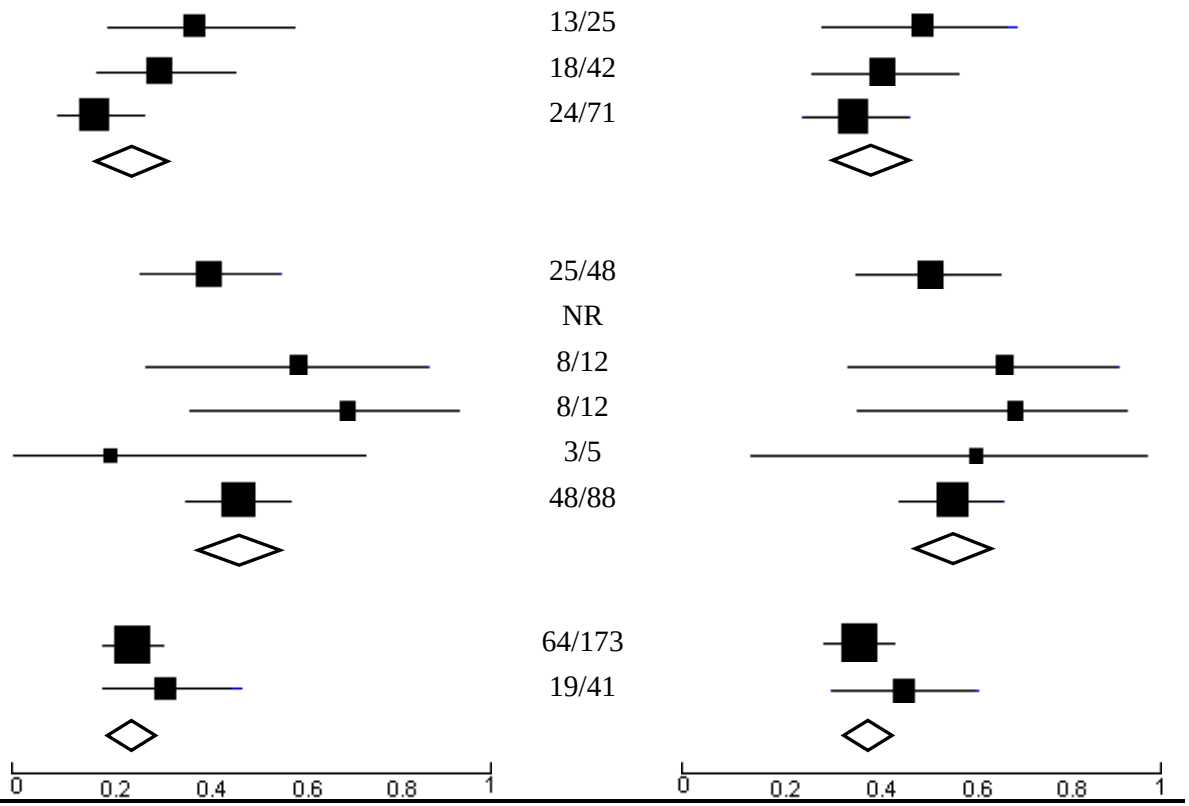
Table 3: Characteristics of studies measuring maternal serum placental protein 13 (PP13) concentrations in first trimester

Reference	Location	Study Design	Participants, study period	Gestational age (weeks)	Study outcomes	Controls
Akolekar et al, 2009* [16]	Kings College Hospital, London, UK	Nested case-control study	Singleton, Mar 2006- Oct 2008	11-13^{+6}	48 early PE (<34 weeks)	416 no complications; and normal outcome matched to the cases for storage time
Audibert et al, 2010* [17]	CHU Sainte-Justine Mother and Child University Hospital, Montreal, Canada	Prospective cohort study	Singleton, nulliparous Nov 2006- June 2008	11-13	40 PE 9 early PE (<34 weeks)	833 unaffected pregnancies
Di Lorenzo et al, 2012 [18]	Institute for Maternal and Child Health, Trieste, Italy	Prospective cohort study	Singleton, Oct 2007-Apr 2009	11-13^{+6}	25 PE 12 early PE (<34 weeks)	2,047 unaffected pregnancies
Karagiannis et al, 2011 [9]	Kings College Hospital, London, UK	Nested case-control study	Singleton, Mar 2006-Sept 2009	11-13^{+6}	173 SGA (<5th centile)	877 no complications, normal outcome, matched to case for storage time
Odibo et al, 2011 [12]	Washington University School of Medicine, St Louis, Missouri, USA	Prospective cohort study	Singleton, Dec 2009-Mar 2010	11-14	42 PE 12 early PE (<34 weeks)	410 unaffected pregnancies (no PE)
Schneuer et al, 2011*# [Sch]	New South Wales, Australia	Prospective cohort study	Singleton, July-Oct, 2006	10-14	71 PE 5 early PE (<34 weeks) 191 SGA (<10th centile) 41 SGA (<3rd centile)	2,423 unaffected pregnancies
Stamatopoulou et al, 2010* [19]	University Hospital Schleswig-Holstein, Kiel, Germany	Nested case-control study	Oct 1998- April 2008	11-13^{+6}	33 PE 15 SGA (<10th centile)	452 normal pregnancies without adverse outcomes, matched for storage time and gestational age
Wortelboer et al, 2010 [15]	Wilhelmina Children's Hospital Utrecht, the Netherlands	Nested case-control study	2004-2006	8-13^{+6}	88 early PE or HELLP syndrome (<34 weeks)	480 no complications during pregnancy, term, same gage at sample date and similar duration of storage time

*Studies assessing normative levels of PP-13 in early pregnancy; PE- preeclampsia, SGA- small for gestational age; # In-house study

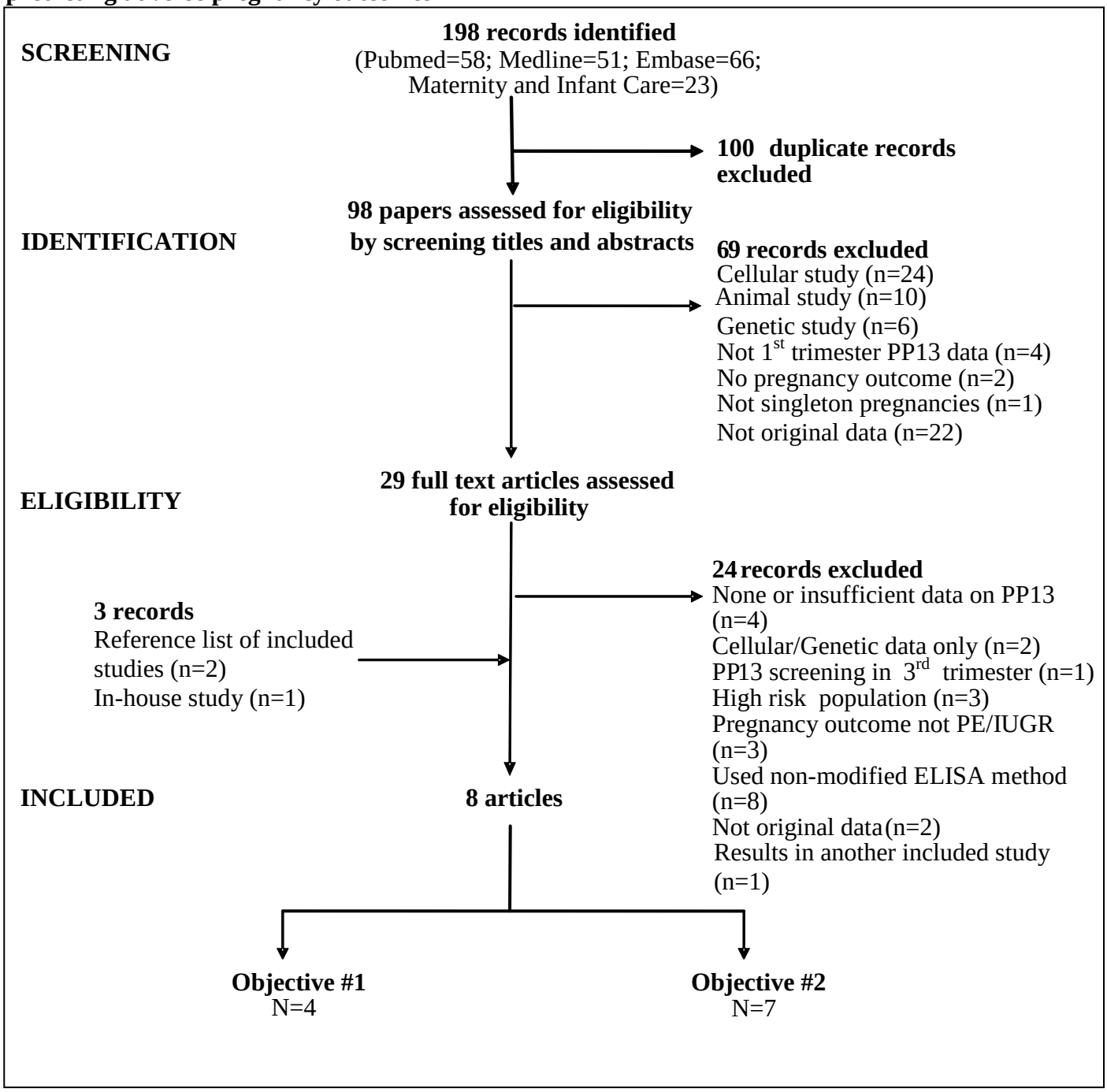
Table 4: AUC and meta-analysis of PP13 in predicting adverse pregnancy outcomes

Outcome Study	**AUC (95% CI)**	**TP/ total cases**	**Sensitivity (95% CI) at 5% FPR**	**TP/ total cases**	**Sensitivity (95% CI) at 10% FPR**
All Preeclampsia					
Audibert et al, 2010	0.52 (0.43 - 0.61)	NR		NR	
Di Lorenzo et al, 2012	NR	9/25		13/25	
Odibo et al, 2011	0.76 (0.62 - 0.82)	13/42		18/42	
Schneuer et al, 2011	0.72 (0.66 - 0.78)	11/71		24/71	
Pooled^					
Early-onset preeclampsia					
Akolekar et al, 2009	0.88 (0.85 - 0.91)	21/48		25/48	
Audibert et al, 2010	0.48 (0.27 - 0.70)	NR		NR	
Di Lorenzo et al, 2012	NR	7/12		8/12	
Odibo et al, 2011	0.85 (0.69 - 1.00)	8/12		8/12	
Schneuer et al, 2011	0.82 (0.63 - 0.99)	1/5		3/5	
Wortelboer et al, 2010	NR	40/88		48/88	
Pooled^					
SGA					
Karagiannis et al, 2011*	NR	43/173		64/173	
Schneuer et al, 2011#	0.83 (0.78 - 0.88)	13/41		19/41	
Pooled^					



SGA: small for gestational age; *Based on SGA <5th centile; # Based on SGA< 3rd centile; NR: not reported AUC: Area under the receiver operating characteristic (ROC) curve; TP: True positives; FPR: False positive rate; ^ Chi-square P>0.05

Figure 1: Flowchart of selection procedure for systematic review of studies of PP13 predicting adverse pregnancy outcomes



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