Prediction and Prevention of Preeclampsia and Other Adverse Pregnancy Outcomes

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Submitted in partial fulfilment of the requirements for the Degree of Doctor of Medicine

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Dedication

To Dan, George, Arthur, Mum, Dad, Nanny and Mr Aquilina

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Abstract

Aim

To assess current methods of prediction of adverse pregnancy outcomes, develop a prediction model and assess diet and life style in preventing preeclampsia.

Methods

Meta-analyses performed to assess the role of abnormal 1st trimester biomarker levels in predicting PE and the predictive accuracy of 2nd trimester UAD indices for stillbirth. A prospective observational study was performed to assess the efficacy of maternal characteristics, biomarkers, arteriography and UADs for predicting adverse pregnancy outcomes. Previously published 1st trimester PE prediction models were validated using data collected from the observational study. A systematic review on the effect of diet and life style based metabolic risk modifying interventions on PE was performed.

Results

The review of biomarkers found that abnormal levels were particularly associated with early onset PE. The stillbirth review demonstrated a three-four fold increased risk of still birth with abnormal UAD. 1045 women were included for analysis in the prospective observational study. Our models' detection rate (false positive rate of 15%) was 72% for PE; 48% PIH; 30 % SGA <10th centile; 57% SGA <5th centile and 67% stillbirth.

In the validation study the observed discrimination ability in the derivation studies ranged from 0.70 to 0.954. When validated against the study cohort, the AUC varied importantly, ranging from 0.504 to 0.833. Dietary interventions were shown to reduce

the risk of PE by 33%, with no reduction in risk with mixed interventions or fatty acid supplementation.

Conclusion

The high heterogeneity of studies in the systematic reviews makes it difficult to draw firm conclusions regarding the use of biomarkers or UADs in screening for pregnancy complications. Our prospective study showed a role for haemodynamics as part of routine 1st trimester screening for assessing the risk of hypertensive disease in pregnancy.

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List of Abbreviations

ADAM-12	A Disintegren and Metalloprotease 12
AFP	Alpha-fetoprotein
Aix	Augmentation index
AUC	Area under the curve
CI	Confidence interval
CMACE	Centre for Maternal and child enquiries
DNA	Deoxyribose nucleic acid
DOR	Diagnostic odds ratio
FPR	False positive rate
GDM	Gestational Diabetes Mellitus
I2	Heterogeneity
IGF	Insulin like growth factor
IQR	Interquartile range
IUD	Intra-uterine Death
IUGR	Intrauterine growth restriction
LR	Likelihood
LBW	Low birth weight
MAP	Mean arterial pressure

MoM	Multiples of the median
MOOSE	Meta-analysis of Observational Studies in Epidemiology
MSAFP	Maternal serum AFP
NICE	National Institute for Clinical Excellence
OR	Odds ratio
PAPP-A	Pregnancy associated plasma protein-A
PE	Preeclampsia
PI	Pulsatility Index
PIH	Pregnancy induced hypertension
PlGF	Placental growth factor
PP13	Placental protein 13
PRISMA	Preferred Reporting Items for systematic reviews and meta-analyses
PSEP	Percentage of separation between the estimated mean of 4^{th} and 1^{st} quartiles of probabilities
PWV	Pulse wave velocity
PWV_{AO}	Pulse wave velocity in the aorta
QUADAS-2	Quality assessment of diagnostic accuracy studies-2

RI	Resistance index
ROC	Receiver operating curve
RR	Relative risk
SBP_{AO}	Systolic blood pressure in the aorta
SD ratio	Systolic/Diastolic Ratio
Sflt-1	Soluble fms-like tyrosine kinase-1
SGA	Small for gestational age
SVEGFR-1	Soluble vascular endothelial growth factor-1
UAD	Uterine artery Doppler
VEGF	Vascular endothelial growth factor
β-hCG	Beta human chorionic gonadotrophin

Chapter 1: Introduction

PE affects around 5% of pregnancies and is a major cause of maternal and perinatal morbidity and mortality.(1) It is a multisystem disease characterised by blood pressure ≥140/90mmHg and protein in the urine (0.3g/24h) after 20 weeks gestation. The only definitive treatment for PE is delivery. An important part of management for the obstetrician is deciding the appropriate time for delivery based on risks of the complications of PE developing, such as HELLP (haemolysis, elevated liver enzymes, low platelets), eclampsia and placental abruption; versus the risks of preterm delivery to the baby.

The last MBRRACE report (published December 2017) showed a maternal mortality rate of 0.13/100 0000 with 3 deaths from PE between 2013-2015.(2) The management of PE and its associated complications gives rise to large health care costs (approximately £9000/person).(3) Patients are often admitted in a tertiary care facility and may need care in an intensive care unit. The neonatal mortality and morbidity associated with the disease is high due to preterm delivery, growth restriction and stillbirth.

Modern medicine is increasingly focusing on preventing disease. Prediction and prevention of complications in pregnancy is an important part of routine antenatal care enabling interventions and treatment to be allocated to those that need it and reducing costs and the potential burden of unnecessary tests and treatments to women whom don't need it. The National Collaborating centre for Women's and Children's Health has issued guidelines on routine prenatal care recommending that at the first visit a

woman's level of risk for PE should be evaluated by a series of maternal characteristics for example age and weight, to identify those at high risk and allow intensive monitoring.(4) However this approach would falsely classify two thirds of women as being high risk and in need of intensive monitoring and prophylactic aspirin therapy, highlighting the need for a better screening test.(5)

The placenta is crucial to the development of preeclampsia. (6) PE is associated with poor placentation and incomplete remodelling of the utero-placental spiral arteries. In normal pregnancy the maternal spiral arteries are converted from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control.(7, 8) In pregnancies complicated by PE, placental abruption and birth of SGA neonates, there is histological evidence of impaired trophoblastic invasion of the spiral arteries and the persistence of high impedance in the uterine vessels, resulting in reduced utero-placental blood flow and placental hypoxia. (9-11)

A number of angiogenic factors have been implicated in the pathogenesis of preeclampsia. Persistent placental hypoxia induces the expression of angiotensin II type I receptor agonistic autoantibodies (AT1-AA) which stimulate the production of sFlt-1, sENG and endothelin-1.(6, 12) sFlt-1 and sENG are anti-angiogenic factors produced by the placenta which are shown to be increased in pregnancies affected by preeclampsia.(13, 14) Soluble sFlt-1 is a splice variant of VEGF receptor-1, which binds to circulating PlGF and VEGF inhibiting their actions and decreasing their concentrations in the uteroplacental and maternal circulation.(15, 16) These angiogenic factors play an essential role in vascular remodelling, consequently a fall in their levels leads to endothelial dysfunction and development of the clinical symptoms of the disease.(17-21)(22-24) The defective angiogenesis leads to the failure of

cytotrophoblasts to convert from an epithelial to an endothelial phenotype and invade maternal spiral arteries.(23) Zhou et al. demonstrated that exogenous sFlt-1 inhibits placental cytotrophoblast invasion in vitro. (23) Maynard et al. showed that exogenous sFlt-1 administered to pregnant rats induces hypertension, proteinuria and glomerular endotheliosis. VEGF also induces nitric oxide and vasodilatory prostacyclins in endothelial cells, suggesting a role in decreasing vascular tone and blood pressure. (22, 25, 26)

Maternal blood levels of sFlt-1, PlGF and VEGF have been shown to correlate with the severity of preeclampsia, with increasing levels of sFlt-1 and decreasing levels of VEGF and PlGF, associated with more severe and early onset disease.(27) Khalil et al. showed that placental concentrations of sFlt-1, sEng and PlGF mirrored maternal serum changes, consistent with the view that the placenta is the main source of these factors in pregnancy.(28, 29) Abnormal levels of these markers can be measured prior to the onset of disease. Decreased levels of PlGF and VEGF have been shown from the end of the first trimester in women that go on to develop preeclampsia.(15) Increased levels of sFlt-1 are not seen until the second trimester.(12, 30, 31) The ratio of sFlt-1/PlGF increases from around 5 weeks prior to disease onset and has been proposed as a predictive tool for preeclampsia.(15, 22) The SaPPPhirE study demonstrated the ratio to have a pooled sensitivity of 80% and specificity of 92%, positive LR of 10.5 and negative LR of 0.22 in predicting preeclampsia in low and high risk women.(16) Currently NICE have recommended measuring the ratio to rule out preeclampsia in women presenting with suspected preeclampsia between 20-34+6 weeks gestation.(32) Khalil et al. looked at longitudinal changes in maternal serum PlGF and sFlt-1 in women at increased risk of preeclampsia. They showed PlGF levels to be significantly lower from 11 and 13 weeks onwards in women who developed early

and late PE respectively. They also showed an increase in the sFlt-1/PlGF ratio from 11 weeks gestation in the early PE group.(12)

In normal pregnancy PlGF steadily increases during the 1st two trimesters and peaks at 29-32 weeks and then declines. In PE PlGF concentrations start to decrease 9-11 weeks before the onset of hypertension and proteinuria.(33) Ghosh et al looked at PlGF and UADs in the early 2nd trimester as a screening tool for PE and individually they were strong predictors for PE but in combination their association with PE was not significant.(33)

Previous studies have examined the use of UADs, maternal characteristics and different biochemical markers either in combination or alone to improve the detection of pregnancies at high risk of complications from poor placentation.(5, 33-37) A systematic review assessing the use of 1st trimester UADs for predicting adverse pregnancy outcome showed high specificities for predicting PE and intrauterine growth restriction but low sensitivities.(38) Many potential biomarkers have been identified but findings have been relatively inconsistent between studies. Detection rates for combinations of multiple markers have varied between 38-100%.(39) There's therefore a need for further studies to assess the predictive value of different markers and find the best combination. (27, 39)

Evidence for an association of raised AFP with PE and other adverse pregnancy outcomes is conflicting.(35, 40) Costa et al. 2008 showed that 2nd trimester AFP levels were significantly higher in a population that went on to have an adverse pregnancy outcome. (40) Whereas Kang et al. 2008 did not see any significant difference.(35) Currently the majority of studies have focused on 2nd trimester UADs and AFP. We

have shown that raised maternal serum AFP in the 2nd trimester is associated with a significant increase in the risk of developing PE and that detection can be improved when combined with 2nd trimester UADs. (41, 42) However, interventions to reduce the incidence of PE have greatest benefit when they are commenced in the 1st trimester indicating the need for an effective method of 1st trimester screening.(43)

Kisspeptin-54 (metastin) was first identified as a suppressor of tumour metastasis and has been described in abundance in the placenta. As levels rise dramatically from 8 weeks gestation coinciding with the time of peak trophoblastic invasion it is thought to play a key role in implantation and development. A small study measured kisspeptin levels between 8-14 weeks of gestation and found that levels were lower in women that had SGA neonates.(44) A subsequent study measured kisspeptin levels at 16-20 weeks gestation and looked at the association with SGA and PE and also found that kisspeptin levels were significantly lower indicating that kisspeptin levels are reduced in conditions associated with poor placentation. (45)

Women who develop PE are at an increased risk of cardiovascular disease in later life.(46) Cardiovascular disease is associated with increased central systolic blood pressure and arterial stiffness. In women with established PE there is an increase in central aortic systolic blood pressure, pulse wave velocity and augmentation index which are measures of arterial stiffness. This increased arterial stiffness has been detected as early as eleven weeks gestation and can be measured non-invasively by using an Arteriograph.(47)

A screening test with adequate sensitivity and specificity that can be performed in the 1st trimester would be the ideal, in order to allow intervention at an earlier stage when it

is likely to be more effective. Implementation of an adequately sensitive and specific screening test would also enable further investigation into more preventative strategies. The current main prophylactic treatment for PE is aspirin. Extensive randomised studies have shown prophylactic low dose aspirin to be associated with a ten percent reduction in PE however a systematic review performed by Bujold et al. has shown 50% risk reduction if treatment if commenced before 16 weeks gestation. (43, 48) The recently published findings of the ASPRE trial showed the administration of low dose aspirin to women at high risk of preterm preeclampsia resulted in a lower incidence of the disease than placebo. (49)

There has been a rise in the number of prognostic models being developed in obstetrics, particularly for PE, however few seem to have been widely implemented. A recent systematic review by Kleinrouweler et al. identified 69 prediction models for PE, only 5 of these had been externally validated and model performance was found to be lower when externally rather than internally validated. They recommended that systematic reviews should be performed to identify and validate existing models to decide whether a new model should be developed or an existing model updated. (50)

Other adverse pregnancy outcomes such as stillbirth and intra-uterine growth restriction are also associated with poor placentation with 60% of stillbirths being linked to placental complications. (51) The stillbirth rate in the UK is currently 5.1 per 1000 and whilst there has been a downward trend over the past ten years, the UK has one of the highest stillbirth rates in the developed world. (52) There is a huge need to reduce this rate particularly focussing on those occurring closer to full term.

The RCOG have published a guideline on the investigation and management of the SGA fetus recommending that women who have 3 or more minor risk factors have

uterine artery Dopplers performed between 20-24 weeks and serial growth scans if these are abnormal. In the guideline PAPP-A<0.415 is considered a major risk factor and it is recommended that these women have serial growth scans regardless of uterine artery Doppler results.(53) Benton et al. looked at maternal plasma PlGF levels and placental histology in women with suspected fetal growth restriction. They found low PlGF identified placental fetal growth restriction with a 98.2% sensitivity and 75.1% specificity. This is clinically relevant as many fetuses are small due to constitutional factors and are otherwise well and at low risk of complications and could be managed expectantly. Conversely, those that are small due to placental problems are at high risk of stillbirth and serious neonatal complications. The ability to discriminate between those that are constitutionally small and those in which there is underlying placental pathology would enable improved management, focusing on those that are at risk of adverse perinatal outcome and reducing the number of scans and unnecessary interventions for those that are constitutionally small. (54)

There is currently no routine screening test or guidance for predicting pregnancies at risk of stillbirth. A systematic review of biomarker and ultrasonic tests noted that none of 16 single, or 5 combined tests, did well as predictors of stillbirth. However, stillbirth attributed to placental dysfunction was moderately to strongly associated with low first trimester PAPP-A and abnormal uterine artery Doppler velocimetry in the second trimester.(55) Akolekar et al. demonstrated that the addition of PlGF improves the performance of screening by maternal factors and other biomarkers. They also found that whilst PAPP-A on its own or with UAD and ductus venosus pulsatility is useful in predicting stillbirth, its contribution was not significant once PlGF was added to the model. Overall their model could predict 42% of all stillbirths and 61% of those due to impaired placentation.(56)

Section 1.1 Aims of thesis

My thesis aims to develop a screening test for the prediction of PE and other adverse pregnancy outcomes as a result of poor placentation; and to validate pre-existing PE models. It also aims to find further preventative therapies. It will address these through the following objectives:

A. To determine the accuracy of biomarkers for the prediction of women at risk of PE and other adverse pregnancy outcomes.

B. To determine the accuracy of UADs for the prediction of PE and other adverse pregnancy outcomes

C. To evaluate the predictive value of maternal characteristics and arteriography for the prediction of PE and other adverse pregnancy outcomes

D. To validate pre-existing PE models

E. To perform systematic reviews assessing the effectiveness of biomarkers, UADs and diet and lifestyle interventions in the prediction and prevention of PE and other adverse pregnancy outcomes

The specific research questions that I have attempted to answer in this thesis are given below and summarised in a structured format in table 1.1.

- Can 1st trimester maternal AFP, placental growth factor, Pregnancy associated plasma protein-A, beta-hCG and UADs in combination with maternal characteristics and arteriography be used for the prediction of women at risk of PE and other adverse pregnancy outcomes?

- Can serial PlGF/AFP levels be used to predict pregnancies affected by PE and other adverse outcomes?
- Can kisspeptin be used as a biomarker for prediction of PE and other adverse pregnancy outcomes?
- Can my primary study data be used to validate pre-existing PE models?
- How accurate are 1st trimester blood biochemical markers for predicting PE?
- Can UADs predict stillbirth?
- Can diet and life style based metabolic risk modifying interventions reduce the incidence of PE?

Table 1.1: Structured Questions for each chapter of this thesis

Chapter Number	**Population**	**Intervention or Test**	**Outcomes**	**Research Design**
Objective A: To determine the accuracy of biomarkers for the prediction of women at risk of PE and other adverse pregnancy outcomes				
5	Pregnant women attending for 1st trimester scan	Maternal blood levels of AFP, PlGF, Kisspeptin Serial levels of PlGF at 20, 28 and 34-36 weeks	PE PIH Placental Abruption Stillbirth SGA GDM	Prospective observational study
Objective B: To determine the accuracy of UADs for the prediction of PE and other adverse pregnancy outcomes				
5	Pregnant women attending for 1st trimester scan	1st trimester UADs	PE PIH Placental Abruption Stillbirth	Prospective observational study

			SGA GDM	
Objective C: To evaluate the predictive value of maternal characteristics and arteriography for the prediction of PE and other adverse pregnancy outcomes				
5	Pregnant women attending for 1st trimester scan	Maternal history and arteriography measured in the 1st trimester	PE PIH Placental Abruption Stillbirth SGA GDM	Prospective observational study
Objective D: To validate pre-existing PE models				
6	Pregnant women in the 1st trimester	Biomarkers, UADs, arteriography, mean arterial pressure	PE	Review of pre-existing PE models and validation of data with my primary study data
Objective E: To perform systematic reviews assessing the effectiveness of biomarkers, UADs and diet and lifestyle interventions in the prediction and prevention of PE and other adverse pregnancy outcomes				
3	Pregnant women in the 1st trimester	Measurement of any blood biomarkers	PE	Systematic review and meta-analysis
4	Pregnant women in the first and 2nd trimester	Measurement of UADs	Stillbirth	Systematic review and meta-analysis
7	All pregnant women	Diet and lifestyle interventions -Fibre -Essential fatty acids -Physical Activity -Dietary advice	PE	Systematic review and meta-analysis

Chapter 2: Methodology

Systematic Reviews

Systematic reviews were carried out with a prospective protocol in line with the current Cochrane recommendations. For reviews containing randomised controlled trials the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines were adhered to and for those containing observational studies MOOSE (Meta-analysis of Observational Studies) guidelines were followed.(57, 58)

Section 2.1 Systematic reviews of Effectiveness (Metabolic risk modifying agents, Biomarkers)

Study Identification and Selection

Questions were focused and well defined detailing population, intervention/test, comparison and study design. Major electronic databases were searched including MEDLINE, EMBASE and Cochrane. Hand searches of reference lists were also performed to identify any relevant articles that were not captured by the electronic searches. Language restrictions were not applied. Study selection was performed in two stages. First the electronic searches were scrutinized and appropriate studies were identified by two reviewers. Copies of the full manuscripts of all citations that were likely to meet the selection criteria were obtained. The full text of these was assessed by both reviewers independently and studies that fulfilled the inclusion criteria were selected. Any disagreements were resolved by discussion with a third reviewer.

Study quality assessment and data extraction

The quality of the selected primary randomized controlled trials was evaluated based on accepted contemporary standards.(59) The risk of bias of the individual studies was assessed in six domains including sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and other potential sources of bias. The quality of observational studies; case-control and cohort, were assessed using the Newcastle-Ottawa scale.(60) Included studies were also assessed for consistency of results (heterogeneity). Individual studies were described by study type, intervention, numbers participating, population examined and study quality. Data was extracted in duplicate using pre-designed data extraction forms.

Data synthesis

Results were summarised as pooled relative risks (RR) or odds ratios (OR) with 95% confidence intervals. Heterogeneity was evaluated and if substantial heterogeneity was noted (I^2>50%) possible causes were explored. To explore for sources of heterogeneity subgroup analyses were performed to see whether variations in clinical factors e.g. population, intervention or study quality affected the estimation of effect. We performed univariable meta-regression analyses followed by multivariable analysis to control for confounding among variables. Sensitivity analysis was also performed. Publication bias was explored for using Harbord's or Eggers test. All analyses were carried out with REVMAN 5.0 and STATA version 12.1 statistical software.

Section 2.2 Systematic Reviews of Test Accuracy (Stillbirth and Doppler review)

Systematic reviews of test accuracy were performed using established methodology in line with the recommendations of the Cochrane Collaboration including those of the Cochrane Methods Working Group on Screening and Diagnostic tests.

Study Identification and Selection

This was performed as detailed in section 1.

Study quality assessment and data extraction

Methodological quality of the selected primary studies was performed using Quality Assessment of Diagnostic Accuracy Studies criteria (QUADAS-2), which consists of four key domains: patient selection, index test, reference standard, flow and timing, which was adapted to the topic as necessary.(61) Each of these domains is assessed for risk of bias and concerns regarding applicability and classified as low or high risk or unclear. Data extraction was performed in duplicate by 2 reviewers on to pre-designed data extraction forms.

Data Synthesis

Sensitivities, specificities and LRs with their 95% confidence intervals for individual studies were derived. A multilevel bivariate random effects model as implemented in the mentandi user-written command developed for Stata statistical software was used to obtain summary estimates of sensitivity and specificity. (62) From these, corresponding LRs and their 95% confidence intervals were also obtained.(63) This model also estimates the correlation between sensitivity and specificity as a random

parameter to represent the counterbalance between sensitivity and specificity due to threshold effect.

Section 2.3 Primary Study

This was a prospective observational study recruiting pregnant women attending the Royal London hospital in the 1st trimester of pregnancy. Written informed consent was obtained from those women agreeing to participate in the study and ethics approval was granted from the East of England ethics committee.

Maternal history and characteristics

Information was collected on age, ethnicity, method of conception, parity, smoking, alcohol and drug use, past medical and obstetric history, family history and drug history. Maternal weight and height were measured and body mass index calculated.

Exclusion Criteria

Women with multiple pregnancies or those affected by chromosomal or structural abnormalities, pregnancy loss under 24 weeks or gestational age more than 14 weeks were excluded.

Arteriograph measurements

Measurements were performed with women in the supine position. The parallel straight line distance between the suprasternal notch and symphysis pubis was measured (Jug-Sy) with a tape measure. This measurement provides an indirect measure of the aortic length. The arteriograph cuff was applied over the brachial artery for

measurement of aortic systolic blood pressure (SBP_{AO} (mmHg)), pulse wave velocity (PWV (m/s)), augmentation index (AIx (%)) and mean arterial pressure (mmHg). The cuff records the early (direct) systolic wave (P1), late (reflected) systolic wave (P2) and diastolic waves (P3) secondary to central pressure changes. The Arteriograph first measures the systolic and diastolic blood pressure oscillometrically. The cuff is then deflated and re-inflated to measure the diastolic pressure and supra-systolic pressure (measured SBP plus 35mmHg). The pressure fluctuations in the brachial artery are detected by the cuff and the signals transmitted wirelessly to a laptop computer which contains software for analysis.

The AIx% was calculated by dividing the pressure difference between the first forward wave due to systole and the second reflected wave (P2-P1) by the pulse pressure [AIx%=(P2-P1)x100/PP]. The aortic PWV (PWV_{AO}) was calculated by dividing the distance between the suprasternal notch and the upper border of the symphysis pubis in metres by the time interval between the onset of the first systolic wave and the onset of the second reflected wave in seconds. Central SBP was estimated based on the relationship between the brachial and SBP_{AO} in relation to the late systolic wave amplitude.

All recordings were made by the same researcher. Results were not given to the women or their doctors and therefore did not influence subsequent care.

UAD measurements

Transabdominal ultrasound was used to measure the uterine artery flow velocity waveforms with colour pulsed Doppler using a Voluson E6 with a 3.5/5 MHz linear array. The high pass filter was set at 100Hz. With the power output kept within safety

guidelines. A sagittal section of the uterus was obtained and the cervical canal and internal cervical os identified. The transducer was then tilted from side to side and colour flow mapping used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os. Pulsed Doppler was then applied with the sampling gate set at 2mm to cover the whole vessel and a reproducible uterine artery waveform was obtained, using an angle of insonation less than 30°. Peak velocity of each waveform needed to be ≥60m/sec. UAD waveforms were obtained from both uterine arteries. In each artery the Pulsatility Index (PI), Resistance index, SD ratios and the presence or absence of a diastolic notch was recorded.

All recordings were made by the same researcher. Results were not given to the women or their doctors and therefore did not influence subsequent care.

Serum biomarkers

Maternal serum was taken and stored for later analysis for the biomarkers AFP and PlGF. Immediate analysis for PAPP-A, β-hCG and kisspeptin were performed.

Outcome measures

The diagnosis of PE and gestational hypertension was made according to the International Society for the Study of Hypertension in Pregnancy criteria. Gestational hypertension is characterised by a systolic blood pressure ≥140mmHg and /or diastolic blood pressure ≥90mmHg on at least two occasions four hours apart developing after 20 weeks gestation with no significant proteinuria. PE is characterised by gestational hypertension with proteinuria ≥300mg in 24hours or 30mg/mmol on urine protein creatinine ratio. Other outcomes examined were stillbirth, defined as fetal death after 24 weeks gestation, placental abruption, gestational diabetes mellitus defined as a

fasting blood glucose level ≥5.6mmol/l or a 2 hour plasma glucose level≥7.8mmol/L and SGA less than the 5th or 10th centiles on customised growth charts.(64)

Statistical Analysis

Haemodynamic parameters and maternal serum biomarkers were converted into multiples of the median (MoM). Logistic regression with stepwise selection was performed to determine multivariate models in the prediction of PE, PIH, stillbirth, SGA <10th and 5th centile. Two final models were used to construct ROC curves and detection rates for specified false-positive rates, and areas under the curve (AUC) were calculated.

Section 2.4 Validation Section

Validation study population

The validation study population was obtained as detailed in section 3. A further cohort of women obtained from previous similar research was added to this population in order to increase numbers for analysis and enable comparison of the models performance in the two groups.

Prognostic models and study selection

Medline was searched from inception until November 2016 without language restrictions. Models were selected for validation if they met the criteria detailed in table 6.1. Models were excluded if they did not include 1 or more of the predictors and if they did not have a regression coefficient.

Quality assessment of prognostic models and data extraction

The Quality in Prognosis studies (QUIPS) tool was used to assess the risk of bias in the included prognostic studies. The bias domains assessed were study participation, attrition, prognostic factor measurement, confounding measurement and account, outcome measurement and analysis and reporting. Each domain was assessed as either low, high or moderate risk of bias. (65) Individual studies were described by study design, variables measured, numbers participating and the number of PE events.

Data synthesis

Baseline characteristics of the validation cohort were described using median and interquartile ranges (IQR) and absolute and relative frequencies. Baseline clinical and demographic characteristics of the women recruited in the two cohorts used in the validation process were compared. We used Mann-Whitney U tests and Chi-squared tests or Fisher exact tests when needed for these comparisons. The comparison of both cohorts was further extended to evaluate the predictive performance of the models within the two groups of women included in the validation cohort.

Validation of the models was performed by applying the regression coefficients that were published in the different derivation studies to our validation population. We obtained predicted probabilities of any PE for all models.

Each models discrimination ability and its calibration were assessed using the area under the ROC curve. AUCs in the validation cohort were compared with the AUC reported by authors of the original models using a Z-test. (66) Evaluation of model calibration was performed by the Hosmer-Lemeshow goodness-of-fit test.

All the analyses were carried out with Stata v13.

Chapter 3: Abnormal blood biomarkers in early pregnancy are associated with PE: A systematic review of effectiveness

3.1 Abstract

Objective

To evaluate the strength of association between abnormal levels of 1st trimester maternal blood biomarkers and the risk of PE.

Methods

Systematic review of literature that assessed the association between any abnormal maternal blood biomarker in the 1st trimester and PE with meta-analysis. Results were summarized as pooled odds ratios with 95% confidence intervals.

Results

Thirty studies (65 538 women) were identified for inclusion. Twenty four studies assessed PE of any onset, 10 studied early onset PE and seven evaluated late onset PE (after 34 weeks of gestation). The biomarkers PAPP-A (OR 2.1, 95% CI 1.6, 2.6), PP13 (OR 4.4, 95% CI 2.9, 6.8), sFlt-1 (OR 1.3, 95% CI 2.9, 6.8), pentraxin (OR 5.3, 95% CI 1.9, 15.0) and inhibin-A (OR 3.6, 95% CI 1.7, 7.6) were significantly associated with any PE. The odds of early onset PE were significantly increased when the biomarkers PlGF (OR 3.4, 95% CI 1.6, 7.2), PAPP-A (OR 4.8, 95% CI 2.5, 22.5), PP13 (OR 7.5, 95% CI 2.5, 22.5), soluble endoglin (OR 18.5, 95% CI 8.4, 41.0) and inhibin-A (OR 4.1, 95% CI 1.9, 8.8) were abnormal. Two biomarkers, soluble endoglin (OR 2.1, 95% CI 1.9, 2.4) and inhibin-A (OR 1.9, 95% CI 1.4, 2.8) were significantly associated with late onset PE.

Conclusion

Abnormal levels of maternal blood biomarkers in the 1st trimester are significantly associated with PE, particularly early onset disease.

Citation of paper arising from this work

Allen RE, Rogozinska E, Cleverly K, Aquilina J, Thangaratinam S. Abnormal blood biomarkers in early pregnancy are associated with PE: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2014; 182:194-201

3.2 Introduction

The National Institute of Clinical Excellence (NICE) recommends routine early pregnancy assessment for risk of PE based on maternal history, and to offer low dose aspirin to those at high risk with early commencement of interventions having most benefit.(4, 48) Previous studies have demonstrated low detection rates for PE with a screening strategy based on maternal history alone, highlighting the need for an effective 1st trimester screening test to allow early delivery of preventative therapies. (67, 68)

Numerous studies have evaluated the role of various placental factors in the early prediction of PE. (69-94) Impaired placental perfusion is thought to lead to placental ischaemia and damage with the release of inflammatory factors contributing to clinical symptoms of the disease. (83-87) Although primary studies have evaluated the role of various biomarkers in early pregnancy, the overall strength of association between abnormal biomarkers in early pregnancy and PE is not clear. (95) In addition the relationship between maternal blood biomarkers and the timing of PE onset needs to be evaluated.

We undertook a systematic review and meta-analysis to evaluate the association between maternal serum biomarkers in the 1st trimester and the development of PE.

3.3 Methods

Identification of studies

We performed a systematic review with a prospective protocol in line with current recommendations. (59) We searched Cochrane, Embase and Medline databases from database inception up to April 2013 to identify relevant citations. Reference lists of all known primary and review articles were examined to identify cited articles that were not captured by the electronic searches. We contacted the authors of primary studies if relevant data were not reported. Language restrictions were not applied. We used the following search term combination and their word variants: 1st trimester AND biomarker (PlGF, β-hCG, AFP, PAPP-A, nitric oxide, SVEGFR-1, sflt-1, inhibin-a, unconjugated oestriol, endoglin, activin-a, PP13, ADAM-12, dimethylarginine, pentraxin-3, P-selectin, adrenomedullin, visfatin, cell free DNA, cell free fetal DNA) AND preeclampsia.

Study selection and data extraction

The eligibility of studies was based on those that had assessed the association between any of the serum or plasma biomarkers in the 1st trimester and PE. Acceptable reference standards for PE were persistent high systolic (≥140mmHg) or diastolic (≥90mmHg) blood pressure and proteinuria (>0.3g/24 hours or a dipstick result of >1+, equivalent to 30mg/dl in a single urine sample or spot urine protein/creatinine ratio >30mg protein/mmol creatinine) of new onset after 20 weeks of gestation, according to the International Society for the Study of Hypertension in Pregnancy criteria. Included studies needed to describe the occurrence of PE conditional on the test result as means and standard deviations for continuous outcomes or in a manner that 2x2 classification tables could be constructed for dichotomous outcomes.(95)

Study selection was performed in two stages as detailed in our paper. (95) The electronic searches were examined and appropriate studies identified. Two independent reviewers (RA and KC) reviewed the full text of identified papers, selected those that fulfilled the inclusion criteria and performed data extraction. Disagreements were resolved with input from a third reviewer (ST). If there were multiple publications of one dataset we only included the most recent or complete paper.

Quality assessment of the included studies

Selected studies methodological quality was assessed using the Newcastle-Ottawa scale.(60) For case control studies, we assessed the risk of bias in: the definition, selection and representativeness of the cases and controls; the comparability of the groups; and the ascertainment and completeness of exposure. For the cohort studies, we evaluated the risk of bias in: the selection and representativeness of the cohorts; their comparability; the exposure and outcome assessment; and the completeness of follow up. Studies were considered to have a low risk of bias if they scored 4 stars for selection, 2 for comparability or 3 stars for exposure or outcome. Studies that scored 0 or 1 star for selection, 0 stars for comparability or 0 or 1 star for exposure or outcome were considered to have high risk of bias. Studies scoring between these were regarded to have medium risk of bias. (95)

Data Synthesis

Data synthesis was performed as documented in our previous paper. Results were summarised as odds ratios with 95% confidence intervals. When the marker concentration was provided as a continuous variable, we expressed the results as standardised mean differences with standard deviation. Results were pooled using an inverse variance weighted random effect approach. Both continuous and dichotomous

data were meta-analysed by converting odds ratios to effect size. This enabled the results to be summarised without loss of data and prevented data being misleading. (96) Publication bias was assessed by funnel plot analysis using Eggers test to evaluate for asymmetry. All analyses were performed with Revman 5.0 and Stata 12.1 statistical software.

3.4 Results

Characteristics of the included studies

1,071 relevant citations were identified with full copies of 88 studies retrieved for further assessment. After full evaluation, 30 studies were included in the review (Fig 3.1, appendix 3.1). (30, 69-94, 97-99) of which seventeen (17/30, 57%) were case control studies and 13 (13/30, 43%) cohort studies. Sample sizes ranged from 45 to 47,922. The markers evaluated in these studies are detailed in figure 3.1.

Fifty percent of the studies specified in detail the inclusion and exclusion criteria. Ten studies were on low risk women and eight included high risk women. The high risk studies included women with previous PE, pre-existing hypertension, diabetes or renal disease. Twelve studies didn't detail the inclusion criteria. Women with multiple pregnancies and fetal anomalies were excluded. The outcomes reported were early, late or any onset PE.

Quality of the included studies

Two thirds of the cohort studies (8/13, 62%) had a medium risk of selection bias, two thirds had high risk of comparability bias (8/13, 62%) and 90% (12/13) were medium risk for outcome assessment. Amongst case control studies, 12%, (2/17) had a high

risk of selection bias, 59% (10/17) were medium risk for comparability bias and almost half (41%) were low risk for exposure assessment (7/17). The quality assessment is provided in figure 3.2, and in Appendix 3.2.

Figure 3.1: Flow chart of study identification and selection

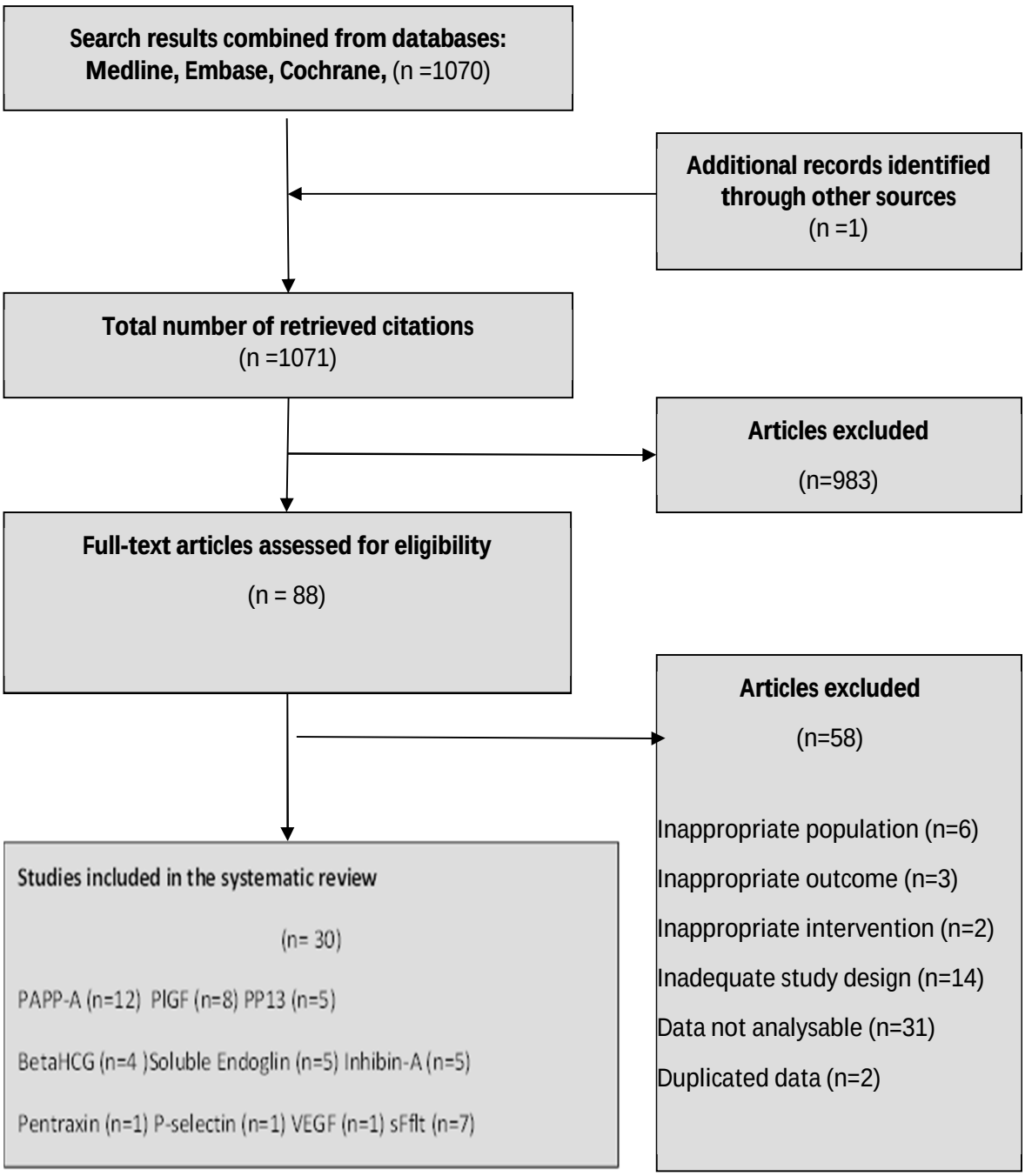


Figure 3.2a: Bar chart summarizing cohort study quality using the Newcastle-Ottawa scale

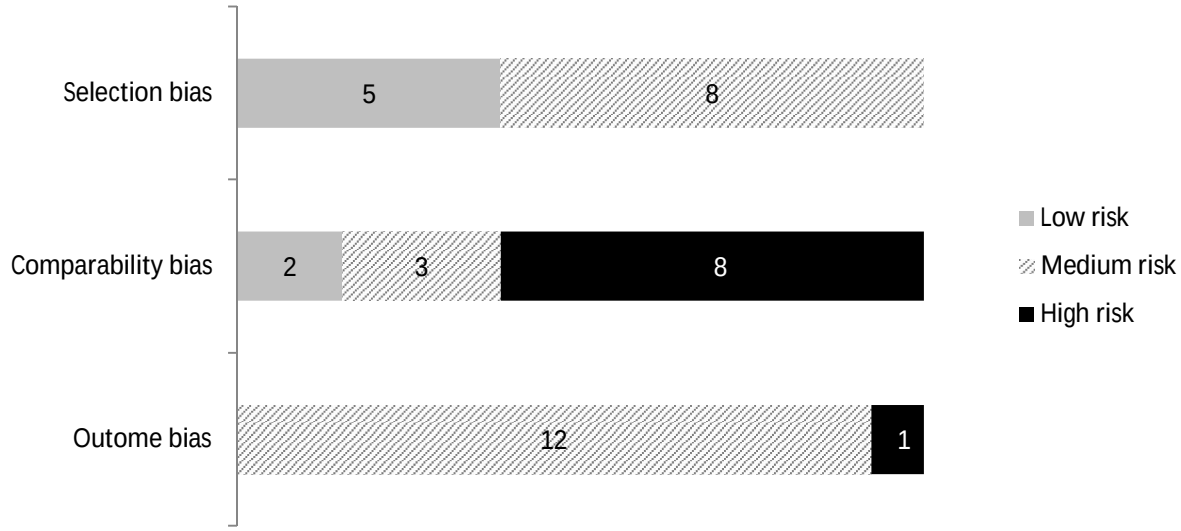
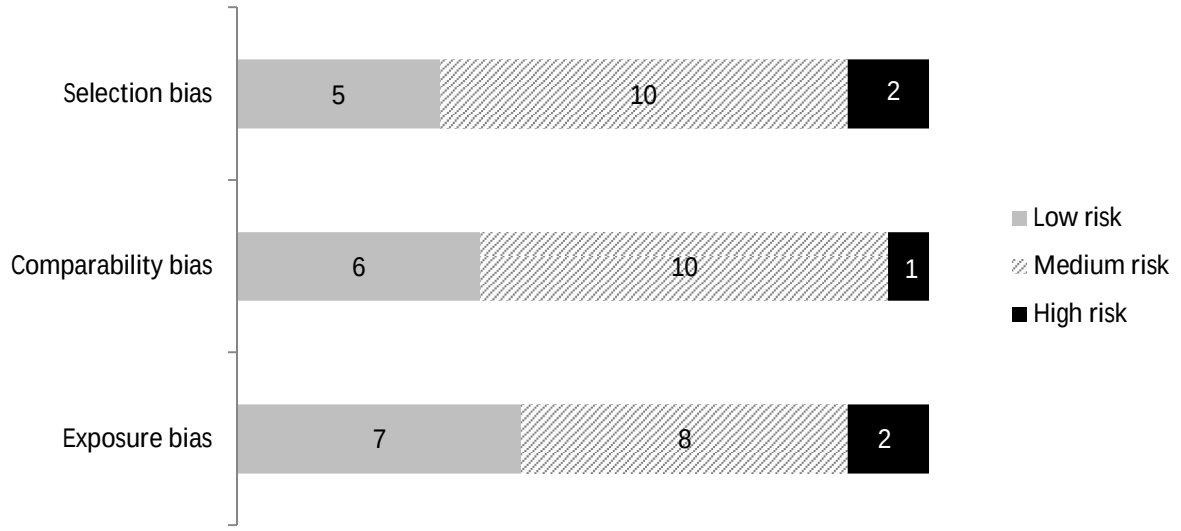


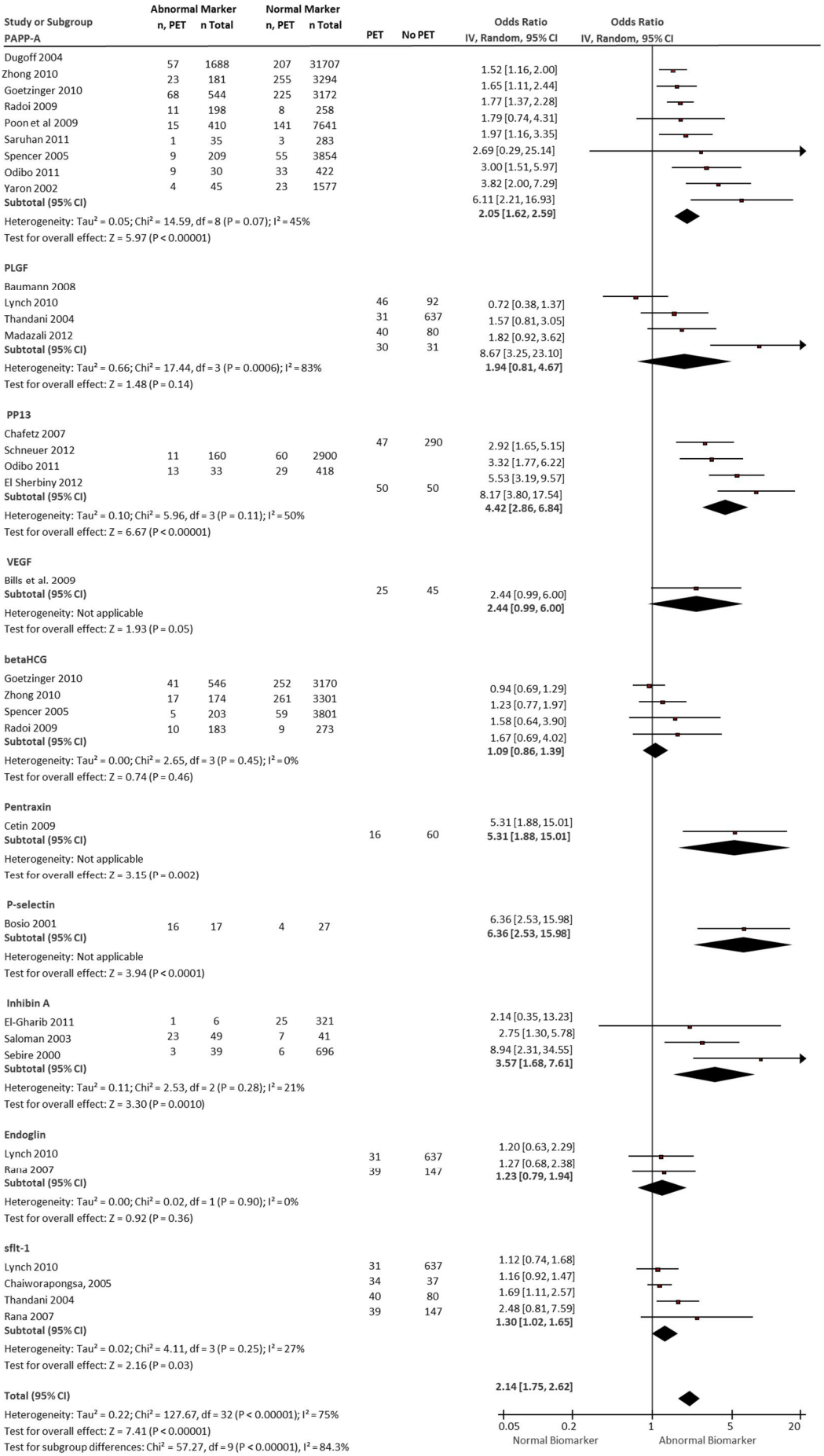
Figure 3.2b: Bar chart summarizing case control study quality using the Newcastle-Ottawa scale



Association between early pregnancy biomarkers and PE

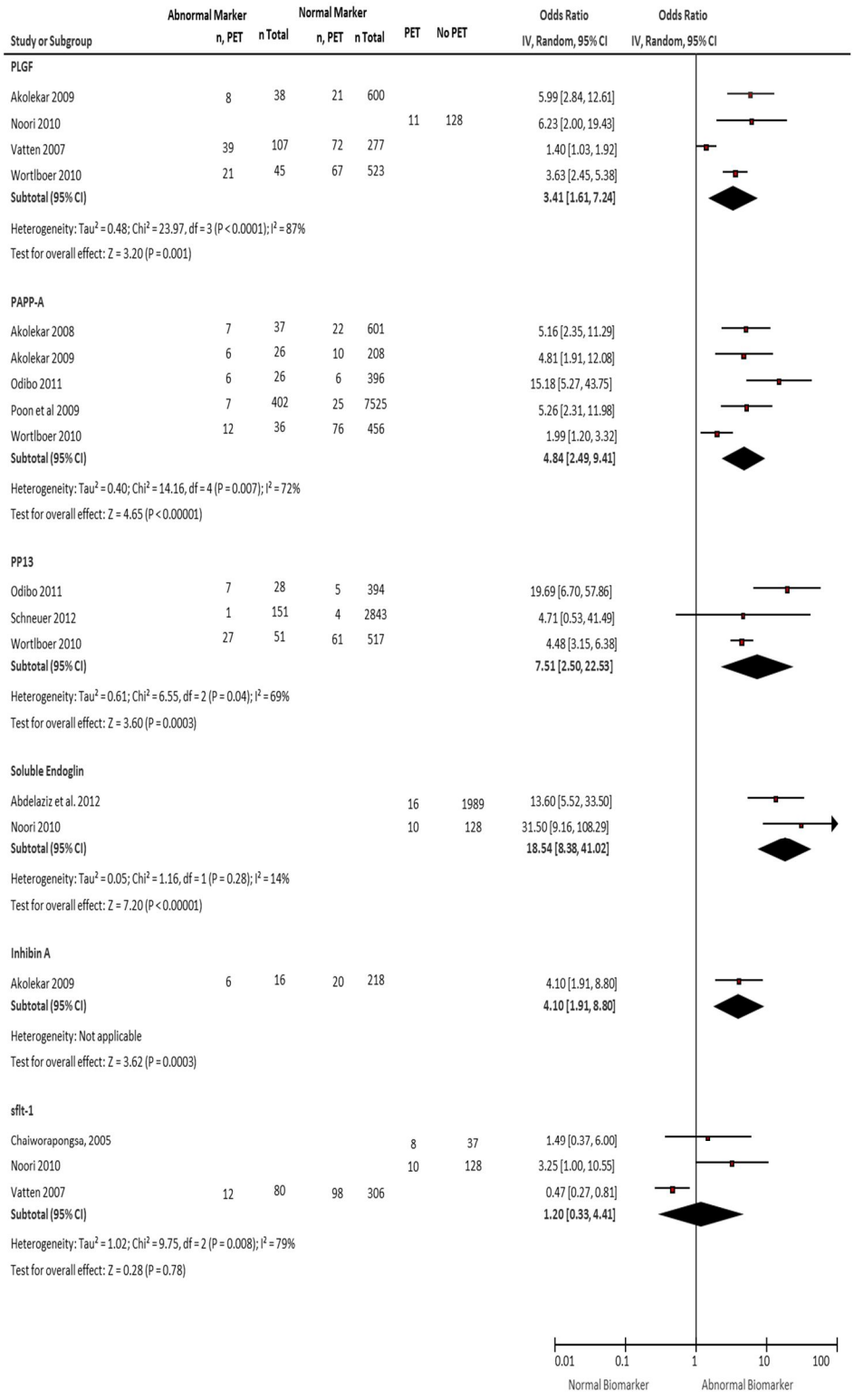
Abnormal 1st trimester maternal biomarkers and PE of any onset were reported in 24 studies which included 61,745 women. Abnormal levels of the biomarkers PAPP-A (9 studies; OR 2.1, 95% CI 1.6, 2.6, I^2 45%), PP13 (4 studies; OR 4.4, 95% CI 2.9, 6.8, I^2 50%), sFlt-1 (4 studies; OR 1.3, 95% CI 2.9, 6.8, I^2 27%), pentraxin (1 study; OR 5.31, CI 1.9, 15.0) and inhibin-A (3 studies; OR 3.57, 95% CI 1.7, 7.6, I^2 21%) were significantly associated with development of PE. There was no significant association for PlGF (4 studies; OR 1.94, 95% CI 0.81, 4.67, I^2 83%), β-hCG (4 studies; OR 1.09, 95% CI 0.86, 1.39, I^2 0%) soluble endoglin (2 studies; OR 1.23, 95% CI 0.79, 1.94, I^2 0%) and VEGF (1 study; OR 2.44, 95% CI 0.99, 6) (see fig 3.3).

Figure 3.3: Forest plot demonstrating the relationship between biomarkers and all PE


Study or Subgroup
Abnormal Marker n, PET n Total
Normal Marker n, PET n Total
PET No PET
Odds Ratio IV, Random, 95% CI
Odds Ratio IV, Random, 95% CI
PAPP-A
Dugoff 2004 57 1688 207 31707 1.52 [1.16, 2.00]
Zhong 2010 23 181 255 3294 1.65 [1.11, 2.44]
Goetzinger 2010 68 544 225 3172 1.77 [1.37, 2.28]
Radoi 2009 11 198 8 258 1.79 [0.74, 4.31]
Poon et al 2009 15 410 141 7641 1.97 [1.16, 3.35]
Saruhan 2011 1 35 3 283 2.69 [0.29, 25.14]
Spencer 2005 9 209 55 3854 3.00 [1.51, 5.97]
Odibo 2011 9 30 33 422 3.82 [2.00, 7.29]
Yaron 2002 4 45 23 1577 6.11 [2.21, 16.93]
Subtotal (95% CI) 2.05 [1.62, 2.59]
Heterogeneity: Tau² = 0.05; Chi² = 14.59, df = 8 (P = 0.07); I² = 45%
Test for overall effect: Z = 5.97 (P < 0.00001)
PLGF
Baumann 2008
Lynch 2010 46 92 0.72 [0.38, 1.37]
Thandani 2004 31 637 1.57 [0.81, 3.05]
Madazali 2012 40 80 1.82 [0.92, 3.62]
Subtotal (95% CI) 30 31 8.67 [3.25, 23.10]
1.94 [0.81, 4.67]
Heterogeneity: Tau² = 0.66; Chi² = 17.44, df = 3 (P = 0.0006); I² = 83%
Test for overall effect: Z = 1.48 (P = 0.14)
PP13
Chafetz 2007 47 290 2.92 [1.65, 5.15]
Schneuer 2012 11 160 60 2900 3.32 [1.77, 6.22]
Odibo 2011 13 33 29 418 5.53 [3.19, 9.57]
El Sherbiny 2012 50 50 8.17 [3.80, 17.54]
Subtotal (95% CI) 4.42 [2.86, 6.84]
Heterogeneity: Tau² = 0.10; Chi² = 5.96, df = 3 (P = 0.11); I² = 50%
Test for overall effect: Z = 6.67 (P < 0.00001)
VEGF
Bills et al. 2009 25 45 2.44 [0.99, 6.00]
Subtotal (95% CI) 2.44 [0.99, 6.00]
Heterogeneity: Not applicable
Test for overall effect: Z = 1.93 (P = 0.05)
betaHCG
Goetzinger 2010 41 546 252 3170 0.94 [0.69, 1.29]
Zhong 2010 17 174 261 3301 1.23 [0.77, 1.97]
Spencer 2005 5 203 59 3801 1.58 [0.64, 3.90]
Radoi 2009 10 183 9 273 1.67 [0.69, 4.02]
Subtotal (95% CI) 1.09 [0.86, 1.39]
Heterogeneity: Tau² = 0.00; Chi² = 2.65, df = 3 (P = 0.45); I² = 0%
Test for overall effect: Z = 0.74 (P = 0.46)
Pentraxin
Cetin 2009 16 60 5.31 [1.88, 15.01]
Subtotal (95% CI) 5.31 [1.88, 15.01]
Heterogeneity: Not applicable
Test for overall effect: Z = 3.15 (P = 0.002)
P-selectin
Bosio 2001 16 17 4 27 6.36 [2.53, 15.98]
Subtotal (95% CI) 6.36 [2.53, 15.98]
Heterogeneity: Not applicable
Test for overall effect: Z = 3.94 (P < 0.0001)
Inhibin A
El-Gharib 2011 1 6 25 321 2.14 [0.35, 13.23]
Saloman 2003 23 49 7 41 2.75 [1.30, 5.78]
Sebire 2000 3 39 6 696 8.94 [2.31, 34.55]
Subtotal (95% CI) 3.57 [1.68, 7.61]
Heterogeneity: Tau² = 0.11; Chi² = 2.53, df = 2 (P = 0.28); I² = 21%
Test for overall effect: Z = 3.30 (P = 0.0010)
Endoglin
Lynch 2010 31 637 1.20 [0.63, 2.29]
Rana 2007 39 147 1.27 [0.68, 2.38]
Subtotal (95% CI) 1.23 [0.79, 1.94]
Heterogeneity: Tau² = 0.00; Chi² = 0.02, df = 1 (P = 0.90); I² = 0%
Test for overall effect: Z = 0.92 (P = 0.36)
sflt-1
Lynch 2010 31 637 1.12 [0.74, 1.68]
Chaiworapongsa, 2005 34 37 1.16 [0.92, 1.47]
Thandani 2004 40 80 1.69 [1.11, 2.57]
Rana 2007 39 147 2.48 [0.81, 7.59]
Subtotal (95% CI) 1.30 [1.02, 1.65]
Heterogeneity: Tau² = 0.02; Chi² = 4.11, df = 3 (P = 0.25); I² = 27%
Test for overall effect: Z = 2.16 (P = 0.03)
Total (95% CI) 2.14 [1.75, 2.62]
Heterogeneity: Tau² = 0.22; Chi² = 127.67, df = 32 (P < 0.00001); I² = 75%
Test for overall effect: Z = 7.41 (P < 0.00001)
Test for subgroup differences: Chi² = 57.27, df = 9 (P < 0.00001), I² = 84.3%
0.05
0.2
1
5
20
Normal Biomarker
Abnormal Biomarker

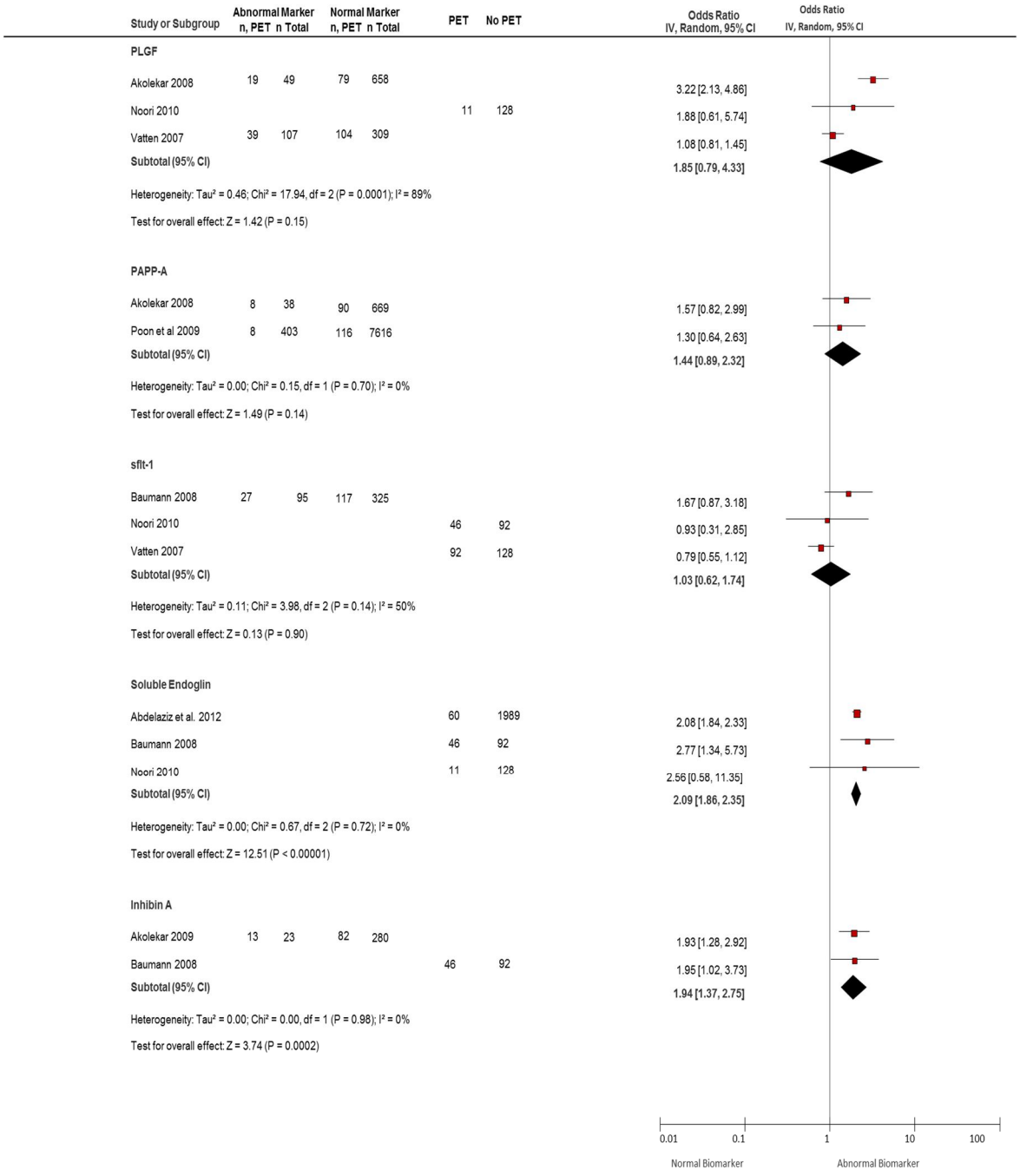
Ten studies (15,760 women) evaluated six biomarkers with the development of PE before 34 weeks of gestation. PlGF (4 studies; OR 3.4, 95% CI 1.6, 7.2, I^2 87%), PAPP-A (5 studies; OR 4.8, 95% CI 2.5, 22.5, I^2 72%), PP13 (5 studies; OR 7.5, 95% CI 2.5, 22.5, I^2 69%), soluble endoglin (2 studies; OR 18.5, 95% CI 8.4, 41.0, 14%) and inhibin-A (1 study; OR 4.1, 95% CI 1.9, 8.8) were significantly associated with early onset PE. sFlt-1 was not. (3 studies; OR 1.2, 95% CI 0.33, 4.41, I^2 79%) (see fig 3.4).

Figure 3.4: Forest plot demonstrating the relationship between biomarkers and early PE



The relationship of late onset PE and abnormal biomarker levels was evaluated in seven studies (12,053 women), examining five biomarkers. A significant association was seen for soluble endoglin (3 studies; OR 2.1, 95% CI 1.9, 2.4, I^2 0%) and inhibin-A (2 studies; OR 1.9, 95% CI 1.4, 2.8, I^2 0%). This association was not seen for abnormal levels of sFlt-1 (3 studies; OR 1.03, 95% CI 0.62, 1.74, I^2 50%), PAPP-A (2 studies; OR 1.44 95% CI 0.89, 2.32, I^2 0%) and PlGF (3 studies; OR 1.85, 95% CI 0.7, 4.33, I^2 89%) (see fig 3.5).

Figure 3.5: Forest plot demonstrating the relationship between biomarkers and late PE



Assessment of publication bias

Egger's test for publication bias was significant for PAPP-A in association with any onset PE (p=0.04) and early onset PE (p=0.01). It was not significant for PlGF, sFlt-1 or PP13. Funnel plots demonstrating these results can be seen in appendix 3.3. We could not assess the risk of publication bias for other biomarkers due to the small number of studies.

3.5 Discussion

This review shows that abnormal levels of maternal haematological biomarkers are associated with PE particularly early onset disease. Amongst the biomarkers, low PAPP-A and high levels of soluble endoglin were most predictive of early onset PE.

Strengths and Limitations

Our review is the first to provide summary estimates of the association between abnormal 1st trimester maternal blood biomarkers and onset of PE. Previous reviews have not quantified the strength of association for all relevant biomarkers limiting direct comparison of the markers. (39, 68, 100) and have only assessed four biomarkers from any trimester of pregnancy. (101) The approach of combining dichotomous and continuous data allowed direct comparison of biomarkers, improving the power of the analysis and avoiding any potential conflict that could arise from two separate analyses and ensuring heterogeneity was detected and not wrongly reported. (96)

Our findings were limited by the amount of heterogeneity which may be due to studies reporting insufficient details of the population characteristics and underlying risk

factors. Many studies did not detail the gestation at onset of PE. Several studies identified could not be included as they reported results as medians with ranges or multiples of the median and despite efforts to contact the authors of relevant studies it was not possible to include all the existing evidence in this meta-analysis.

Biomarkers and PE

The underlying mechanism for the development of PE is thought to be impaired trophoblastic invasion of the maternal spiral arteries and their conversion from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control. (7, 8) Impaired placental perfusion is thought to lead to placental ischemia and damage causing release of inflammatory factors which leads to platelet activation, endothelial dysfunction and consequently development of PE symptoms. (17, 19-21, 99) Dysregulation and imbalance of sFlt-1, VEGF and PlGF; placental pro-angiogenic and anti-angiogenic vasoactive agents; have a significant role in the pathogenesis.(102)

The pre-symptomatic levels of angiogenic biomarkers, appear to be linked to severity and timing of PE onset, especially for markers such as PlGF and PAPP-A, that are significantly associated with early onset, but not late onset disease.(27) For markers such as sFlt-1, there was a positive association with early onset PE, but not late onset. Some studies have suggested that PlGF levels are already significantly lower in the 1st trimester in women who develop PE but that changes in sFlt-1 occur later. (103) Evidence from studies measuring serial levels of angiogenic biomarkers have shown conflicting results which is likely to account for the high heterogeneity seen with our analysis. Vatten et al. demonstrated lower levels of sFlt-1 in the 1st trimester in women developing preterm PE with levels substantially higher than controls in the 2nd

trimester. [38] Rana et al. demonstrated that 1st trimester levels of sFlt-1 were non-significantly higher in women whom later developed PE but were significantly higher in the 2nd trimester. [27] The levels of some markers such as VEGF are known to be too low for detection in the 1st trimester. This could account for the paucity of studies on this marker, for which no association was demonstrated for PE.(39)

Overall biomarkers were better for the prediction of early onset than late or any onset PE supporting the concept of two distinct disease entities with different pathophysiologies.(100) Early onset PE being associated with inadequate placentation leading to an imbalance of pro- and anti-angiogenic factors with histology from placentae demonstrating abnormal villous and vascular morphology. Histology and morphology in late onset disease is not dissimilar to controls and disease is thought to be related to impaired glucose metabolism with a hyperdynamic low peripheral resistance maternal cardiovascular profile. In this situation it is unsurprising that there is a poor correlation between biomarker levels and late onset disease as trophoblastic invasion in the 1st trimester is likely to be normal. The mechanism of inadequate placentation being associated with early onset PE but not late onset, is supported by the sensitivity of UADs as a screening tool for early PE but not late onset disease.

Implications for clinical practice and research

Our review has identified which biomarkers are associated with the onset of PE, particularly early onset disease. The promising markers identified by this review, will allow the role of additional biomarkers to existing clinical prediction models to be assessed. Incorporating these biomarkers to current clinical prediction models will be the initial step in the promotion of individualised medicine practice allowing stratification of women early in their pregnancy, for targeted management. The only treatment that

has currently been found to have significant benefit in the prevention of PE is aspirin.(104)

Any study on biomarkers should be standardised in its methodology enabling easier comparison and meta-analysis. The study population needs to be well defined, as well as the outcomes, with clear differentiation between late and early onset disease and its severity. This cohort of high risk women could subsequently be evaluated in future randomised trials assessing the effectiveness of interventions to prevent PE.

Chapter 4: Predictive accuracy of UAD indices for stillbirth: a systematic review and meta-analysis

4.1 Abstract

Objective

The aim of this review was to assess the predictive accuracy of UAD for stillbirth.

Methods

MEDLINE, EMBASE and Cochrane databases were searched without language restrictions from inception until March 2015. Studies that were included were those that assessed the association of abnormal UAD parameters and stillbirth. Two independent reviewers performed study selection, data extraction and quality assessment. Study results were pooled and summary estimates of sensitivity, specificity, LRs and their 95% confidence intervals were obtained. Diagnostic odds ratio was used to summarise overall test accuracy.

Results

340 relevant citations were returned form the literature searches with 34 considered in full. Thirteen 2nd trimester (85, 846 women, 508 stillbirths) studies met our search criteria, and two first (n=9935, 66 stillbirths) were included in the review. For 2nd trimester studies bivariate pooled estimate for sensitivity was 65% (95% CI 38 – 85%) and for specificity it was 82% (95% CI 72– 88%). The positive LR was 3.5 (95% CI 2.3 – 5.5) and negative LR 0.43 (95% CI 0.22 – 0.85). The diagnostic odds ratio was 8.3 (95% CI 3 – 22.4). Pooled estimates for 1st trimester studies could not be obtained due to the low number of studies and participants found.

Conclusions

2nd trimester abnormal UAD indices are associated with a three to four fold increase in the risk of stillbirth. It is difficult to make any firm conclusions due to high heterogeneity. There is a role for individual patient data meta-analysis to define which Doppler parameter and threshold value should be measured.

Citation of paper arising from this work

R. E. Allen, M. Morlando, B. Thilaganathan, J. Zamora, K. S. Khan, S. Thangaratinam, A. Bhide. Predictive accuracy of 2nd trimester UAD indices for stillbirth: a systematic review and meta-analysis. USOG 2016. Vol 47 (1) 22-27.

4.2 Introduction

Stillbirth is defined as a baby born without signs of live after 23+6 weeks of pregnancy. There has been a downward trend in the rate of stillbirths in the UK over the past 10 years. The current rate is 5.1/1000. (52)

The last CMACE report developed a new classification for conditions arising in pregnancy that caused or were associated with stillbirth. . This classification enabled a reduction in the number of stillbirths being reported as unexplained from 50% to 23%. They found the main causes or associated factors of stillbirth were antepartum or intrapartum haemorrhage (13%), intra-uterine growth restriction (IUGR – 10%) and specific placental conditions (9%). PE was associated with 4.7% of stillbirths. (52) These conditions are all associated with impaired placentation which can be measured indirectly by UADs demonstrating an increase in pulsatility index. Some studies have reported the association of elevated pulsatility/resistance index or notches with stillbirth. (5-7) The performance of UADs for prediction of stillbirth has varied across these studies and there's also been variation in the reporting of outcomes and the measurements that have been used.

The aims of this review are:

- Assess the association between UADs and stillbirth and determine their predictive value
- To compare the predictive value of UADs for stillbirth at different gestations and in high/low risk populations

4.3 Methods

We undertook the systematic review with a prospective protocol in line with current recommendations.(59) We searched MEDLINE, EMBASE and Cochrane databases from inception until March 2015 without language restrictions. Reference lists of primary and review articles were examined to capture those missed by electronic searches. No language restrictions were applied. The authors of primary studies were contacted to obtain relevant data if it was not reported.

We used the following combination of search terms: pregnan*, gravid*, “uterine artery dopplers”, Doppler, stillbirth, IUD, “intra-uterine death”. Studies that evaluated the association of UAD with stillbirth were selected in a two stage process. Firstly the electronic searches were scrutinized and appropriate studies identified. Secondly, two independent reviewers reviewed the full text of identified papers and selected the studies that fulfilled the inclusion criteria. Data was extracted in 2x2 tables for the incidence of stillbirth in women with abnormal UADs. We used the individual author’s definition of abnormal UAD. Appendix 4.1 shows the data collection sheet used.

All included studies methodological quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies criteria (QUADAS-2), which consists of four key domains: patient selection, index test, reference standard, flow and timing.(61) Each of these domains is assessed for risk of bias and classified as low or high risk or unclear.

Data for true positives, true negatives, false positives and false negatives was extracted from primary studies and used to estimate sensitivities, specificities and LRs with their 95% confidence intervals for individual studies. Results for studies that were performed in the same trimester were pooled. A multilevel bivariate random effects model as implemented in the mentandi user-written command developed for Stata statistical software(62) was used to obtain summary estimates of sensitivity and specificity. From these indices corresponding LRs and their 95% confidence intervals were derived. (63) This model also estimates the correlation between sensitivity and specificity as a random parameter to represent the counterbalance between sensitivity and specificity due to threshold effect. Due to the low number of studies included in the review we analysed the effect of covariates (screening general population versus high risk population) on diagnostic accuracy as measured by Diagnostic Odds Ratio using meta-regression weighted by the inverse of within study variance of log (DOR). Meta-DiSc software was used for this analysis.(105)

4.4 Results

We found 340 relevant citations. After screening titles and abstracts 34 were considered for further assessment. Full evaluation of these resulted in the identification

of 13 2nd trimester studies (n = 85,846) including 508 stillbirths and two 1st trimester studies (n=9935, 66 stillbirths) (Fig. 4.1, Table 4.1, appendix 4.2).

Quality of the included studies

The quality of the included studies is shown in table 4.2 and figures 4.2a and b. All of the 2nd trimester studies had a low risk of bias and concerns regarding applicability for flow and timing, reference standard (stillbirth) and index test (UAD assessment). Six of the 13 (46%) 2nd trimester studies had a high risk of bias and high concerns for applicability regarding patient selection. Both 1st trimester studies were assessed as low risk for these domains.

Table 4.1. Characteristics of the included studies

Author/year	**Risk status high/low**	**Definition of abnormal UtA Doppler**	**No of included women**	**No of stillbirths**	**Stillbirth rate/1000**
Madazali et al. 2014	High	SD ratio>2.6 and bilateral notching	65	3	46
Jamal et al. 2013	Low	Mean PI >95th percentile and/or bilateral notches	435	5	11.5
Poon et al 2013	Low	PI MoM >90th centile	65, 819	306	4.6
Singh et al. 2012	Any	Mean PI>90th centile	14, 997	135	9
Iacovella et al. 2012	Any	Mean RI>90th	9859	62	6.3

		centile			
Filippi et al. 2011	High	Mean PI >1.45, bilateral or unilateral notching	159	2	12.5
Proctor et al. 2009	High	Mean PI >1.45	90	15	166
Fratelli et al. 2008	High	Bilateral notching or mean RI>0.8	76	4	50
Schwarze et al. 2005	Any	Unilateral or bilateral notches	346	2	5.7
Axt-Fliedner et al. 2005	High	Both RI >0.58	52	4	77
Albaiges et al. 2000	Any	Bilateral notches or mean PI >1.45 or	1757	6	3.4
Coleman et al. 2000	High	Mean RI >0.7	116	2	17.2
Bewley et al. 1991	Any	Mean RI >95th centile	925	12	12.9
Steel et al. 1990	Any	Mean RI >0.58	1014	11	10.8
Fleischer et al. 1986	High	Systolic diastolic ratio >2.6	71	5	70.4

Figure 4.1: Flow chart of study selection

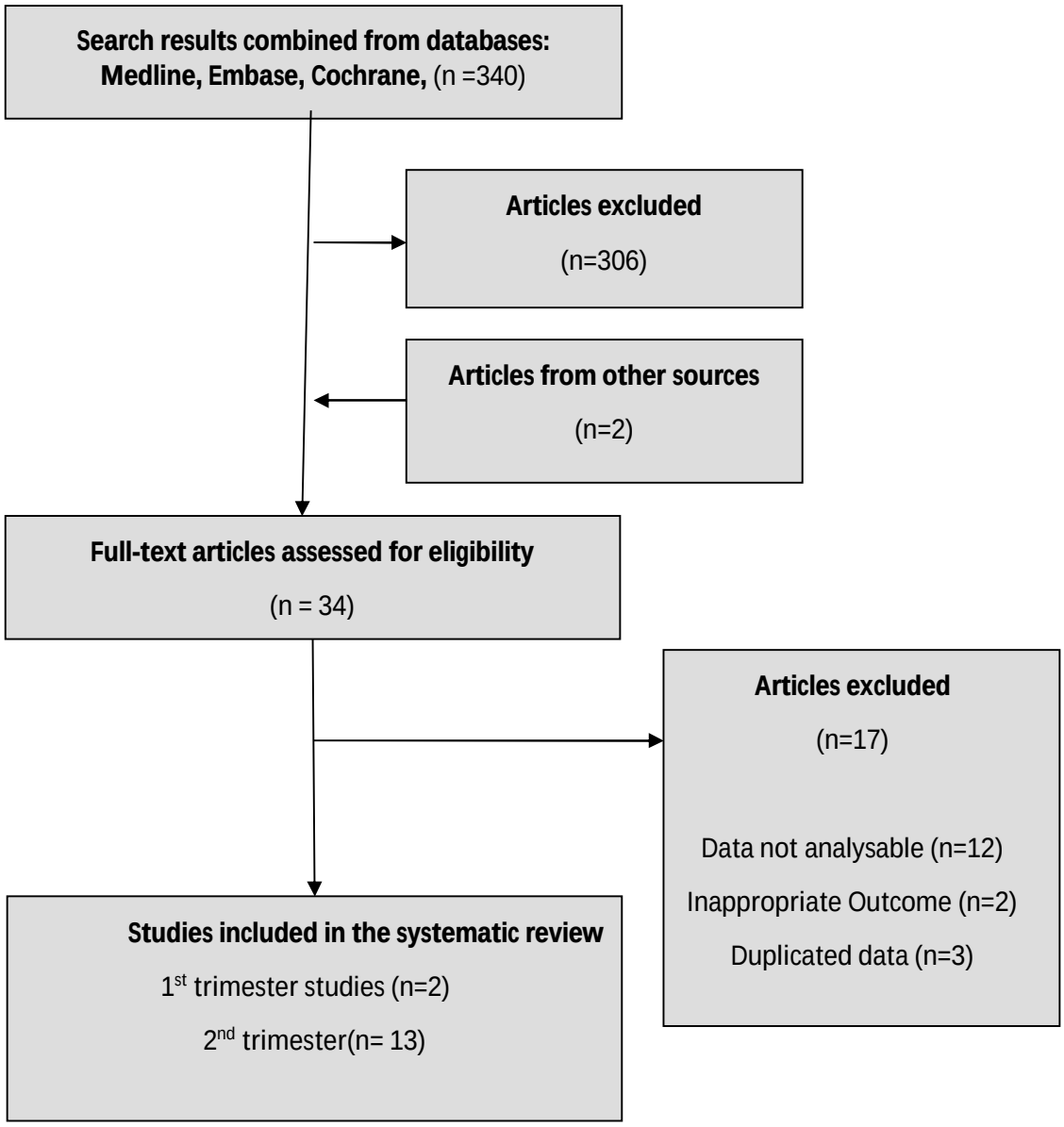


Figure 4.2a: Quality assessment of 1st trimester studies

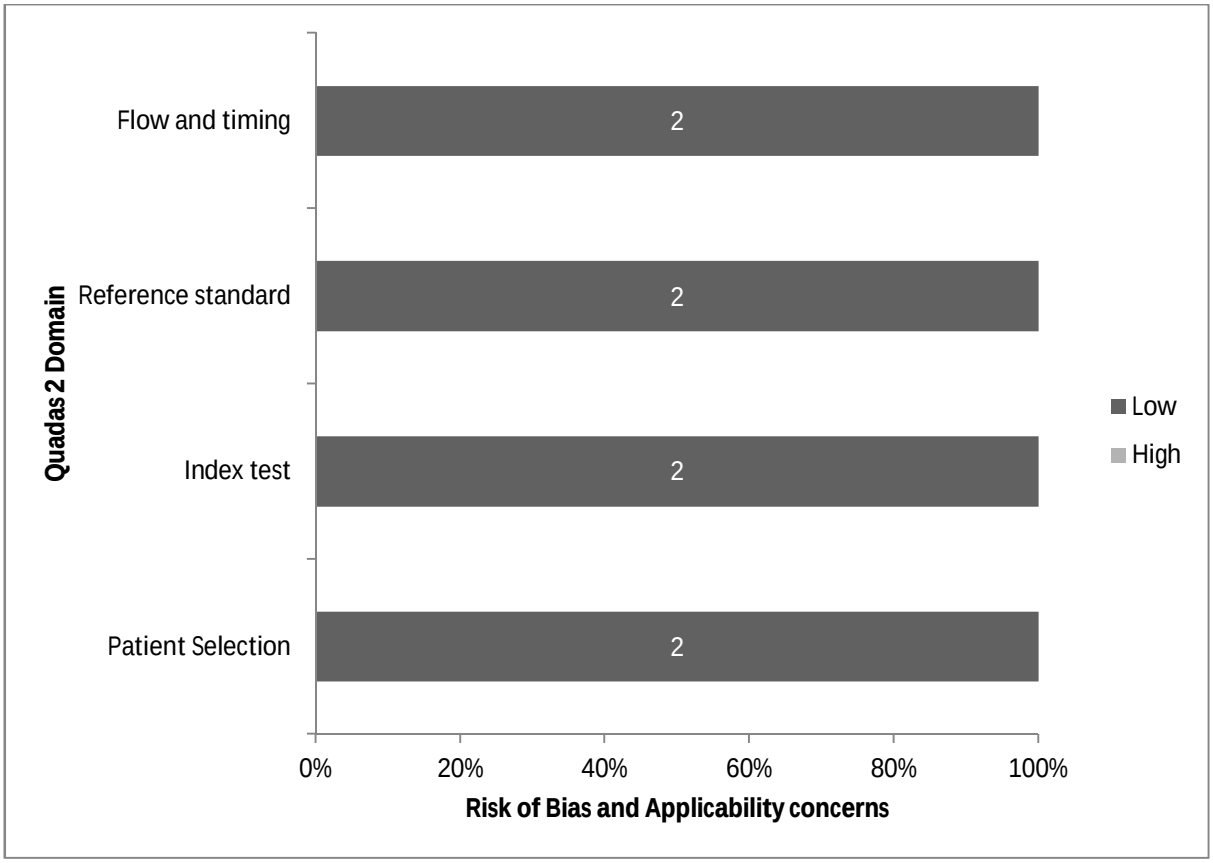


Fig 4.2b Quality assessment of 2nd trimester studies

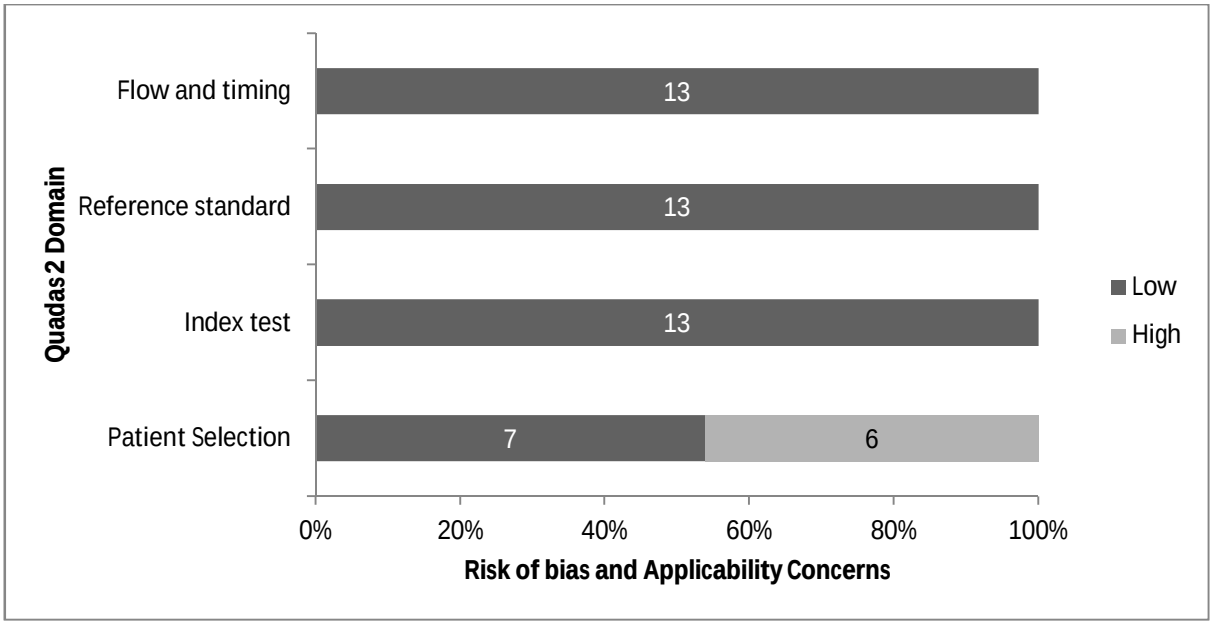


Table 4.2: Quality assessment of included studies using QUADAS-2 criteria

Study	Year	Risk of Bias				Applicability Concerns		
		Patient Selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Madazali	2014	High	Low	Low	Low	High	Low	Low
Jamal	2013	Low	Low	Low	Low	Low	Low	Low
Poon	2013	Low	Low	Low	Low	Low	Low	Low
Singh	2012	Low	Low	Low	Low	Low	Low	Low
Iacovella	2012	Low	Low	Low	Low	Low	Low	Low
Filippi	2011	High	Low	Low	Low	Low	Low	Low
Proctor	2009	High	Low	Low	Low	High	Low	Low
Fratelli	2008	Low	Low	Low	Low	Low	Low	Low
Axt Fliedner	2005	High	Low	Low	Low	High	Low	Low
Schwarze	2005	High	Low	Low	Low	High	Low	Low
Coleman	2000	Low	Low	Low	Low	High	Low	Low
Albiages	2000	Low	Low	Low	Low	Low	Low	Low
Bewley	1991	Low	Low	Low	Low	Low	Low	Low
Steel	1990	Low	Low	Low	Low	Low	Low	Low
Fleischer	1986	High	Low	Low	Low	High	Low	Low

Characteristics of the included studies

Thirteen 2nd trimester studies were included.(106-118) Six studies were on high-risk women with co-morbidities such as hypertension, medical disorders, previous intrauterine growth restriction, placental abruption or stillbirth.(106-110, 118) Seven were on an unselected group of women. Two studies were in women whom had abnormal biochemistry on Downs syndrome screening, including low PAPP-A and oestriol or high β-hCG/ AFP or Inhibin A. Doppler results were concealed from the clinicians in 4 of the studies(110, 111, 114, 117), open in 7 (110, 113) (106-108, 112, 115)and they were not documented in two(116, 118). In 4 studies some of the women received aspirin or heparin (106, 108, 115, 118). Women on these medications were excluded in one study. (116) There was no documentation regarding the use of these

drugs in 8 of the studies. (107, 109-114, 117) The abnormal uterine artery parameters assessed varied between studies and included notching (unilateral/bilateral) (4 studies), mean pulsatility index (PI) >1.45 (3 studies), mean PI >90th centile (2 studies), mean PI >95th percentile (1 study) mean resistance index (RI) >0.7 (1 study), mean RI >0.58 (2 studies), mean RI>95th centile (1 study) or systolic diastolic ratio >2.6 (2 studies). Although different uterine artery parameters were used, the various UAD indices cut-offs are highly correlated. For example, PI of 1.45 corresponds to the 95th centile at 21-22 weeks, (119) mean RI of 0.73 and systolic to diastolic ratio of 4.05 at 20 weeks corresponds to the 95th centile.(120) In general, all the cut-offs used represent Doppler indices at the upper extreme of the reference range.

Two 1st trimester studies were included.(121, 122) One was on high risk women with a previous history of adverse pregnancy outcomes due to placental problems such as IUGR, PE, stillbirth or medical conditions including chronic hypertension, renal disease, thrombophilia.(122) In both studies clinicians were blinded to the Doppler results. There was no documentation regarding the use of anti-thrombotic or platelet agents in one of the studies and in one study they were not administered. In 1 study a mean RI >0.8 or bilateral notching was used as a cut off in the other study a cut off of mean RI >90th centile was used.

Performance of 1st trimester UADs for predicting stillbirth

The sensitivity of UADs in the 2 individual studies ranged from 14.5-100%. The specificity ranged from 64-91%. Due to the limited number of studies and participants and the high heterogeneity observed we could not obtain a pooled estimate of sensitivity and specificity.

Performance of 2nd trimester UADs for predicting stillbirth

We divided the studies into subgroups based on whether the study populations were unselected or at high risk of developing complications. The sensitivity of UAD in the 13 individual studies ranged from 25- 100%. The specificity ranged from 33-94%. Bivariate pooled estimate for sensitivity was 65% (95% CI 38 –85%) and for specificity, it was 82% (95% CI 72– 88%). The positive LR was 3.5 (95% CI 2.3 –5.5) and negative LR 0.43 (95% CI 0.22 – 0.85). Summary ROC curve and summary point are shown in Fig 4.3. The prediction ellipse covers entirely the region of poor diagnostic performance (close to the diagonal of the ROC plane) denoting a high inter-study heterogeneity in both accuracy indices with smaller studies tending to give higher sensitivities but having almost no impact on pooled sensitivity given their small number of stillbirths. The diagnostic odds ratio provides an overall summary of test accuracy, which is 8.3 (95% CI 3 – 22.4).

Subgroup analysis of the high risk group (n=6) showed that the average sensitivity was 90% (95% CI 36 – 99 %) with a pooled specificity of 69% (95% CI 54 – 81%). The positive LR was 2.9 (95% CI 1.8 – 4.9), negative LR 0.14 (95% CI 0.1 – 1.8) and diagnostic OR 21.3 (95% CI 1.2 – 380). The analysis demonstrated high heterogeneity which along with the small number of stillbirths makes it impossible to draw any conclusions about sensitivity of the test (Fig 4.4). The prediction ellipse and the confidence ellipse covers the whole ROC space.

Figure 4.3: Summary ROC curve for the performance of 2nd trimester UADs in predicting stillbirth

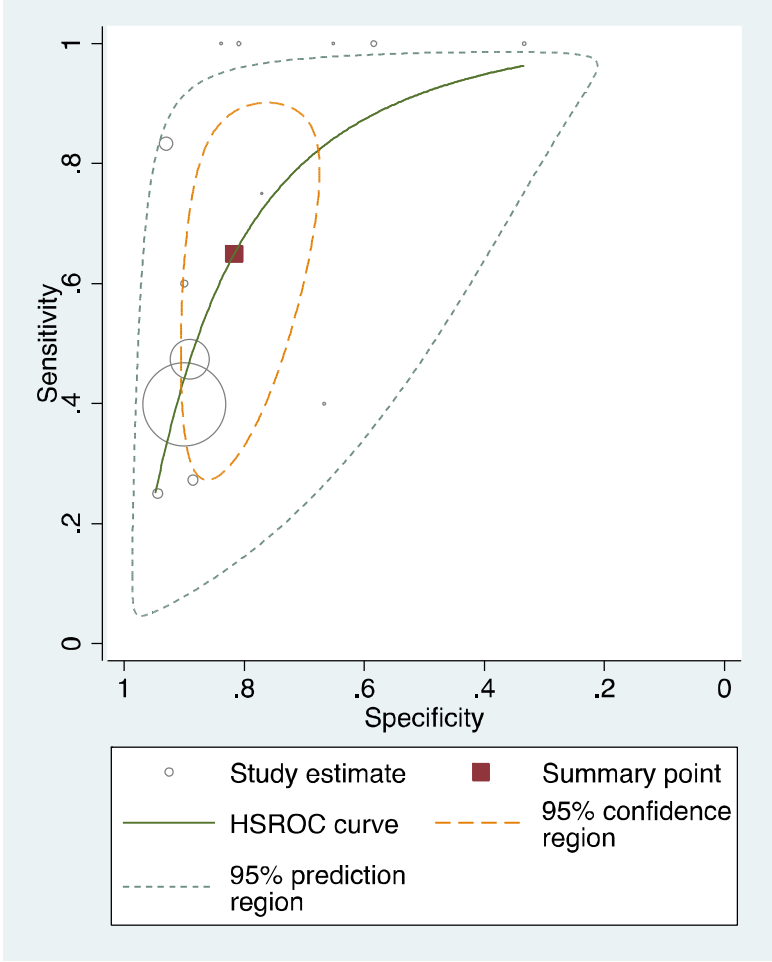
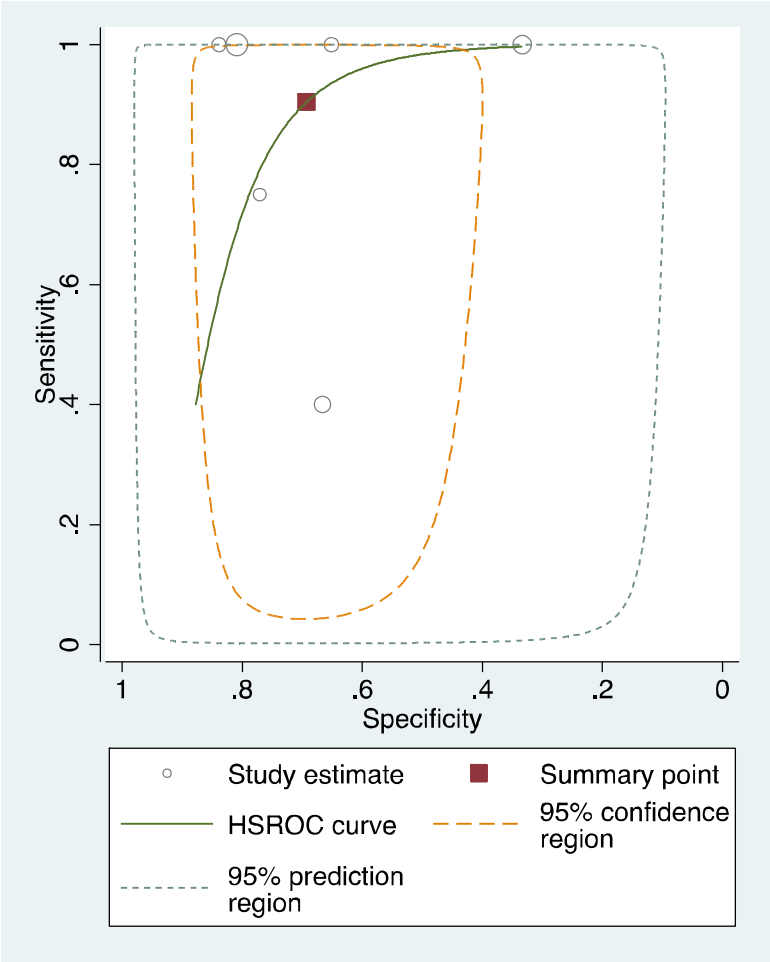
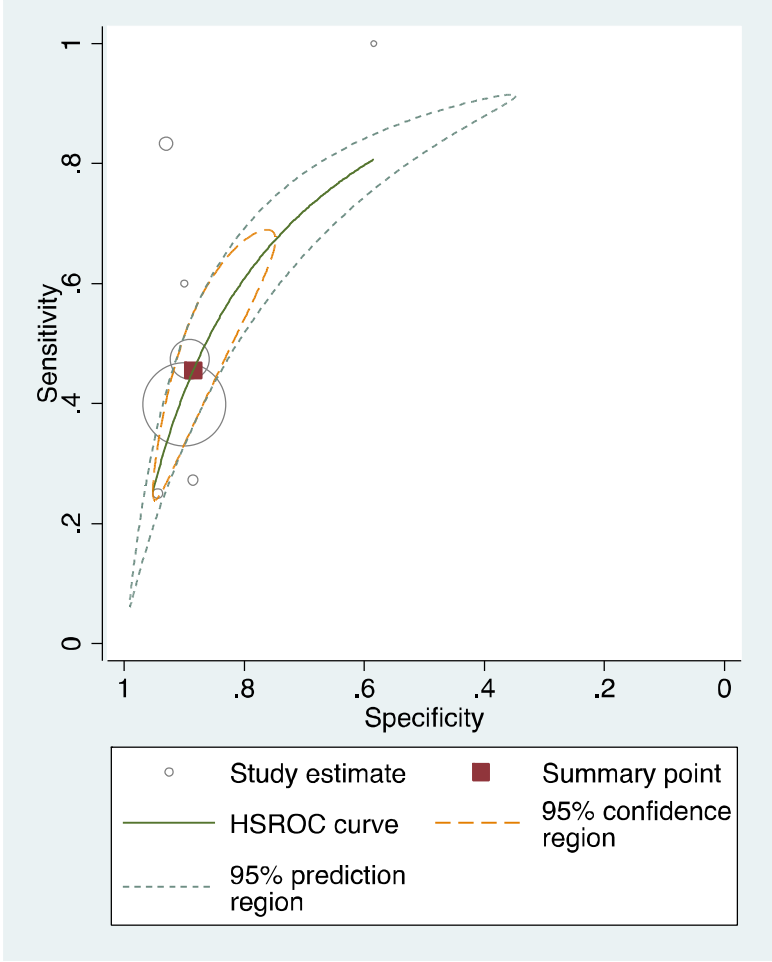


Figure 4.4: Subgroup analysis summary ROC curve for the performance of 2nd trimester UADs in predicting stillbirth in a high risk population



In the seven 2nd trimester studies that performed UAD in an unselected population the prediction ellipse covers almost entirely the range of specificity being narrower for sensitivity (Fig 4.5). Pooled sensitivity is 46%, (95% CI: 32 – 60%), pooled specificity is 88% (95% CI: 82 – 93%). The positive LR was 3.96 (95% CI: 3.1 – 5.0), negative LR 0.62 (95% CI: 0.50 – 0.75) and diagnostic OR 6.4 (95% CI: 5.2 – 8.0).

Figure 4.5: Subgroup analysis summary ROC curve for the performance of 2nd trimester UADs in predicting stillbirth in an unselected population



4.5 Discussion

Main Findings

The results of the meta-analysis have shown that abnormal 2nd trimester UAD indices in an unselected population increases the likelihood of stillbirth by a factor of three to four. Specificity was relatively high but sensitivity varied between studies. The high heterogeneity demonstrated could be caused by several factors defined a-priori, including the background risk of the populations recruited, differences in reported rates of stillbirth, different abnormal uterine artery parameters measured and different

chosen positivity thresholds.

Strengths and limitations

Included studies were of good quality reducing the risk of bias and the literature search was performed without language restrictions. Our study is limited by the heterogeneity. The populations tested varied between those that were high risk due to previous obstetric or medical history and those that were performed on the general pregnant population. There was a large amount of inter-study variation between the UAD parameters measured and their cut off values. We performed a subgroup analysis in order to control for these factors.

Unfortunately we were unable to include some studies in our analysis due to difficulty obtaining primary data despite contacting the authors. Stillbirth has a variety of causes. Congenital structural abnormalities and infections are unlikely to have placental failure as an underlying cause and therefore no association with abnormal UAD. Therefore, the utility of UAD testing would be limited to those potentially avoidable stillbirths with placental failure as the underlying cause. A meta-analysis of UAD for the prediction of perinatal death was reported in 2000 which was based on four studies, perinatal death rather than stillbirth was reported. (123) The positive and negative LRs in that study were 4.0 and 0.6, not too different to those found in our meta-analysis.

Interpretation

The stillbirth rate in the UK is 5.2 per 1000. There has been a downward trend over the past ten years but it remains one of the highest stillbirth rates in the developed world.(52) There is an urgent need to reduce this focussing on deaths occurring at or near term. There are no routine screening tests for detecting pregnancies at increased

risk of stillbirth. The policy of routine induction of labour from 41 weeks' gestation was specifically introduced by the National Institute of Clinical Excellence (NICE) to prevent stillbirth, however it is yet to change the prevalence of term stillbirths.(124) Parity, racial origin, smoking, low educational level and previous adverse pregnancy outcome are all associated with stillbirth. The prevalence of these risk factors in the UK (nulliparity 43%, smoking 19% and low education 49.6%) means that their combined prevalence is likely to result in the majority of the population being identified as screen positive. These risk factors have modest adjusted odds ratios between 1.6 and 2.9 for stillbirth with very low sensitivities. In contrast, risk factors with higher odds ratios such as abruption (adjusted odds ratio of 18.9) have low (1%) population prevalence. (125)

Identification of a test with modest sensitivity, low false positive rate and a high positive LR has the potential to be useful for guiding intervention. This review has shown that UAD has these qualities if performed in the 2nd trimester. UAD indices are relatively easy to measure and can be performed at the time of the 20 week anomaly scan. The additional cost is estimated to be £18-25 and an extra 5 minutes of time.(126) Pregnancies that are screen positive could then have closer surveillance by regular scans to check growth, liquor volume, umbilical and fetal Dopplers.

Stillbirths occurring at the limits of viability are not preventable by elective delivery. Those occurring nearer term may well be preventable, since it is possible to deliver electively without increasing the risks of operative delivery.(127) Unfortunately, except the study of Poon et al, there was insufficient data provided in the other published individual studies to enable subgroup analysis based on timing of stillbirth. An individual patient data meta-analysis would allow this to be assessed as well as determining which Doppler parameter and threshold value should be measured. Subsequent studies should assess whether intervention in screen positive women

could lead to a reduction in stillbirths, and if performance of UAD testing for stillbirth prediction could be enhanced by the addition of maternal serum biomarkers.

Chapter 5: Prospective observational study to determine the accuracy of 1st trimester serum biomarkers and UADs in combination with maternal characteristics and arteriography for the prediction of women at risk of PE and other adverse pregnancy outcomes

5.1 Abstract

Objectives

Assess efficacy of biomarkers, arteriography and UADs for predicting hypertensive disease of pregnancy, SGA, placental abruption and stillbirth.

Methods

Prospective 1st trimester study. Transabdominal ultrasound was used to assess uterine artery blood flow. Arteriography was measured non-invasively and systolic blood pressure in the aorta (SBPAO), pulse wave velocity (PWV), augmentation index (Aix) and mean arterial pressure (MAP) were measured. Maternal serum was taken and levels of PlGF, AFP, PAPP-A and β-hCG determined. Haemodynamic parameters and maternal serum biomarkers were converted into multiples of the median (MoM). Logistic regression with stepwise selection performed to determine multivariate models. 2 final models were used to construct ROC curves and detection rates for specified false-positive rates, and areas under the curve (AUC) were calculated.

Results

1045 women were left for analysis after exclusions (n=205, 16.4%). 14 (1.3%) developed PE, 23 (2.2%) PIH, 64 SGA <5th centile (6.1%), 118 SGA <10th centile (11.3%), 3 stillbirth (0.29%). Aix (p<0.027), SBPAO (p=0.002) and MAP (p=0.03) along with mean uterine artery PI (p=0.03), Afro-Caribbean ethnicity (p=0.013) and a history

of hypertension (p=0.047) were significantly associated with the development of PE. Detection rate was 72% for a FPR of 15%, AUC 0.81, 95% CI 0.69-0.93. SBPAO (p=0.015), MAP (p=0.001) and maternal weight (p=0.001) were the only parameters significantly associated with PIH. Detection rate 48% for a 15% FPR, AUC 0.76, 95% CI 0.65-0.86. Low PAPP-A, PlGF and maternal hypertension were significantly associated with SGA <10th centile (p=0.007, 0.004 and 0.03 respectively), with a detection rate of 30% for a fixed FPR of 15%, AUC 0.608, 95% CI 0.54-0.68). For SGA <5th centile there was a significant association with low PlGF (p=0.00), PAPP-A (0.011), South Asian ethnicity (p=0.047) and maternal smoking (p=0.018). Detection rate 57% for a 15% fixed FPR, AUC 0.73, 95% CI 0.65-0.80). High AFP (p=0.041), low PlGF (p=0.036) and maternal smoking (p=0.025) were significantly associated with stillbirth with a detection rate of 67% for a 15% FPR. AUC 0.92, 95% CI 0.82-1.0).

Conclusions

In our population with a low prevalence of PE (1.3%) there was no demonstrable association between 1st trimester maternal serum biomarkers and hypertensive disease of pregnancy and between increased uterine artery PI and PIH. There was a significant association between low levels of PlGF and PAPP-A and SGA, and low levels of PlGF and AFP and IUD. The use of our model improved the prediction of preeclampsia from that based on current NICE guidelines.

Citations of papers arising from this work

Allen R, Bestwick J, Thangaratinam S, Aquilina J. Maternal blood biomarkers, arteriography and uterine artery Doppler (UAD) for the prediction of hypertensive disease in pregnancy. Ultrasound in Obstetrics and Gynecology. 2016. Vol 48 Suppl 1:344

Allen R and Aquilina J. Prospective observational study to determine the accuracy of first trimester serum biomarkers and uterine artery Dopplers in combination with maternal characteristics and arteriography for the prediction of women at risk of preeclampsia and other adverse pregnancy outcomes. Journal of Maternal Fetal and Neonatal medicine 2017. Aug 3: 1-18

5.2 Introduction

Assessment of pregnancies at risk of PE and intra-uterine growth restriction is made at the booking appointment based on clinical risk factors such as previous obstetric history and age.(4) However it is thought that this approach could falsely classify two thirds of women as being high risk exposing them to unnecessary interventions.(5)

Currently a low pregnancy associated plasma protein-A (PAPP-A) ≤0.4 Multiples of the Median (MoMs) on 1st trimester screening is considered a major risk factor for having a SGA baby and it is therefore recommended that these women have serial scans from 26-28 weeks gestation to assess fetal growth and placental blood flow. (53)

Many maternal serum biomarkers have been investigated as potential screening tests for women at high risk of developing complications related to poor placentation such as PE, gestational hypertension and IUGR. A systematic review by Kuc et al. showed detection rates between 38-100% depending on combinations of multiple markers but concluded that prospective studies were needed to evaluate them in clinical practice. (39)

Based on existing studies, our systematic review and our research on AFP in the 2nd trimester we decided to assess the role of AFP in the 1st trimester in combination with PlGF, UADs and arteriography as a predictive test for detecting pregnancies at high risk of developing PE and other adverse outcomes.(41, 42, 95) We also assessed PAPP-A and β-hCG in those women whom had participated in the combined screening test programme for trisomy 21, and kisspeptin in a further small subset of women.

5.3 Materials and Methods

This was a prospective observational study recruiting an unselected population of pregnant women attending the Royal London Hospital for dating or nuchal translucency scans in the 1st trimester of pregnancy (11-14 weeks gestation) between January 2013 and July 2014. Written informed consent was obtained from those women agreeing to participate in the study. Ethics approval was granted by East of England Research Ethics Committee. Maternal characteristics and medical history were recorded. Arteriography, UAD measurements and maternal serum samples were taken.

1st trimester serum kisspeptin levels were measured in a subset of women. Selection was random and dependent on availability of staff to process the samples and test kits.

A further subset of women with mean UAD PI ≥1.96 were asked to return for serial monitoring of maternal serum PlGF and AFP at 20, 28 and 34-36 weeks gestation. A cut off of 1.96 for mean PI was used as per Costa's paper as it was felt that these women were more likely to develop complications secondary to placental insufficiency. (40)

Maternal history and characteristics

Information was collected on age, ethnicity, method of conception, parity, smoking, alcohol and drug use, past medical and obstetric history, family history and drug history. Maternal weight and height were measured, and BMI calculated.

Arteriograph measurements

These were performed as documented in chapter 2.

UAD measurements

These were performed as documented in chapter 2.

Biomarker measurements

Ten mls of maternal blood was taken in gold top gel tubes with no additives. The blood samples were allowed to clot and then centrifuged with removal of serum into aliquots which were stored at -80^{o}C until analysed. 50µl samples were extracted for measurement of PlGF, 10 µl for AFP, 15 µl for PAPP-A and 10 µl for β-hCG. Elecsys test kits from Cobas were used and analysed by a Roche Cobas e601. The lower limit for detection of AFP was 0.5iu/ml, 3pg/ml for PlGF, 4mIU/L for PAPP-A and <0.1iu/L for β-hCG. Tests of repeatability were performed to determine between batch imprecision and revealed a coefficient of variation of 2.07% for AFP and 3.89% for PlGF. AFP and PlGF measurements were performed on an additional 1141 saved serum samples from a previous cohort of women who were recruited for similar research at our hospital.

Kisspeptin samples were taken in a lithium heparin blood tube containing 200ul of aprotinin to inhibit plasma breakdown of peptides. Within a minute of taking the sample it was placed in a centrifuge, set to 4000rpm for 4 mins and spun at 4c. Immediately after this the plasma was separated into 3 eppendorf containers and frozen at -80c. Samples were analysed at Imperial College by Waljit Dhillo's group using radioimmunoassay. There was never more than 1 minute left between each step as if

there's a delay of more than 5min during a step there is a risk of kisspeptin breakdown or an interference effect, and results may be uninterpretable.

Samples were analysed by an assessor blind to the clinical outcomes.

Outcome measures

As defined in chapter 2. Outcome data was obtained from the hospital maternity records, where data was missing the woman was contacted directly.

Statistical Analysis

Haemodynamic parameters, maternal serum biomarkers, UAD mean PI and MAP were converted into multiples of the median (MoM). PlGF, AFP, PAPP-A, β-hCG and MAP MoMs were adjusted for gestational age, weight and ethnicity. The biomarkers were also adjusted for smoking status. PAPP-A and β-hCG were in addition adjusted for IVF. Mean PI MoMs were adjusted by the crown-rump measurement and maternal height in meters. SBPAO, Aix75 and pulse wave velocity MoMs were adjusted for weight, parity and maternal age. Aix75 and SBP_{AO} were also adjusted for ethnicity. Aix75 was further adjusted by maternal height.

Univariate analysis was initially performed to determine variables with a significant association with our outcomes. Logistic regression with stepwise selection was then performed to determine multivariate models in the prediction of PE, PIH, stillbirth and SGA. Variables were included in the models based on a significant p value ($p<0.05$) on multivariate analysis only. Final models were used to construct ROC curves and

detection rates for specified false-positive rates, and areas under the curve (AUC) were calculated.

The statistical software package STATA version 12 was used for all data analyses.

5.4 Results

Of the 1250 women recruited 1045 women were left for analysis after exclusions (n=205, 16.4%) (see figure 5.1 for explanation). 14 (1.3%) developed PE, 23 (2.2%) PIH, 64 SGA <5th centile (6.1%), 118 SGA <10th centile (11.3%) and 3 had a stillbirth (see figure 5.1). There were no cases of placental abruption. The maternal characteristics of the various outcome groups are shown in table 5.1.

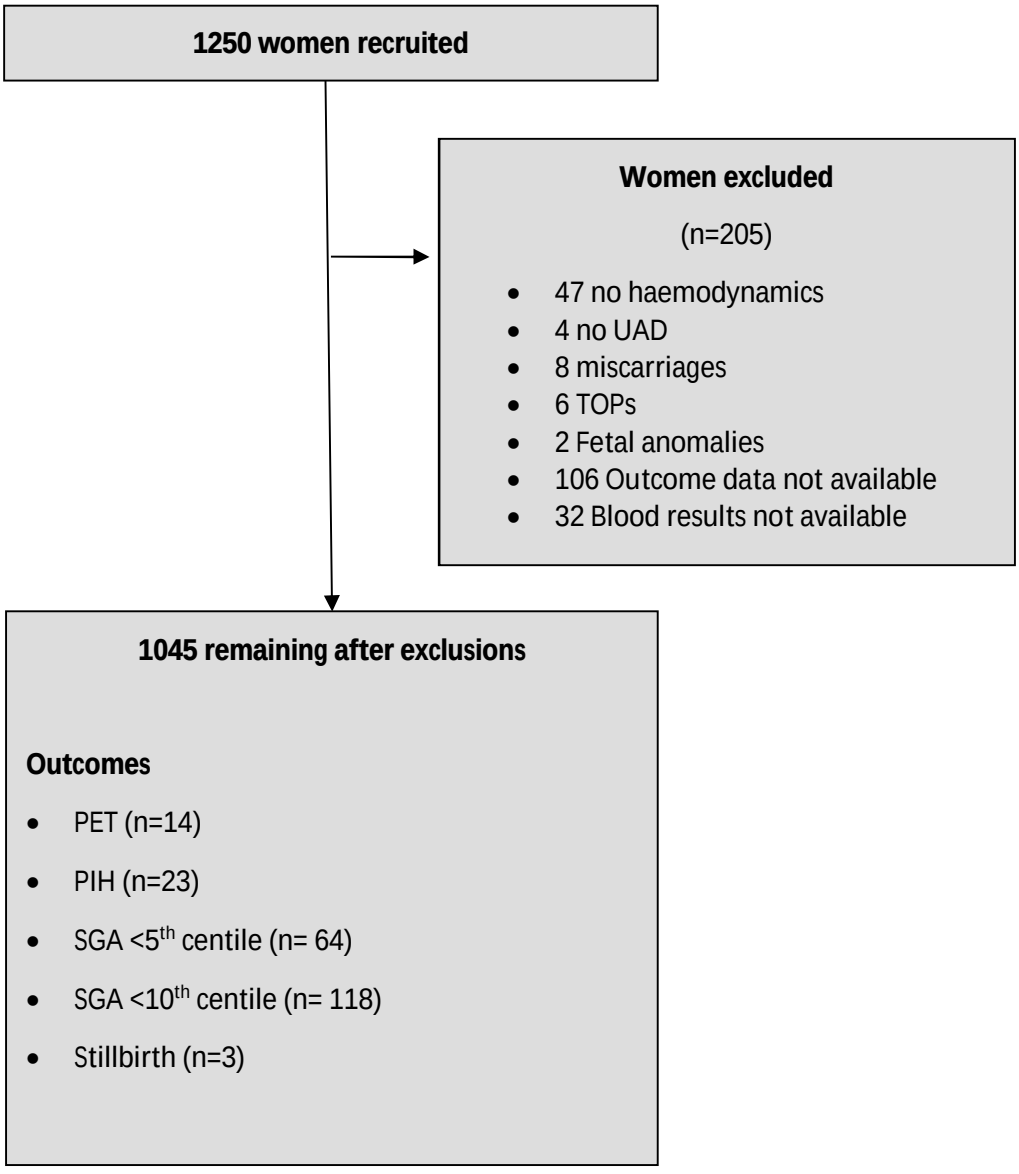
5.4.1 PE

Aix ($p<0.027$), SBP_{AO} ($p=0.002$) and MAP ($p=0.014$) along with mean uterine artery PI ($p=0.03$), Afro-Caribbean ethnicity ($p=0.013$) and a history of hypertension ($p=0.047$) were significantly associated with the development of PE. There was no statistically significant association between levels of AFP ($p =0.6$) PlGF ($p= 0.98$) PAPP-A ($p= 0.52$) and β-hCG ($p=0.27$). The detection rate for PE was 72% for a FPR of 15%, LR 4.8, AUC 0.81, 95% CI 0.69-0.93. The detection rate using the current NICE recommendations, based on maternal characteristics, was 43% for FPR 11%, LR 3.9.

PlGF and AFP data was also obtained for a cohort of 1141 women recruited prospectively for research in the 1st trimester of pregnancy (from 2010-2012) for prediction of PE and had serum saved. This gave us a total sample size of 2186

including 56 cases of PE. Demographic data for this population and comparison to my population are shown in chapter 6, table 6.3. This additional analysis was performed to see if results correlated with those above given the small number of preeclamptics in the initial sample. Univariately, PlGF MoM was then found to be significantly associated with PE (OR=0.16, 95% CI 0.04-0.72, p=0.016) but wasn't significant in the multivariate model. The findings for AFP remained unchanged.

Figure 5.1: Flow chart



5.4.2 PIH

SBP_{AO} (p=0.015), MAP (p=0.001) and maternal weight (p=0.001) were the only parameters significantly associated with PIH. Detection rate for PIH was 48% for a 15% FPR, AUC 0.76, 95% CI 0.65-0.86. For PIH the strongest marker was PlGF (p = 0.07) compared to AFP (p= 0.83), PAPP-A (p= 0.54) and βhCG (p value=0.39) but none achieved statistically significant correlations.

5.4.3 SGA

Low PAPP-A and PlGF were significantly associated with SGA <10th centile (p=0.007 and 0.004 respectively) but not AFP (p=0.754), βhCG (p=0.779) and mean PI (p=0.135). None of the haemodynamic parameters showed a statistically significant association with SGA <10th centile. A history of maternal hypertension was also a significant factor in the development of an SGA baby <10th centile (p=0.03). On multivariate analysis the detection rate was 30% for a fixed FPR of 15%, AUC 0.608, 95% CI 0.54-0.68).

For SGA <5th centile there was a significant association with low PlGF and PAPP-A (p=0.00 and 0.011 respectively) South Asian ethnicity (p=0.047) and maternal smoking (p=0.018). The detection rate was 57% for a 15% fixed FPR, AUC 0.73, 95% CI 0.65-0.80).

Table 5.1: Maternal characteristics of control and outcome groups

Maternal characteristic	Control (n=855)	PE (n=14)	PIH (n=23)	SGA <5th centile n=(64)	SGA<10th centile (n=118)	IUD (n=3)
Maternal age (years, median (range))	30 (15-45)	31.5 (24-39)	34 (24-48)	30 (18-41)	31 (18-41)	35 (20-37)
Weight(kg, median (range))	61.5 (37-135)	61.5 (45-106)	69 (44-104)	61 (47-135)	62.5 (35-135)	73 (58-77)
BMI	23 (15-51)	23 (19-41)	25 (17-36)	24 (18-45)	24 (16-45)	26 (24-26)
Ethnicity						
Caucasian	342	5	8	17	37	1
Afro-Caribbean	83	6	5	9	15	0
South Asian	304	3	6	28	46	2
Oriental	89	0	3	5	13	0
Mixed/other	37	0	1	5	7	0
Parity						
Nulliparous	482	8	17	30	59	1
Parous-no previous PE	365	3	6	31	53	2
Parous-Previous PE	8	3	0	3	6	0
Family history of PE (mother/sister)	24	1	1	2	5	0
Smoker	27	1	1	5	5	1
Alcohol	11	0	0	1	3	0
Recreational Drugs	2	0	0	0	0	1
Conception						
Spontaneous	17	13	23	62	116	3
IVF	838	1	0	2	2	0
Medical History						

None	679	12	20	50	93	3
Essential hypertension	5	1	1	3	4	0
Lupus /Antiphospholipid syndrome	4	0	0	0	0	0
Renal disease	0	1	0	0	2	0
Diabetes Mellitus	9	0	0	1	2	0
Asthma	24	0	2	5	8	0
Hyperthyroidism	2	0	0	1	2	0
Hypothyroidism	19	0	0	4	5	0
Sickle cell disease	0	0	0	0	0	0
Medication during pregnancy						
None	716	12	19	53	95	2
Aspirin	19	1	2	2	7	1
Anti-thrombotics	4	0	0	0	1	0
Anti-hypertensives	4	0	0	2	2	0
AEDs	2	0	0	0	0	0
Thyroxine	23	0	0	3	4	0
Beta-mimetics	11	0	2	3	5	0
Immunosupressants	4	1	0	0	1	0
Insulin	5	0	0	1	0	0
steroids	1	0	0	0	3	0
Anti-thyroid drugs	2	0	0	0	0	0

5.4.4 Stillbirth

High AFP (p=0.041) and low PlGF (p=0.036) were significantly associated with stillbirth, as was a maternal history of smoking (p=0.025) with a detection rate of 67% for a 15% FPR. AUC 0.92, 95% CI 0.82-1.0. No association was seen with haemodynamic parameters, increased uterine artery PI (p=0.59) or PAPP-A (p=0.44) and β-hCG (p=0.21).

See table 5.2 for comparison of median MoMs in affected and unaffected pregnancies. See appendix 5.1 for full details of the logistic regression models, the univariate and multivariate odds ratios for prediction of each outcome and ROC curves. Appendix 5.2 summarises the sensitivity of the screening tool at various false positive rates.

Table 5.2: Comparison of median MoMs in affected and unaffected pregnancies

		PE	PIH	IUD	SGA5	SGA10
AIX50	Affected	1.09	1.03	0.96	1.03	1.00
	Unaffected	1.00	1.00	1.00	1.00	1.00
	p-value	0.027	0.104	0.364	0.201	0.555
PWV	Affected	1.03	1.05	1.16	1.00	1.01
	Unaffected	1.00	1.00	1.00	1.00	1.00
	p-value	0.848	0.133	0.439	0.786	0.603
SBPAO	Affected	1.11	1.05	1.00	1.01	1.00
	Unaffected	1.00	1.00	1.00	1.00	1.00
	p-value	0.002	0.015	0.635	0.331	0.858
MAP	Affected	1.08	1.09	1.03	0.99	0.99
	Unaffected	1.00	1.00	1.00	1.00	1.00
	p-value	0.014	0.001	0.390	0.625	0.335
Mean UAD PI	Affected	1.08	1.00	1.14	1.06	1.04
	Unaffected	1.00	1.00	1.00	1.00	1.00
	p-value	0.030	0.889	0.593	0.087	0.135
AFP	Affected	0.92	1.05	1.83	1.10	1.03
	Unaffected	1.00	1.00	1.00	0.99	1.00
	p-value	0.601	0.836	0.041	0.075	0.754
Free β-hCG	Affected	0.83	0.91	0.65	1.05	0.90
	Unaffected	0.96	0.96	0.96	0.96	0.96
	p-value	0.266	0.399	0.206	0.522	0.779
PAPPA	Affected	0.76	0.93	1.24	0.87	0.90
	Unaffected	1.02	1.02	1.02	1.02	1.03
	p-value	0.524	0.542	0.438	0.011	0.007
PlGF	Affected	0.98	0.79	0.66	0.79	0.89
	Unaffected	1.00	1.00	1.00	1.01	1.01
	p-value	0.981	0.071	0.036	0.000	0.004

No corrections were made for multiple testing

5.4.5 Kisspeptin results

Sixty two primiparous women had serum kisspeptin levels measured in the 1st trimester. Of these only one developed PE, 2 PIH, one SGA<5th centile, 2 SGA <10th centile and 5 GDM. No significant association between kisspeptin and any of the outcomes were found. Table 5.3 summarises the odds ratios and p-values.

Table 5.3: Kisspeptin results

Outcome	**OR**	**95% CI**	**p-Value**
PE	0.87	(0.68-1.12)	0.291
PIH	0.99	(0.85-1.16)	0.947
IUD	-	-	-
SGA <5th centile	0.88	(0.68-1.13)	0.3
SGA<10th centile	0.85	(0.69-1.03)	0.092

5.4.6 Serial PlGF and AFP

Serial results were obtained for 73 women. In this cohort of women there were 3 cases of PE, 4 PIH, 12 SGA<5th centile and18 SGA<10th centile. There was a significant association between low serial PlGF levels and SGA<5th (p=0.037) and 10th centiles (p=0.014). There was no significant association with any of the other outcomes. Serial levels of AFP weren't associated with any of the outcomes (see appendix 5.3).

5.5 Discussion

This was a prospective cohort study with complete outcome data for 1045 unselected women who attended for their 1st trimester scan at the Royal London Hospital over an 18 month time frame. All haemodynamic and UAD measurements were performed by the same assessor and biomarker levels were measured independently by assessors

who were blinded to the other variable results and outcome data. The population attending the Royal London is ethnically diverse with a large number of South Asian women. We managed to capture a large cross sample of our population which is demonstrated in table 5; our findings should therefore be widely applicable.

In our study population there was a low incidence of PE (1.3%) and PIH (2.2%). We found that our model, combining maternal characteristics with biochemical, biophysical parameters and haemodynamics improved the detection of women at risk of developing preeclampsia to that of the current NICE recommendations based on maternal characteristics alone. In contrast to some other studies we didn't find any association between abnormal levels of the 1st trimester biomarkers and PE/PIH. (30, 69, 73, 76-79, 85-87, 94, 99, 128) There was a significant association between low levels of PlGF (p=0.036) and high levels of AFP (p=0.041) and IUD, and low levels of PlGF (p=0.004) and PAPP-A (p=0.007) and SGA <10th centiles and SGA <5th centile (p=0.011 and p=0.00 for PlGF and PAPP-A respectively).

AFP is an oncofetal glycoprotein produced from the second month of pregnancy, initially by the yolk sac and from the third month by the fetal liver and gastrointestinal tract. It has been used as a screening test for open neural tube and abdominal wall defects. Maternal serum AFP rises from weeks 10 to 32 and then declines. Fetal to maternal transfer of AFP is thought to be via two routes, transplacental and transmembranous. Unexplained mid trimester MSAFP elevations have been associated with an increased risk of SGA, preterm delivery, placental abruption and PE.(129-131) In the first and 2nd trimester evaluation of risk (FASTER) study involving 33 000 women, MSAFP >2MoM was associated with placental abnormalities as well as IUGR (OR 2.7), miscarriage (OR 4.4) and intrauterine fetal death (OR 5.8) (129).

Walters et al. reported that 13% of women with elevated MSAFP developed PE compared to 1% of the women with normal MSAFP.(130) Williams et al (1992) compared 201 women with unexplained elevated MSAFP (greater than and including 2.0 MoM) with 211 women with normal MSAFP. A significant association was found between elevated MSAFP and PE, adjusted risk ratio (ARR) being 3.8.(131) The association between AFP and adverse pregnancy outcome has been attributed to a breakdown in the placental barrier resulting in an increase in the diffusion of alpha-fetoprotein from fetal to maternal serum. This is supported by the finding that fetal maternal haemorrhage of <0.1ml can cause a substantial elevation in MSAFP. (132) In a small morphometric study of placentae associated with raised MSAFP, a normal concentration of amniotic fluid AFP, and a normally formed fetus, the mean total placental volume, volume of parenchyma and villous surface area were increased, there were more areas of infarction and a lower fetal-placental weight ratio compared with the control group. (133) Boyd et al. propose that the reason 2nd trimester AFP may be raised in combination with a normally formed fetus can be attributed mainly to changes in the placenta which has the property of responding to an adverse environment by increasing its surface of exchange.

From previous studies, including our own which demonstrated AFP as a promising 2nd trimester marker, we were disappointed that this study did not show it to have a role in 1st trimester screening for hypertensive disease. (41, 42) At the commencement of this research there were no other publications that we could find assessing the association of 1st trimester AFP levels and PE. However during this research a paper was published in 2016 which demonstrated an association between raised AFP levels in the 1st and 2nd trimester and PE, with values being inversely related to gestational age at delivery. They concluded that measurement of serum AFP at 11-13 and 19-24 weeks improved the prediction of preterm PE provided by maternal factors alone. They did

identify that its performance as a screening test was superior at 19-24 weeks than in the 1st trimester and that measurement alone in the 1st trimester is unlikely to improve the performance of screening provided by a combination of maternal factors, uterine artery PI, MAP and PlGF.(134) We hypothesise that our finding of no significant association between increased MSAFP and PE may be because endothelial damage in the 1st trimester is still not established. However we did show a significant association between increased 1st trimester AFP and IUD. This would be an interesting area for future research.

PlGF is a member of the vascular endothelial growth factor subfamily and is expressed by trophoblast cells. It is a pro-angiogenic protein involved in the regulation of placental vascular development and maternal endothelial function during pregnancy. Changes in levels of PlGF or its inhibitory receptor have been implicated in the development of PE.(15, 22, 24, 135) We demonstrated an association between low PlGF and SGA. Previous research supports this finding.(85, 136) Our research does not support previous researcher's evidence for demonstrating serial low levels of PlGF for predicting pregnancies at risk of PE. (91, 137) However there were only 3 preeclamptics in the sample who had serial levels measured. Previous studies were retrospective case control so were able to select a large number of preeclamptics for measurement of PlGF levels. They also found that the levels were lower in those with preterm rather than term PE. Our systematic review showed abnormal levels of PlGF to be significantly associated with early but not late or any onset PE.(95) It is likely that our study didn't demonstrate an association between both one off 1st trimester or serial levels of PlGF because the numbers of early onset PE were so low leading us to assess all preeclamptics together.

We found an association between low PlGF and stillbirth supporting findings by Akolekar et al. In our three cases of stillbirth two weighed less than the 10th centile on customised growth charts. Placental histology was abnormal in two cases. In one case the placenta weighed less than the 3rd centile for gestational age with delayed maturation and chronic decidiutis; this patient also had PIH. The other was from the normally grown fetus which also showed delayed maturation. These findings suggest that at least two thirds of the cases of stillbirth were placentally mediated and supports the use of placental biomarkers such as PlGF for the prediction of stillbirth.

PAPP-A is a syncitiotrophoblast derived insulin like growth factor binding protein protease that increases the bioavailability of insulin like growth factor. IGF is believed to play a role in fetal growth by mediating trophoblast invasion to the decidua and regulating steroidogenesis and glucose and amino acid transport in the chorionic villi. It is therefore not surprising that a low PAPP-A is associated with a higher incidence of PE and other adverse pregnancy outcomes secondary to poor placentation such as growth restriction. (73, 138) Our research did not support other researchers or our systematic reviews findings of an association with PE. Our systematic review showed a significant association between low PAPP-A with early and any onset PE. Previous research has shown a stronger association between low PAPP-A and early onset PE than late onset.(37) Studies on placental pathology have suggested that early onset disease is more likely to be associated with abnormal villous and vascular morphology whereas in late onset disease the placental morphology and histology are not dissimilar to those in controls.(139, 140) There is evidence that late onset disease is more likely to be related to impaired glucose metabolism and a hyperdynamic low peripheral resistance maternal cardiovascular profile. We showed a low PAPP-A to be significantly associated with SGA, supporting the findings of other researchers. (141)

Several previous studies have reported an association between increased 2nd trimester levels of β-hCG and adverse pregnancy outcomes including PE, SGA, preterm delivery and miscarriage. Other studies found that decreased levels in the 1st trimester were predictive of PE. (142) In our study abnormal 1st trimester levels of β-hCG were not significantly associated with any of the outcomes we assessed supporting our systematic review findings and findings by Spencer (2005) and Smets (2008).(44, 99) hCG is produced by the syncitiotrophoblast of the placenta and consists of alpha and beta subunits. It has been postulated that low β-hCG between 10-14 weeks may be a consequence of impaired placentation and a smaller placental mass and that subsequently high 2nd trimester levels may develop as a result of hypoperfusion–related stimulation. The increased hCG in PE may be associated with reactive hyperplasia of cytotrophoblastic cells and functional alteration of the syncitiotrophoblast leading to increased leakage into the maternal circulation.(142, 143) Our findings of no association between abnormal levels and adverse pregnancy outcome would support this hypothesis.

Kisspeptin-54 (metastin) was first identified as a suppressor of tumour metastasis and has been described in abundance in the placenta. As levels rise dramatically from 8 weeks gestation coinciding with the time of peak trophoblastic invasion it is thought to play a key role in implantation and development. A small study measured kisspeptin levels between 8-14 weeks of gestation and found that levels were lower in women that had SGA neonates. (44) A subsequent study measured kisspeptin levels at 16-20 weeks gestation and looked at the association with SGA and PE and also found that kisspeptin levels were significantly lower indicating that kisspeptin levels are reduced in conditions associated with poor placentation. (45) These studies were retrospective case control and did not assess its use as a biomarker for PE in the 1st trimester. Kisspeptin and its association with PE were only assessed in the 2nd trimester study.

We had the opportunity to measure kisspeptin levels in a small sample of our study population due to a separate research collaboration. We did not demonstrate any significant association between low kisspeptin and any adverse outcomes. Although our study's prospective it was hampered by a low prevalence of outcomes in the unselected population whom had kisspeptin measured.

In order to properly assess the role of kisspeptin as a biomarker for PE and other adverse pregnancy outcomes secondary to poor placentation it would need to be performed on a larger scale, ideally on at least all of the women that were recruited. Limitations to measuring kisspeptin; mainly expense of the test and the need to process samples within minutes of being taken from the patient, will have huge implications on its suitability as a biomarker within a prognostic model that could be used routinely in the NHS and therefore it is highly unlikely that it would currently be implemented within such a screening tool.

There was a significant association between a high mean UAD PI and MAP and PE but not PIH supporting previous findings. (47, 94, 144). Our study findings support previous findings by Khalil et al for the role of arteriography for predicting women at risk of hypertensive diseases of pregnancy demonstrated by a significant increase in augmentation index, SBPAO and MAP in our preeclamptic and PIH groups. We did not demonstrate any association between abnormal arteriography and SGA which is supported by previous research findings which did not demonstrate ay significant difference in haemodynamic parameters in SGA without associated PE.(145) Khalil suggests that in women with increased arterial stiffness and higher $SBP_{AO,}$ PE is not mediated by impaired placental perfusion and function. Unlike with UADs and biomarkers which have been shown to be more strongly associated with early onset PE

the effectiveness of arteriography is unrelated to gestational age at onset of disease. These findings support the concept of two separate pathophysiologies for the development of PE, one based on a predisposition to cardiovascular disease that under the physiological stress of pregnancy manifests as early or late PE, the other resulting in early PE due to impaired trophoblastic invasion of the maternal spiral arteries.(47)

Despite one researcher recruiting a large number of diverse women there was a lower incidence than expected of the outcomes we were assessing in our study population. A power calculation was performed prior to study commencement (see appendix 5.4), and the number of women needed to gain statistical power were recruited, however the lower incidence than expected of the various outcomes we were assessing may have affected our findings leading to type 2 error.

5.6 Conclusions

Our model demonstrates an improvement in the detection of preeclampsia from that of the NICE guideline which is currently used. Our findings do not support a role for the routine use of 1st trimester maternal serum biomarkers for predicting women at risk of hypertensive disease in pregnancy. However, my research does support previous findings in the role of PlGF and PAPP-A for predicting women at risk of having an SGA baby. (54, 141) PAPP-A is already routinely measured as part of the combined screening test and has been incorporated in to RCOG guidance on SGA. (53) Haemodynamics show a lot of promise for the prediction of hypertensive disease, from the findings of our study and Khalil's. It is also inexpensive (after the initial purchase of the machine), quick and easy to perform after training.

The finding of high 1st trimester AFP levels in association with stillbirth raises the possibility of combining it with other biomarkers e.g. PlGF, and using it as a screening tool. The increased PlGF in association with stillbirth supports findings by Akolekar et al. (56) Markers can be used in conjunction with biophysical parameters, either 1st trimester or 2nd trimester Doppler.

Our model measures variables reflective of placental function (PAPP-A, PlGF, β-hCG, kisspeptin) or increased resistance in the uteroplacental circulation (UADs), as our study had a very low incidence of early PE we combined all cases to form one group of any onset PE, as the majority of this group were made up of late onset cases this may explain why our study has not shown any association between 1st trimester abnormal biomarker levels and PE.

Our study findings have been limited from the low prevalence of outcomes in our group. Larger studies are needed to clarify the efficacy of biomarkers in 1st trimester prediction models for PE. It is clear that the value of these markers is limited with late onset disease but show promise in terms of early onset and more severe forms of PE. Larger studies are needed to justify the introduction of these markers into routine clinical practice as part of a prediction model for preterm PE and then in screening for other uteroplacental problems.

This is the first study that links a high 1st trimester AFP with stillbirth. Larger studies are needed to ascertain the true value of this association.

Chapter 6: External validation of pre-existing 1st trimester PE prediction models

6.1 Abstract

Objective

To validate the increasing number of prognostic models being developed for PE using our own prospective study.

Methods

A systematic review of literature that assessed biomarkers, UAD and maternal characteristics in the 1st trimester for the prediction of PE was performed and models selected based on predefined criteria. Validation was performed by applying the regression coefficients that were published in the different derivation studies to our cohort. We assessed the models discrimination ability and calibration.

Results

Twenty models were identified for validation from 11 studies. The discrimination ability observed in derivation studies (Area under the Curves) ranged from 0.70 to 0.96 when these models were validated against the validation cohort, these AUC varied importantly, ranging from 0.504 to 0.833. Comparing Area under the Curves obtained in the derivation study to those in the validation cohort we found statistically significant differences in several studies.

Conclusion

There currently isn't a definitive prediction model with adequate ability to discriminate for PE, which performs as well when applied to a different population and can differentiate well between the highest and lowest risk groups within the tested population. The pre-existing large number of models limits the value of further model development and future research should be focussed on further attempts to validate existing models and assessing whether implementation of these improves patient care.

Citation of paper arising from this work:

Allen R, Zamora J, Arroyo-Manzano D, Velauthar L, Allotey J, Thangaratinam S, Aquilina J. External Validation of pre-existing first trimester preeclampsia prediction models. European Journal of Obstetrics and Gynecology and Reproductive Medicine. 2017. Vol 217. 119-125

6.2 Introduction

There has been a rise in the number of prognostic models being developed in obstetrics, particularly for PE, but few have been implemented. Risk assessment is currently performed at the booking appointment and is based on maternal characteristics alone. However this approach is thought to falsely classify two thirds of women as being high risk and in need of intensive monitoring and prophylactic aspirin therapy, highlighting the need for a better screening test.(5)

Modern medicine is increasingly focussing on preventing disease. As a result it is important to identify patients at increased risk of illness. This could be based on a single risk factor or a combination of multiple predictors. Combining predictors into a prognostic model is likely to allow better risk assessment than the use of single risk factors.

In recent years there has been a massive amount of studies published looking at various biophysical and biochemical markers alone or in combination for the prediction of PE. A recent systematic review by Kleinrouweler et al. identified 69 prediction models for PE, only 5 of which had been externally validated and model performance was found to be lower when externally rather than internally validated. They recommended that systematic reviews should be performed to identify and validate existing models to decide whether a new model should be developed or an existing model updated. Following this the better models should be applied in clinical practice and their influence on patient outcomes evaluated.(50)

As a result of this review we have decided to externally validate pre-existing PE models using data obtained from our own prospective study to predict PE risk. We focussed on 1st trimester prediction models as interventions to reduce the risk of PE, mainly aspirin, have been shown to be more effective if commenced prior to sixteen weeks gestation.

6.3 Methods

Objectives

To validate clinical prediction rule for PE from models reported in obstetric literature. (30, 36, 37, 76, 79, 146-151)

Validation study population.

The data from two cohorts of pregnant women attending the Royal London Hospital for their 1st trimester dating scan who were recruited for research into 1st trimester screening for PE over two time periods (2010-2012 and Jan 2013-July 2014) were combined. Women with multiple pregnancies and fetal anomalies were excluded. Ethics approval was obtained from the East of England research ethics committee. Information was collected on age, ethnicity, method of conception, parity, smoking, alcohol and drug use, past medical and obstetric history, family history and drug history. Maternal weight and height were measured and BMI calculated as was blood pressure and MAP. Transabdominal ultrasound was used to measure the uterine artery flow velocity waveforms with colour pulsed Doppler using a Voluson E6 with a 3.5/5 MHz linear array, the method for which has previously been described in chapter 2. Maternal serum was taken and the biomarkers PAPP-A, PlGF, β-hCG and AFP measured.

Prognostic models and study selection

We searched Medline from inception until November 2016 without language restrictions. Full details of the search strategy are given in our previous paper.(50) Models were selected for validation if they met the criteria detailed in table 6.1. Models were excluded if they did not include 1 or more of the predictors and if they did not have a regression coefficient.

Table 6.1: Selection criteria for inclusion in validation of prognostic models

Criteria	
Population	Pregnant women in the 1st trimester
Predictors	Maternal clinical characteristics at booking – Maternal characteristics: Age, BMI, ethnicity, smoking, medical history particularly hypertension, diabetes, antiphospholipid syndrome or SLE. Previous obstetric history: parity, previous hypertensive disease, family history of PE, previous miscarriages, stillbirth or SGA fetus; Current pregnancy: early pregnancy bleeding, systolic and diastolic blood pressure Biochemical markers (1st trimester)– PAPP-A, PlGF, AFP, HCG, kisspeptin Ultrasound markers (1st trimester)– UAD (resistance index, pulsatility index, unilateral or bilateral notching)
Outcomes	Early onset (<34 weeks), late onset (≥ 34 weeks) and any onset PE
Study design	Reporting of a regression coefficient

Quality Assessment of Prognostic Models

The Quality in Prognosis studies (QUIPS) tool was used assess the risk of bias in the included prognostic studies. The bias domains assessed were study participation, attrition, prognostic factor measurement, confounding measurement and account, outcome measurement and analysis and reporting. Each domain was assessed as either low, high or moderate risk of bias. (65)

Statistical analyses

We describe baseline characteristics of the validation cohort using median and interquartile ranges (IQR) and absolute and relative frequencies. We compared baseline clinical and demographic characteristics of the women recruited in the two cohorts used in the validation process. We used Mann-Whitney U tests and Chi-squared tests or Fisher exact tests when needed for these comparisons. The comparison of both cohorts was further extended to evaluate the predictive performance of the models within the two groups of women included in the validation cohort.

Validation of the models included in this review was performed by applying the regression coefficients that were published in the different derivation studies to our cohort. We obtained predicted probabilities of any PE for all models. Since the prevalence of PE in the derivation cohorts were slightly different to the validation one, we recalibrated the regression coefficients of the original models by computing a shrinkage correction coefficient. This allows adjustment of predicted probabilities to the prevalence of PE in the validation cohort. (152) We based all analyses on the shrunk probabilities.

For every model, we assessed its discrimination ability and its calibration. We assessed discrimination ability computing the Area under the ROC curve. We compared AUCs in the validation cohort with the AUC reported by authors of the original models using a Z-test. (66) We evaluated model calibration by the Hosmer-Lemeshow goodness-of-fit test.

Predictive information of a model is the degree of separation of the predictive probabilities estimated for the highest and lowest risk of PE groups. We computed

PSEP statistic as the difference between the mean predicted probability of the high risk group (4th quartile) minus the mean predicted probability for low risk group (1st quartile). The higher the PSEP, the better the predictive information of the model.(153)

All the analyses were carried out with Stata v13.

6.4 Results

Sample description

We analysed 2,186 women. These women were obtained from 2 cohorts recruited over a 4 year period (2010-2014) at the Royal London Hospital. The incidence of PE was 2.4% (n=52). The median age (IQR) was 29 years (26; 33), body-mass index 23.7 (21.1; 27.0) kg/m^2 and 802 (36.8%) had a BMI ≥25 kg/m^2. 824 (37.7%) women were Caucasian, 233 (10.7%) Black, 117 (5.3%) Chinese, 942 (43.3%) Indian or Pakistani, 64 (2.92%) mixed and 0.18% unknown. The population attending the Royal London is ethnically diverse with a large number of South Asian women. The high proportion of women recruited who were of a non-Caucasian ethnicity demonstrates that we managed to capture a large cross sample of our population which has hopefully avoided the introduction of bias.

Among all analysed women we observed 17 (0.8%) who had previous PE, 25 (1.1%) had pre-existing diabetes mellitus, 21 (0.9%) history of hypertension, 426 (19.5%) had a family history of PE and 1006 (46.0%) were multiparous. Other variables are described in table 6.2.

A comparison of the cohorts is shown in table 6.3. We observed statistically significant differences in the median age (p<0.001), BMI (p=0.008), proportion of women with BMI ≥ 25 kg/m^2 (p=0.004) and in the proportion of women with a family history of PE (p<0.001) and previous PE (p<0.001) (table 6.3).

Table 6.2: Baseline variables of validation cohort

	Validation cohort	
	Analysed women (N)	Estimate
Age [1]	2,180	29.00 (26.00; 33.00)
BMI [1]	2,180	23.70 (21.10; 27.00)
FhCG1T [1]	929	32.00 (21.60; 49.90)
PAPP-A (mIU/L) [1]	929	3853 (2351; 6041)
PAPP-A (MoM) [1]	1,932	1.01 (0.69; 1.43)
PlGF (pg/ml) [1]	2,101	57.33 (43.60; 78.37)
PlGF (MoM) [1]	2,082	1.00 (0.77; 1.34)
MAP (mmHg) [1]	2,186	84.00 (78.00; 90.00)
MAP (MoM) [1]	2,179	1.00 (0.94; 1.07)
Mean PI [1]	2,186	1.45 (1.20; 1.72)
Mean PI (MoM) [1]	2,174	1.00 (0.84; 1.18)
BMI (≥25 kg/m^2) [2]	2,180	802 (36.79%)
Family Hx PE (Yes) [2]	2,186	426 (19.49%)
Diabetes Mellitus (Yes) [2]	2,186	25 (1.14%)
Multiparous (Yes) [2]	2,186	1006 (46.02%)
IVF(Yes) [2]	2,186	36 (1.65%)
Past PE (Yes) [3]	2,186	17 (0.78%)
Hypertension (Yes) [3]	2,186	21 (0.96%)

Caucasian (Yes) [2]	2,186	826 (37.79%)
Black (Yes) [2]	2,186	233 (10.66%)
Other racial origin (Yes) [2]	2,186	1127 (51.56%)
Renal disease (Yes) [2]	2,186	2 (0.09%)
Thrombophilic condition (Yes) [2]	2,186	5 (0.23%)

[1] Reported as median (P_{25}; P_{75})
[2] Reported as count (percentage)

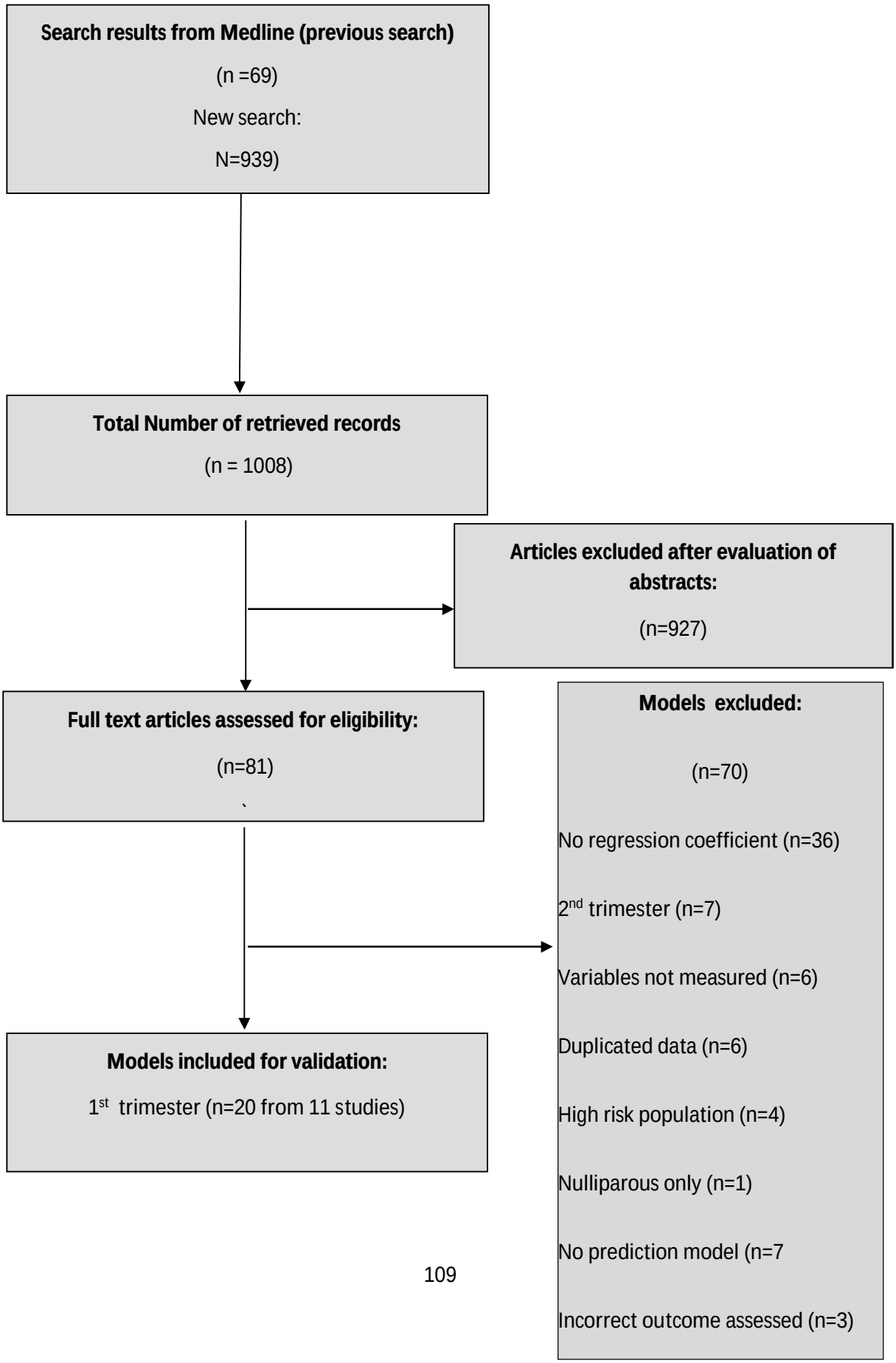
Table 6.3: Baseline variables comparison between validation cohorts

	Velauthar		Allen		
	Analysed women	Estimate	Analysed women	Estimate	P-Value
Age [1]	1138	29.00 (25.00; 33.00)	1042	30.00 (26.00; 33.00)	<0.001
BMI [1]	1137	23.88 (21.31; 27.34)	1043	23.53 (21.00; 26.37)	0.008
FhCG1T [1]	-	-	929	32.00 (21.60; 49.90)	-
PAPP-A (miU/L) [1]	-	-	929	3853 (2351; 6041)	-
PAPP-A (MoM) [1]	1003	1.01 (0.67; 1.45)	929	1.02 (0.71; 1.42)	0.520
PlGF (pg/ml) [1]	1059	61.50 (46.72; 85.20)	1042	53.01 (39.91; 71.53)	<0.001
PlGF (MoM) [1]	1040	1.00 (0.78; 1.30)	1042	1.00 (0.75; 1.40)	0.685
MAP (mmHg) [1]	1143	81.33 (75.00; 86.67)	1043	87.00 (81.00; 93.00)	<0.001
MAP (MoM) [1]	1137	1.00 (0.94; 1.07)	1042	1.00 (0.93; 1.07)	0.539
Mean PI [1]	1143	1.45 (1.22; 1.71)	1043	1.47 (1.17; 1.74)	0.221
Mean PI (MoM) [1]	1136	1.00 (0.86; 1.19)	1038	1.00 (0.80; 1.17)	0.002

BMI (≥25 kg/m^2) [2]	1137	448 (39.40%)	1043	354 (33.94%)	0.008
Family Hx PE (Yes) [2]	1143	413 (36.13%)	1043	13 (1.25%)	<0.001
Diabetes Mellitus (Yes) [2]	1143	14 (1.22%)	1043	11 (1.05%)	0.709
Multiparous (Yes) [2]	1143	547 (47.86%)	1043	459 (44.01%)	0.071
IVF (Yes) [2]	1143	15 (1.31%)	1043	21 (2.01%)	0.198
Past PE (Yes) [3]	1143	0 (0.00%)	1043	17 (1.63%)	<0.001
Hypertension (Yes) [3]	1143	11 (0.96%)	1043	10 (0.96%)	0.993
Caucasian (Yes) [2]	1143	400 (35.00%)	1043	426 (40.84%)	0.005
Black (Yes) [2]	1143	125 (10.94%)	1043	108 (10.35%)	0.660
Other racial origin (Yes) [2]	1143	618 (54.07%)	1043	509 (48.80%)	0.014
[1] U Mann-Whitney	N	Median (P_{25}; P_{75})	N	Median (P_{25}; P_{75})	
[2] Chi^2 and [3] Fisher exact test	N	n (%)	N	n (%)	

Study selection

Figure 6.1: Selection of the models included for validation.



Quality Assessment

Quality assessment was performed by two reviewers (JA) and (RA). There was a moderate risk of bias in the study participation domain for Akolekar but a low risk of bias for all the other domains and in all domains for the other studies. See table 6.4.

Validation

Models being validated are listed in Table 6.5. Sample sizes of these models ranged from 359 and 8,189 with prevalence of PE ranging between 0.4% and 14.7%. The size of the validation cohort depends on data availability for predictors included in the different models. So, sample size in this validation cohort ranges from 929 for the model of DiLorenzo 2012 (Early PE model) and 2,186 for DiLorenzo 2012 (Late PE model), Goetzinger 2010, Plasencia 2008 (Late PE model), Baschat 2014 (early PE model) and Poon 2009b (Early PE model) for which all available data is included in the analysis as no missing values are present for all predictors included in the models.

The discrimination ability observed in derivation studies (AUCs) ranged from 0.70 (Goetzinger 2010) to 0.96 (Scazzocchio 2013, Early PE model); when these models are validated in the validation cohort, these AUC varied importantly. AUCs ranged from 0.504 (DiLorenzo 2012 Early PE model) to 0.833 (Poon 2009b Early PE model). When comparing AUCs obtained in the derivation study to those in the validation cohort we found statistically significant differences in several studies (Akolekar 2008 Early PE model, Plasencia 2008 Early and Late PE model, Poon 2009a Early and Late PE model, Poon 2009b Early PE model, Scazzocchio 2013, Early PE model, Baschat 2014 early and late models).

Table 6.4: Quality assessment of the included studies

Study			**Bias Domains**			
	Study Participation	Study Attrition	Prognostic Factor measurement	Confounding Measurement and Account	Outcome Measurement	Analysis and Reporting
Akolekar 2008	Moderate	Low	Low	Low	Low	Low
Plasencia 2008	Low	Low	Low	Low	low	low
Goetzinger 2010	Low	Low	Low	Low	Low	Low
DiLorenzo 2012	Low	Low	Low	Low	Low	Low
Poon 2008	Low	Low	Low	Low	Low	Low
Poon 2009a	Low	Low	Low	Low	Low	Low
Poon 2009b	Low	Low	Low	Low	Low	Low
Poon 2009c	Low	Low	Low	Low	Low	Low
Parra-Cordero 2013	Low	Low	Low	Low	Low	Low

Baschat 2014	Low	Low	Low	Low	Low	Low
Scazzocchio 2013	Low	Low	Low	Low	Low	Low

Table 6.5: Summary of models and AUC-ROC comparison between derivation studies and validation study

Study	**PE Screening**	**Derivation study**					**Validation study**			**P-Value (AUC ROC Comp-arison)**
		Study design	Variables included	Analysed women	PE events (%)	AUC-ROC (95% CI)	Analysed women	PE events (%)	AUC-ROC (95% CI)	
Akolekar, 2008	Early	Case-control	PlGF, PAPP-A UADs History of chronic hypertension Ethnicity	638	29 (4.5%)	0.941 (0.889;0.994)	1,888	44 (2.33%)	0.718 (0.648; 0.788)	<0.001
Akolekar, 2008	Late	Case-control	PlGF, UADs History of chronic hypertension BMI Ethnicity Family history of PE(mother)	707	98 (13.9%)	0.817 (0.773;0.861)	2,076	48 (2.31%)	0.737 (0.665; 0.810)	0.064
DiLorenzo, 2012	Early	Prospective cohort	PlGF, hx of chronic hypertension, βhCG	2,059	12 (0.6%)	0.893 (N.R)	929	14 (1.51%)	0.504 (0.314;	-

									0.694)	
DiLorenzo, 2012	Late	Prospective cohort	UADs	2,060	13 (6.3%)	N.R	2,186	52 (2.38%)	0.704 (0.643; 0.765)	-
Goetzinger, 2010	Overall	Retrospective cohort	PAPP-A, history of chronic hypertension/diabetes, ethnicity, BMI	3,716	293 (7.9%)	0.70 (0.65;0.72)	2,186	52 (2.38%)	0.648 (0.570; 0.726)	0.233
Plasencia, 2008	Early	Prospective cohort	UADs, history of chronic hypertension	2,617	22 (0.8%)	0.931 (0.904;0.958)	2,174	52 (2.39%)	0.706 (0.643; 0.770)	<0.001
Plasencia, 2008	Late	Prospective cohort	UADs, BMI, ethnicity, previous history of PE, family history of PE(mother)	2,666	71 (2.7%)	0.779 (0.726;0.832)	2,168	52 (2.40%)	0.659 (0.587; 0.730)	0.008
Poon, 2008	Overall	Prospective cohort	Ethnicity, BMI, previous hx PE, family history of PE(mother)	4,522	104 (2.3%)	0.852 (N.R)	2,180	52 (2.39%)	0.711 (0.641; 0.780)	-
Poon, 2009a	Early	Prospective cohort	PAPP-A, UADS, ethnicity, history of chronic hypertension. family history of PE(mother)	7,927	32 (0.4%)	0.905 (0.898;0.911)	1,924	44 (2.29%)	0.765 (0.698; 0.833)	<0.001

Poon, 2009a	Late	Prospective cohort	PAPP-A, ethnicity, BMI, previous history of PE, family history of PE(mother)	8,019	124 (1.5%)	0.790 (0.781;0.799)	1,926	44 (2.28%)	0.691 (0.611; 0.771)	0.016
Poon, 2009b	Early	Prospective cohort	UADs, MAP, ethnicity, history of chronic hypertension, previous history of PE, history of fertility treatment	8,098	37 (0.5%)	0.954 (0.919;0.989)	2,168	52 (2.40%)	0.833 (0.783; 0.884)	<0.001
Poon, 2009b	Late	Prospective cohort	UADs, MAP, age, BMI, ethnicity, history of chronic hypertension, previous history of PE, family history of PE(mother)	8,189	128 (1.6%)	0.863 (0.855;0.870)	2,162	52 (2.41%)	0.828 (0.777; 0.880)	0.187
Poon, 2009c	Early	Prospective cohort nested in case-control	PlGF, PAPP-A, UADs, BMI, previous history of PE, MAP	7,538	34 (0.5%)	N.R	1,888	44 (2.33%)	0.824 (0.771; 0.877)	-
Poon, 2009c	Late	Prospective cohort nested in case-control	PlGF, UADs, ethnicity, BMI, previous history of PE, family history of PE, MAP	7,627	123 (1.6%)	N.R	1,888	44 (2.33%)	0.811 (0.752; 0.869)	-
Parra-Cordero, 2012	Early	Case-Control	BMI, smoking, UAD PI MoM, log PlGF MoM	359	17 (4.73%)	?? (??; ??)	2,076	48 (2.31%)	0.702 (0.636; 0.768)	??

Parra-Cordero, 2012	Late	Case-Control	BMI, UAD PI MoM, log PlGF MoM	359	53 (14.7%)	?? (??; ??)	2,076	48 (2.31%)	0.644 (0.562; 0.726)	??
Scazzocchio, 2013	Early	Prospective cohort	Parity, history of hypertension, renal disease, previous PE	5,170	26 (0.5%)	0.96 (0.94; 0.98)	2,168	52 (2.40%)	0.831 (0.786; 0.876)	<0.001
Scazzocchio, 2013	Late	Prospective cohort	Parity, history of hypertension/diabetes, thrombophilic condition previous history of PE,	5,170	110 (2.12%)	0.71 (0.658; 0.763)	1,925	44 (2.29%)	0.699 (0.612; 0.785)	0.830
Baschat, 2014	Early	Prospective cohort	History of diabetes/hypertension, MAP	2,441	18 (0.73%)	0.83 (0.74; 0.91)	2,186	52 (2.38%)	0.624 (0.546; 0.701)	<0.001
Baschat, 2014	Late	Prospective cohort	Parity, history of hypertension, previous history of PE, MAP, PAPP-A	2,441	108 (4.42%)	0.82 (0.78; 0.86)	1,932	44 (2.28%)	0.631 (0.548; 0.713)	<0.001

Abbreviations:
PE: preeclampsia
AUC-ROC: Area Under the Curve of Receiver Operating Characteristics
N.R: No reported

Appendix 6.1 compares the AUC-ROC between the two validation cohorts that were combined. All the p-values, except for the Baschat study, are non-significant demonstrating that the models performance was the same when tested by two different validation cohorts from our hospital.

The predicted probabilities of PE obtained after applying all models to the validation cohort are shown in table 6.6.

Table 6.6- Estimated probabilities on validation's cohort

		Validation's cohort			
Study	PE Screening	PE events	Analyzed women	Estimated probability Mean (SD)	PSEP (means Q_1;Q_4)
Akolekar, 2008	Early	44 (2.3%)	1,888	0.0233 (0.0802)	8.55% (0.02%; 8.57%)
Akolekar, 2008	Late	48 (2.3%)	2,076	0.0231 (0.0363)	6.03% (0.32%; 6.36%)
DiLorenzo, 2012	Early	14 (1.5%)	929	0.0151 (0.0472)	4.41% (0.17%; 4.58%)
DiLorenzo, 2012	Late	52 (2.4%)	2,186	0.0238 (0.0041)	1.03% (1.88%; 2.91%)
Goetzinger, 2010	Overall	52 (2.4%)	2,186	0.0238 (0.0157)	4.59% (1.27%; 5.87%)
Plasencia, 2008	Early	52 (2.4%)	2,174	0.0239 (0.0661)	8.07% (0.07%; 8.14%)
Plasencia, 2008	Late	52 (2.4%)	2,168	0.0240	5.73%

				(0.0304)	(0.45%; 6.18%)
Poon, 2008	Overall	52 (2.4%)	2,180	0.0239 (0.0294)	5.13% (0.50%; 5.63%)
Poon, 2009a	Early	44 (2.3%)	1,924	0.0229 (0.0568)	7.44% (0.10%; 7.54%)
Poon, 2009a	Late	44 (2.3%)	1,926	0.0228 (0.0242)	4.85% (0.54%; 5.39%)
Poon, 2009b	Early	52 (2.4%)	2,168	0.0240 (0.0685)	8.49% (0.03%; 8.52%)
Poon, 2009b	Late	52 (2.4%)	2,162	0.0241 (0.0393)	6.70% (0.24%; 6.94%)
Poon, 2009c	Early	44 (2.3%)	1,888	0.0233 (0.0873)	8.94% (0.01%; 8.95%)
Poon, 2009c	Late	44 (2.3%)	1,888	0.0233 (0.0553)	7.59% (0.10%; 7.70%)
Parra-Cordero, 2012	Early	48 (2.3%)	2076	0.0231 (0.0260)	3.68% (1.03%; 4.71%)
Parra-Cordero, 2012	Late	48 (2.3%)	2076	0.0231 (0.0265)	4.20% (0.88%; 5.08%)
Scazzocchio, 2013	Early	52 (2.4%)	2168	0.0240 (0.0712)	8.34% (0.05%; 8.39%)
Scazzocchio, 2013	Late	44 (2.3%)	1925	0.0229 (0.0214)	3.61% (0.98%; 4.59%)
Baschat, 2014	Early	52 (2.4%)	2186	0.0238 (0.0036)	0.66% (2.10%; 2.76%)
Baschat, 2014	Late	44 (2.3%)	1932	0.0228 (0.0196)	3.60% (0.90%; 4.50%)

Abbreviations:
SD: Standard deviation

PSEP: Percentage of separation between the estimated mean of 4th and 1st quartiles of probabilities (Q1; Q4) and its means of probability
Q_1 and Q_4: Means of probability of quartile 1st; and quartile 4th

The overall mean probability of PE in the validation cohort ranges from 1.5 to 2.4%, reflecting the prevalence of PE in the cohort. PSEP statistic (the difference between the mean predicted probabilities in Q1 and Q4) was extremely low, ranging from as low as 0.66% (Baschat 2014, early PE model) to 8.94% (Poon 2009c Early PE model) (Table 6.6). Akolekar (Late PE model), Goetzinger 2010, Poon 2008, Poon 2009a (Late PE model), Poon 2009b (Late PE model), Poon 2009c (Late PE model), Baschat 2014 (late PE model), Scazzocchio 2013 (late preeclampsia model) and Parra-Cordero 2012 (early PE model) presented good calibration as assessed by Hosmer-Lemeshow goodness-of-fit test (Appendix 6.2).

6.4 Discussion

A good prediction model should be highly discriminatory for the condition being predicted and there should be minimal difference between the expected and observed incidence of the condition when calibration is performed.

Main Findings

Our study has demonstrated poorer performance of the models for prediction of PE when applied to our external validation cohort. This difference was statistically significant in nine out of thirteen studies where we could compare the validation-derivation AUCs (see Table 6.4). All the derivation studies have reported AUCs that demonstrate that they have produced good models for predicting PE. However, when these models were applied to the validation cohort several were shown to perform

poorly (Di Lorenzo 2012, Early model, Goetzinger 2010, Plasencia 2008, Late, Scazzocchio 2013, late, Baschat 2014 early and late and Poon 2009a late) demonstrated by an AUC <0.7. On assessment of the calibration data the models that have assessed late or any onset of PE have calibrated better against the validation cohort with non-significant Hosmer-Lemeshow P values.

The low PSEP statistic may reflect the low incidence of PE particularly in the validation cohort. It also indicates that the models were unable to identify what variables e.g. UADs, a particular biomarker or maternal characteristic, are key to predicting women at high risk of developing PE reflecting that a definitive 1st trimester predictive test has still not been found. The finding that the models have not performed as well when validated externally as internally is unsurprising and confirms previous findings by Kleinrouweler et al. and Oliveira.(50, 154)

Models assessing early and late onset PE appeared to perform better for early PE with higher AUC values. This supports previous findings indicating two distinct pathophysiologies for early and late PE with early onset being associated with inadequate placentation and an imbalance between pro-and anti-angiogenic factors. Variables such as UADs and biomarkers would therefore be expected to perform better for early onset disease. The better calibration data for those models assessing late or any onset PE may be due to the higher incidence of late/any onset PE in the validation cohort.

Some of the models performed significantly better than others. We examined the studies in greater detail to see if we could find an explanation for this. Akolekar, Parra-Corderro and Goetzinger had a higher incidence of PE in their study population than

the other models and the validation cohort.(30, 69, 76) Goetzinger concluded that their model was only modestly effective in its predictive ability which was confirmed by our validation study. Di Lorenzo performed poorly with a low discriminative ability and poor calibration against the validation cohort. There was a low number of preeclamptics in the study population despite the large number of participants overall.(148) However these factors should not have caused a problem as we used an adjusted probability by the prevalence in each study. Plasencia and Baschat demonstrated lower AUCs in the validation cohort and performed significantly worse for both early and late PE with poor calibration.(146, 150) There was a comparable incidence of PE and overall study participants to the validation cohort however the ethnicity of the women was different, with a three times greater number of Afro-Caribbeans than the validation cohort. There were also a higher proportion of women with pre-existing chronic hypertension in the Baschat study.(150) The Poon studies had a large number of participants and a similar incidence of PE as the validation cohort.(36, 37, 79) There seems to be an improved detection rate with an increase in the number of variables included in the model. With Poon 2009c appearing to perform best.(37) This confirms previous conclusions made by Oliveira and Farina et al. (154, 155)

There have been a small number of other studies that have attempted to externally validate PE prediction models. These studies have also been limited by small numbers of preeclamptics as in our own study. Park et al. validated the Fetal Medicine Foundation multiple logistic regression algorithm in an Australian population. They concluded that the FMF algorithm could effectively be applied to their population. However they reported that their findings were limited by the low number of women whom developed early PE.(156) Skrastad et al. also externally evaluated the FMF algorithm and the PREDICTOR model by Perkin Elmer. They excluded multips and women who were taking aspirin or anticoagulants. They concluded that both models

had a similar, but only modest performance in their prediction of PE. Their validation population was also limited by the low number of early preeclamptics; they defined early PE as delivery before 37 weeks which differs from that of 34 weeks used by the majority of studies. They reported that they only had 1 preeclamptic requiring delivery before 34 weeks which led to them omitting three of the seven risk estimates of the PREDICTOR model. This study was also limited by the relatively small study population (520 compared to 2186 in our study sample).(157) The ethnic make-up of the included women varies considerably from our study population and this highlights the value of externally validating models in several different populations. Oliveria et al. used data from their prospective observational study to validate 8 PE prediction models. Their findings were comparable to ours. They found 7 of the 8 models underperformed when applied to their population.(154)

Oliveira were surprised to find that the predictive accuracy of 1st trimester PE prediction models was not better when there was a higher population prevalence of PE. They suggest that pre-test probability of PE plays a minor role in the predictive accuracy of multimarker algorithms.(154) This reassures us that these prediction models should be able to be applied to our population that has a relatively low incidence of PE.

Strengths and limitations

The strengths of our study include the large number of women prospectively recruited over a four year time period and the measurement of many variables which enabled us to use our data to validate a large number of pre-existing models. We have done a thorough statistical analysis comparing discriminatory and calibration data. Brunelli et al. performed a systematic review assessing the quality of 1st trimester prediction

models for PE. They found frequent methodological deficiencies which may limit their reliability and validity. The main issues highlighted were lack of external validation of models and overfitting where the number of events per variable is fewer than the commonly recommended 10 events per predictor. In their data all but one prediction model for early PE was based on a sample with less than 10 events per predictor. A low event per predictor rate may bias correlation coefficients of the model both positively and negatively affecting the interpretation of their performance.(158) Oliveira also commented on overfitting as a possible explanation as to why the models they assessed didn't perform as well when externally validated.(154) A more conservative approach, such as ours, which shrinks the parameter estimates toward 0 and cross validates model prediction in patient subsets has been found to produce better performing models.

Some of the models found in our search were excluded from our study as the actual prognostic model was not reported in the published paper and we could therefore not apply it to our population. The low incidence of PE in our validation cohort of 1.51-2.41%, and as a result, not differentiating between early and late onset PE, unlike many of the models, may have affected our evaluation of their performance. However the incidence of PE in our population is similar to that in the Poon studies, which from our validation, have demonstrated better discriminatory and calibration data and higher PSEP values than the other models. The Poon model may also perform better when applied to our population than some of the other models as it was developed in a similar UK population.

Future Research

Our validation study has demonstrated that there doesn't appear to currently be a definitive PE prediction model with adequate ability to discriminate for the condition, which performs as well when applied to a different population and can differentiate well between the highest and lowest risk groups within the tested population. The already large number of models assessing various variables alone or in combination limits the value of further development of models and further research would be better focussed on further attempts to validate existing models and assessing whether implementation of the models improves patient care. Evaluation of the models performance may be different if applied to a different cohort. Randomised trials could be performed allocating one group of women to intervention based on the standard risk assessment of PE based on NICE guidelines and the other to intervention based on predicted risk following application of the prognostic model. Outcomes in the different groups could then be measured to see if they were improved in those whom the prognostic model was applied. Some of the models we assessed were excluded from our study as the actual prognostic model was not reported in the published paper. Consensus based guidelines have been published recently advising authors on issues to report when developing a model. It is important that any future published models present their findings in way that allows application and validation of the model.

Chapter 7: Effect of diet and life style based metabolic risk modifying interventions on PE: A meta-analysis

7.1 Abstract

Objective

To evaluate the effect of dietary and lifestyle interventions with the potential to modify metabolic risk factors on the risk of PE.

Methods

We searched MEDLINE, EMBASE and Cochrane from inception until February 2013. Randomised trials in pregnant women evaluating the effect of dietary and lifestyle interventions with the potential to modify metabolic risks such as obesity, hyperlipidaemia, hyperglycaemia and hypertension on the risk of PE were included. Two independent reviewers selected studies, extracted data and assessed quality. Results were summarised as pooled relative risks (RR) for dichotomous data.

Results

Eighteen studies (8712 women) met our search criteria for inclusion. Six studies evaluated diet (2695 women), six studied mixed interventions with diet, physical activity and lifestyle (1438 women) and six assessed essential fatty acid supplementation (4579 women). The interventions overall reduced the risk of PE (RR 0.81, 95% CI 0.69, 0.94; p=0.006 I^2=0%) compared to the control group. Dietary interventions reduced the risk of PE by 33% (RR 0.67, 95% CI 0.53 to 0.85; p=0.001; I^2=0%). There was no reduction in the risk of PE with mixed interventions (RR 0.93, 95% CI 0.66 to 1.32, p=0.68, I^2=0%) or fatty acid supplementation (RR 0.92, 95% CI 0.71 to 1.18;

p=0.49, I^2=15%). Meta-regression showed a borderline impact of gestational diabetes status (p=0.05) on the observed effect.

Conclusion

Dietary and lifestyle interventions have the potential to reduce the risk of PE. The effect of additional therapeutic interventions in women with GDM on PE is not known.

Citation of paper arising from this work:

Allen, R., Rogozinska, E., Sivarajasingam, P., Khan, K. S., & Thangaratinam, S. (2014). Effect of diet-and lifestyle-based metabolic risk-modifying interventions on PE: a meta-analysis. *Acta obstetricia et gynecologica Scandinavica*, *93* (10) 973-985

7.2 Introduction

PE remains a leading cause of maternal deaths worldwide (1) and in developed countries, it is the main cause of maternal admissions to intensive care. (159) It is also associated with an increased risk of perinatal mortality and is the cause of approximately 10% of stillbirths and 15% of preterm births.(160, 161) Obesity, dyslipidaemia, diabetes and hypertension are independent risk factors for PE.(162) PE, characterised by insulin resistance, widespread endothelial damage and dysfunction and increased systemic inflammatory response, shares metabolic risk factors with cardiovascular diseases such as myocardial infarction, stroke and death in the long term. (163) A systematic review has shown that higher levels of serum triglycerides was associated with, and precedes the onset of PE.(164)

Interventions that reduce cardiovascular events by modifying metabolic risk factors also have the potential to reduce the risk of PE. Currently, low dose aspirin, recommended as a prophylactic measure has been shown to reduce PE risk by 10%.(43, 165) Interventions based on diet and lifestyle are effective in reducing gestational weight gain with potential to reduce other adverse outcomes such as PE and gestational diabetes.(166) A large Norwegian observational study of 23,000 pregnant women showed that the risk of PE was reduced by a third in women on a diet rich in vegetables, vegetable oil, fibre and fruits by comparing them to women with a high intake of processed meat, salty snacks and sweet drinks. (167)The risk of PE is increased by 2.9 times in obese women compared to those of normal weight, and the odds were increased by 7, when the systolic blood pressure was measured on the highest fifth compared to lowest fifth centile.(168)

Our previous review on weight management interventions in pregnancy had a narrow focus on gestational weight gain and PE. (166) Given the association of PE with metabolic risk factors such as raised lipids and blood sugar, we have widened the scope of our research question. We undertook a systematic review to collate the evidence on the effect of non-pharmacological metabolic risk modifying interventions in preventing PE.

7.3 Methodology

We carried out a systematic review with a prospective protocol in line with current recommendations and PRISMA guidelines.(59) We searched Cochrane, Embase and Medline databases from database inception up to February 2013 to identify relevant citations. The search was undertaken in two steps. First, we updated our previous search on diet and lifestyle interventions in pregnancy to identify relevant new papers published until 2013. (The search strategy and search terms have been published previously). (9) Next, we undertook a separate search to identify studies that evaluated other relevant interventions that influence metabolic risk factors such as essential fatty acids and fibre.

Identification of Studies

We used the following search terms for the intervention (diet, fiber, dietary fibre, bran, isaphagula husk, methylcellulose, psyllium, fatty acids, fish oil, exercise) and combined with those for outcomes (preeclampsia, pre-eclampsia, PIH, gestational hypertension, hypertension). (The full search criteria is given in appendix 7.1). Reference lists of all known primary and review articles were examined to identify cited articles that were not captured by the electronic searches. Language restrictions were not applied.

Study selection and data extraction

Randomised controlled trials in pregnant women evaluating the effect of dietary and lifestyle interventions with the potential to modify metabolic risks such as obesity, hyperlipidaemia/glycaemia and hypertension on the risk of PE were included. Study selection was performed in two stages. Interventions that included both diet and physical activity with or without behavioural modification were considered to be lifestyle interventions. We excluded non-randomised and animal studies. Firstly the electronic searches were scrutinised and appropriate studies were identified by two reviewers (RA and SS). Secondly, the two independent reviewers reviewed the full text of the identified papers and selected the studies that fulfilled the inclusion criteria. Any disagreements were resolved by discussion with a third reviewer (ST). Data were extracted, in duplicate, by dual teams of two independent reviewers (EA, ST; and RA, SS) in 2x2 tables for the effect of intervention on PE.

Quality assessment of the included studies

We evaluated the quality of the selected studies based on accepted contemporary standards.(59) The risk of bias of the individual studies was assessed in six domains: sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and other potential sources of bias.

Data Synthesis

Results were summarised as pooled relative risks (RR) with 95% confidence intervals (CI) for dichotomous data. The treatment effects of the studies were pooled separately for the various intervention subgroups. Heterogeneity of treatment effects was evaluated graphically with forest plots and if substantial heterogeneity was noted (I^2

>50%) possible causes were explored. To explore for sources of clinical heterogeneity we took into consideration the type of intervention and body mass index as explanatory variables. Additionally, we examined the impact of study quality on estimation of effect according to individual quality items (random sequence generation, allocation concealment, performance bias, detection bias and attrition bias). We initially performed univariable meta-regression analyses followed by multivariable analysis, which controlled for confounding among variables. Sensitivity analysis was performed by excluding studies on women with diabetes in pregnancy.

We explored for publication bias by producing funnel plots which were tested for their asymmetry using Harbord's test. All analyses were carried out with Revman 5.0 and Stata version 12.1 statistical software.

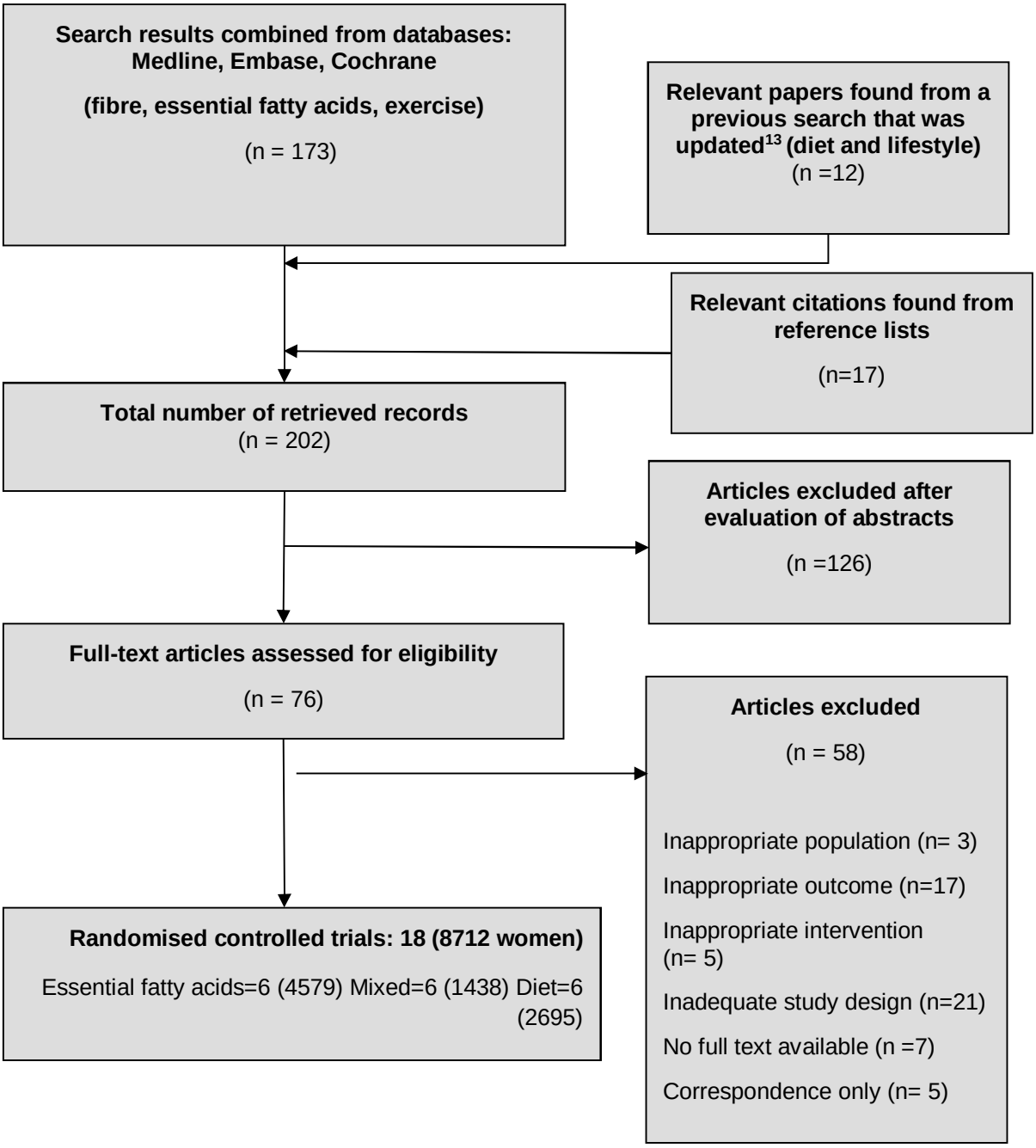
7.4 Results

Study selection

We included 18 randomised trials (8712 women) from a total of 202 citations (Fig 7.1). Of these, 173 studies were identified from our search on metabolic risk factor modifying interventions such as fibre and essential fatty acids. A further 29 studies were obtained by updating our previous search on diet and lifestyle based interventions (166). From the 202 papers evaluated, we included 18 studies (8712 women) in the review (Fig. 7.1) (169-185) Six studies (2695 women) (169, 171, 177, 178, 182, 183) evaluated mainly diet based interventions, six (1438 women) (170, 172, 179, 180, 185, 186) studied mixed interventions such as diet, physical activity and lifestyle and six (4579 women) (173-176, 181, 184, 187) studied lipid lowering agents such as essential fatty acids. The main reasons for exclusion of studies were inappropriate study design or PE

not assessed as an outcome (Fig 7.1). No RCTs examined the effect of only physical activity on the risk of PE.

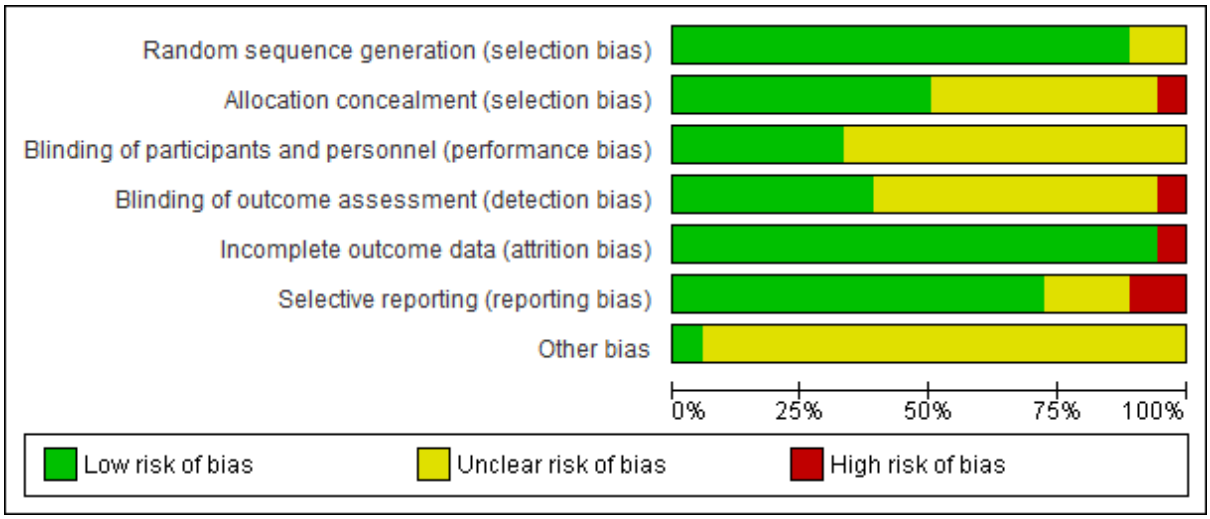
Fig 7.1: Flow chart of study selection



Quality of the included studies

The quality of the included studies is shown in Fig 7.2. Sequence generation was low risk in 89% (16/18) of the studies and unclear in the remaining 11% (2/18). Allocation concealment was low risk in 50% (9/18), high risk in 6% (1/18) and unclear in 44% (7/18). The risk of selective reporting was low risk in 72%, high in 11% and unclear in 17%. Performance bias was low risk in 33% (7/18) and unclear in 67%. The risk of bias for blinding in objective outcome assessments was unclear in thirteen (10/18, 55%) of the studies. 17 (94%) studies adequately addressed the problem of incomplete outcome data.

Fig 7.2: Quality of RCTs included in systematic review



Characteristics of the included studies

The included studies comprised of women with various body mass index (BMI). Seven studies included mainly obese women (BMI≥30 kg/m^2).(170-172, 178, 182-184) Ten studied women with any BMI.(169, 174-177, 179-181, 184, 186) Maternal weight was not specified in one study. (173) The effect of interventions was evaluated on pregnant women at risk of gestational diabetes mellitus in three studies.(169, 178, 182) In two of

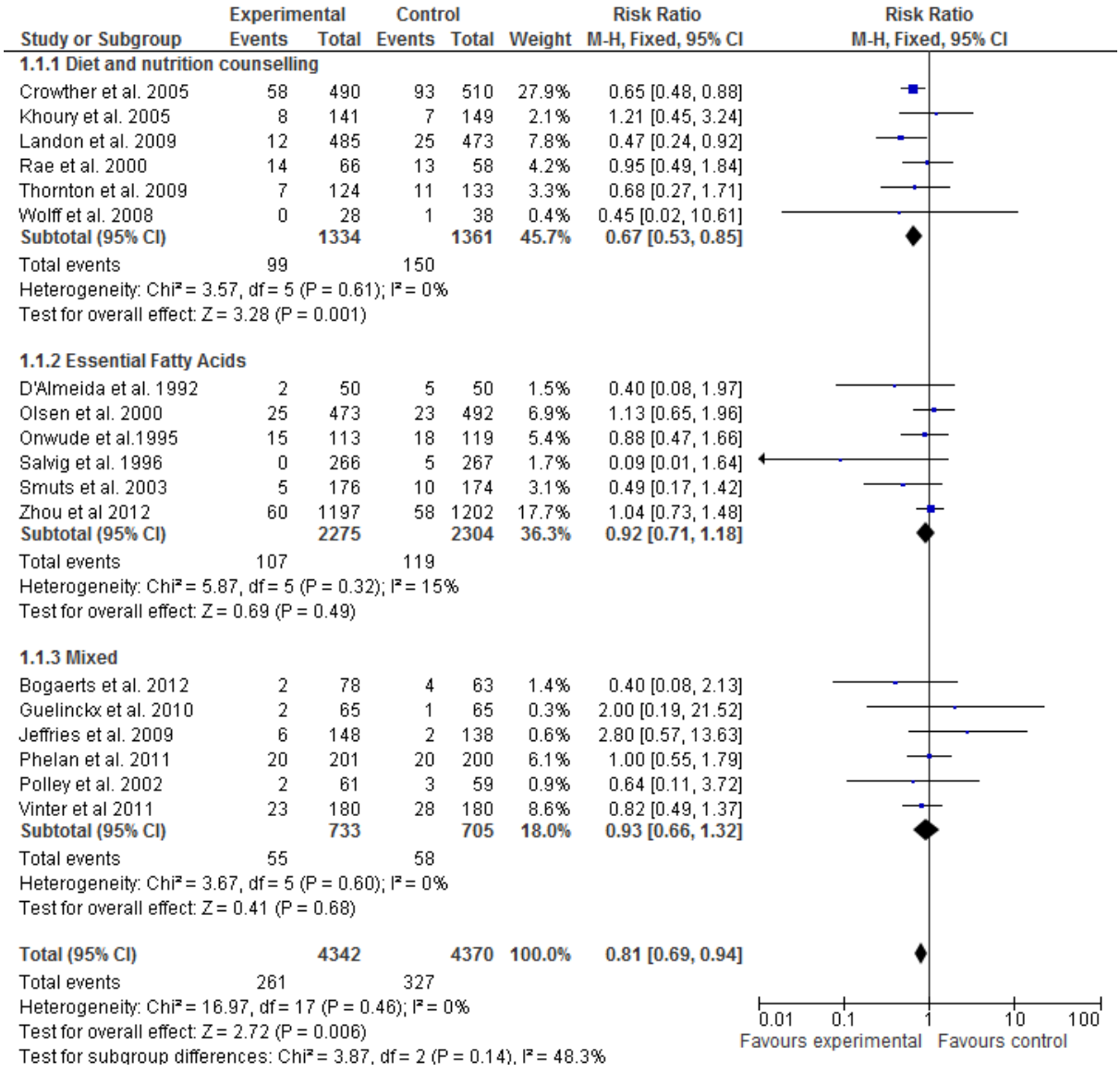
the studies that included women with GDM, insulin was prescribed as an adjunct to diet and lifestyle in 7.8% and 20% of women in the intervention group and 0.4% and 3% of women in the control group respectively.(169, 178) None of the women in the mixed or essential fatty acids intervention groups had impaired glucose tolerance. Three of the studies (50%) in the mixed intervention group included mainly obese women. Seven studies assessed the effect of interventions in women with no pre-existing risk factors for the development of PE.(170, 174, 177, 179, 181, 182, 186) Three in women with risk factors which included previous pregnancies affected by IUGR, PIH, PE, stillbirth or abnormal UAD measurements in the index pregnancy. (173, 175, 176) Eight studies did not report the risk status of the participants.

The diet based interventions included an energy restricted or cholesterol lowering diet, healthy dietary advice provided by a trained nutritionist and use of food diaries. Six studies assessed a combination of both diet and lifestyle such as dietary guidance, gym membership and personalised graphs of weight gain during pregnancy. The lipid lowering agents included essential fatty acids such as fish oil and gamma linoleic acid. Five studies examined fish oils (eicosapentanoic acid and/or docosahexanoic acid) alone and one study looked at docosahexanoic acid in combination with gammalinoleic acid. (173) The control group included women who were provided routine antenatal care. The interventions were commenced in the 1st trimester in seven studies evaluating mixed interventions (n=5) and essential fatty acids (n=2); (172, 173, 179, 180, 184-186) in the 2nd trimester in ten studies on interventions such as diet (n=5) and essential fatty acid (n=5) (169, 171, 175-177, 179, 181-184); and in the third trimester in one study with essential fatty acids (174) that was continued until delivery. In two studies, in diet (n=1) and mixed (n=1) group, the intervention was commenced in any trimester (see appendix 7.2). (170, 178)

Effect of interventions on PE

The interventions significantly reduced the risk of PE (RR 0.81, 95% CI 0.69 to 0.94; *p*=0.006) compared to the control group with no heterogeneity (I^2= 0%) (Fig 7.3). Amongst interventions, those based mainly on diet showed a significant reduction in PE by 33%, compared to the controls (RR 0.67, 95% CI 0.53 to 0.85; *p*=0.001) with no heterogeneity (I^2=0%). Pooled estimate of the six studies on mixed interventions (diet and lifestyle) did not show a difference in the rates of PE between the two groups (RR 0.93, 95% CI 0.66 to1.32, *p*=0.68; I^2=0%). There was no significant reduction in PE (RR 0.92, 95% CI 0.71 to1.18; *p*=0.49, I^2=15%) in women on essential fatty acids. Sensitivity analysis after excluding women with gestational diabetes showed that the reduction in PE did not persist by combining all interventions (RR 0.91, CI 0.75, 1.11, *p*=0.37) or in the diet only group (RR 0.86, 95% CI 0.45, 1.64, *p*=0.64). Sensitivity analysis after excluding studies where the intervention was commenced after the 1st trimester showed no significant reduction in PE (RR 0.95, 95% CI 0.74, 1.22, *p*=0.69).

Fig 7.3: Effect of diet based, mixed and essential fatty acid interventions on the risk of PE



Meta-regression analysis did not show any significant impact of explanatory variables such as the type of intervention (p=0.11) BMI category (p=0.39), or components of study quality on the effect of interventions on the risk of PE. A borderline significance (p=0.05) was observed for gestational diabetes (Table 7.1). Harbord's test for funnel plot asymmetry to explore for publication bias was not significant (p=0.545). See fig 7.4.

Fig 7.4: Funnel plot to explore for publication bias

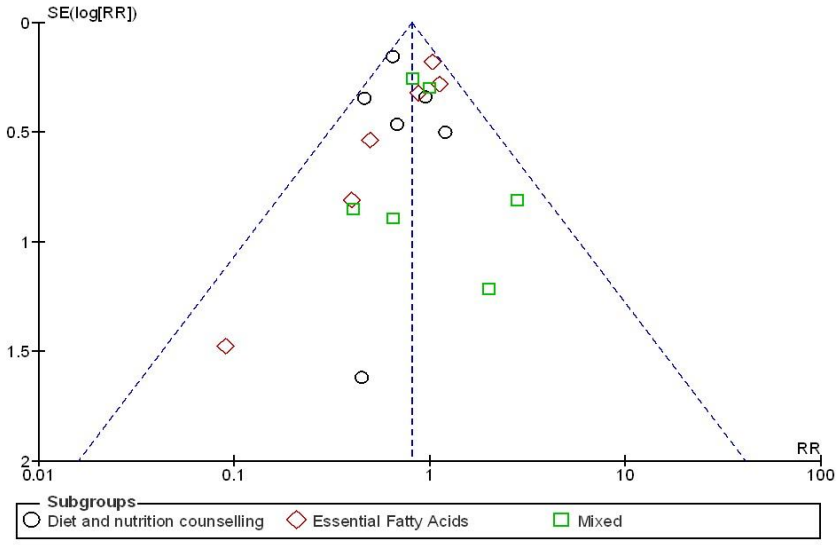


Table 7.1: Exploration of heterogeneity in estimation of effect of diet and life style based metabolic risk modifying interventions on PE (univariable analysis)

Heterogeneity outcomes	**Coefficient (SE)**	***P* - value**
Clinical features		
Intervention type (diet vs mixed vs fish oil)	0.18 (0.11)	0.11
BMI category (mixed vs overweight & obese)#	-0.18 (0.21)	0.39
DM status (diabetic vs healthy)	0.35 (0.17)	0.05
Study quality		
Random sequence generation*	0.31 (0.16)	0.07
Allocation concealment	0.10 (0.19)	0.60
Performance bias	0.13 (0.20)	0.53
Detection bias	0.27 (0.17)	0.13

Attrition bias	0.26 (0.16)	0.13

** mm (method-of-moments) estimate used for estimation of between study variance instead of **reml** due to data unclear data convergence*

7.5 Discussion

Main Findings

Dietary and lifestyle interventions in pregnancy may reduce the risk of PE. Amongst interventions, those based on diet were more effective in reducing the risk compared to mixed interventions or with essential fatty acid supplementation in pregnancy. It is likely that this benefit was driven by studies on women with GDM.

The reduction in PE was observed with diet based intervention and not with mixed diet and physical activity based intervention. This could be due to the increased compliance with a simple intervention with one component compared to a complex intervention incorporating diet and physical activity. (166) Unfortunately, we were unable to compare compliance between groups as this data was not reported by the majority of the studies. Where it was reported (three of the essential fatty acids studies and three of the mixed group studies) the actual number complying with the intervention was not given in the majority of cases but was reported to be similar between groups. The risk of PE is known to be reduced by a third with a healthy balanced diet comprising vegetables, compared to a diet predominantly on processed food. (167) An increase in dietary total fibre intake reduces the level of triglycerides which in turn is associated with a reduction in PE. (188) It has also been shown that diets based on low fat meat and dairy products, whole grains, fruit, vegetables and fish from second trimester until delivery is effective in reducing maternal total and low density lipoprotein (LDL) cholesterol in the intervention compared to control group. (177)

The studies that evaluated dietary intervention were comprised mostly of women with pre-existing metabolic risk factors such as obesity and impaired glucose tolerance, compared to the other studies. The combination of less weight gain and better glycaemic control may have contributed to the reduction in PE. Existing randomised evidence do not suggest a significant association between reduction in gestational weight gain and lowered risk of PE (189) The reduction in PE was not observed after excluding studies with GDM, suggesting a significant role for glycaemic control on the outcome.

Fish oil with eicosapentanoic acid and docosahexanoic acid, increase the peroxismal β-oxidation of fatty acids and reduce hepatic fatty acid production, thereby reducing the availability of substrates for triglycerides. Although in the non-pregnant state, supplementation with fish oil is known to significantly reduce the levels of triglycerides and cholesterol in both normal and dyslipidaemic population, this reduction was not observed in pregnancy supplemented with fish oil after 20 weeks of gestation. (190) It is possible that the absence of beneficial effect of omega 3 fatty acids in reducing PE is due to its ineffectual action on metabolic risk factors in pregnancy. Although both essential fatty acids and mixed intervention subgroups did not show any beneficial effect, the type of intervention did not have a significant impact on the outcome as shown by our meta-regression.

Our review is the first, to our knowledge, to specifically evaluate the role of dietary and lifestyle interventions with potential to modify metabolic risk factors on the risk of PE. We undertook a detailed search strategy and captured the relevant studies without any language restrictions. We have provided the clarity necessary to judge the effects of

the interventions by assessing the quality of the individual studies. The findings were limited by the variation in the components, intensity, compliance, timing and delivery of complex interventions such as those based on diet and mixed type. The studies on essential fatty acids varied in the dose and type of fatty acid supplementation, the baseline levels of n-3 fatty acids in the participants, and the duration and timing of the supplementation. No studies performed a direct comparison of the various interventions, thereby restricting the ranking of interventions based on effectiveness. The studies did not always report the effect of intervention on all the relevant metabolic risk factors such as gestational weight gain, gestational diabetes and lipid levels.

The two studies on women with gestational diabetes had additional intervention such as insulin. Although, both randomised groups received insulin, the proportion in the intervention group was higher than the control group in both studies. Hence, we cannot rule out the possibility that insulin use could have been an important contributor to the beneficial effect observed.

We restricted our systematic review to those interventions that had a proven role in reducing underlying metabolic risk factors. Inclusion of other interventions such as zinc and calcium, that have a potential modifying effect on risk factors, could have led to a different conclusion. Although serum zinc levels were lower in women with PIH, there is no robust evaluation of the role of zinc in preventing PE. (191)

7.6 Conclusion

There is a need to evaluate the effect of dietary interventions in women with pre-existing metabolic risk factors on PE. A large randomised trial involving women with metabolic risk factors will quantify the effect of diet based intervention with precise estimates. There is a scope for further research on the effect of the interventions that modify metabolic factors such as lipid levels, blood pressure and glycaemic control on PE. Individual patient data meta-analysis of interventions of published trials will allow us to evaluate the differential effects of the intervention in various subgroups of women, and to undertake additional analysis by excluding women with added interventions such as insulin. The optimal timing, components of the dietary intervention, mode of delivery and setting need to be determined to help make recommendations for clinical practice.

Chapter 8: Conclusion

Section 8.1 Summary of Findings

This chapter summarises the results of the individual chapters. Detailed results are provided in each individual chapter. The table below is the table of my research objectives given in the introduction chapter with an additional column which includes a summary of the main results.

Table 8.1 Structured questions of the chapters of the thesis with results

Chapter Number	Population	Intervention or Test	Outcomes	Research Design	Results
Objective A: To determine the accuracy of biomarkers for the prediction of women at risk of PE and other adverse pregnancy outcomes					
	Pregnant women attending for 1st trimester scan	Maternal blood levels of AFP, PlGF, β-hCG, PAPP-A and Kisspeptin Serial levels of PlGF and AFP at 20, 28 and 34-36 weeks	PE PIH Placental Abruption Stillbirth IUGR and SGA	Prospective observational study	No significant association found between any of the biomarkers and PE/PIH or gestational diabetes mellitus. Low PAPP-A and PlGF were significantly associated with SGA <10th centile (p=0.007 and 0.004 respectively). High AFP (p=0.041) and low PlGF (p=0.036) were significantly associated with stillbirth. There was a significant association between low serial PlGF levels and SGA<5th (p=0.037) and 10th centiles (p=0.014). No association between serial AFP levels and any of the outcomes.
Objective B: To determine the accuracy of UADs for the prediction of PE and other adverse pregnancy outcomes					
	Pregnant women attending for 1st trimester scan	1st trimester UADs	PE PIH Placental Abruption Stillbirth IUGR and SGA	Prospective observational study	Increased mean UAD PI (1.08 MoM) was significantly associated with PE (p=0.03) but not with any of the other outcomes assessed.

Objective C: To evaluate the predictive value of maternal characteristics and arteriography for the prediction of PE and other adverse pregnancy outcomes					
	Pregnant women attending for 1st trimester scan	Maternal history and arteriography measured in the 1st trimester	PE PIH Placental Abruption Stillbirth IUGR and SGA	Prospective observational study	Aix (p<0.027), SBPAO (p=0.002) MAP (p=0.014), Afro-Caribbean ethnicity (p=0.013) and a history of hypertension (p=0.047) were significantly associated with the development of PE. SBPAO (p=0.015), MAP (p=0.001) and maternal weight (p=0.001) were the only parameters significantly associated with PIH. All haemodynamic parameters were not significantly associated with SGA <10th or 5th centiles. Maternal hypertension was significantly associated with SGA <10th centile (p=0.03). For SGA<5th centile there was a significant association with South Asian ethnicity (p=0.047) and maternal smoking (p=0.018). Maternal smoking was significantly associated with stillbirth (p=0.025). The haemodynamic parameters augmentation index, pulse wave velocity, MAP and SBPAO were significantly associated with GDM (p=0.025, 0.048, 0.007 and 0.019 respectively) along with maternal South Asian or Oriental ethnicity (p=<0.001 and 0.024).
Objective D: To validate pre-existing PE models					
	Pregnant women in the 1st trimester	Biomarkers, UADs, arteriography, mean arterial pressure	PE	Review of pre-existing PE models and validation of data with my primary study data	The validation cohort included 2186 women. The incidence of PE was 2.4%. Fourteen models were identified for validation. Sample sizes of these models ranged from 638 and 8,189 with prevalence of PE ranging between 0.4% and 13.9%.The discrimination ability observed in derivation studies (Area Under the Curves) ranged from 0.70 to 0.954 when these models

					were validated against the validation cohort, these AUC varied importantly, ranging from 0.504 to 0.833.
Objective E: To perform systematic reviews assessing the effectiveness of biomarkers, UADs and diet and lifestyle interventions in the prediction and prevention of PE and other adverse pregnancy outcomes					
	Pregnant women in the 1st trimester	Measurement of any blood biomarkers	PE	Systematic review and meta-analysis	1071 citations, 30 studies included in the systematic review. Markers assessed were PAPP-A, PlGF, PP13, β-hCG, soluble endoglin, inhibin-A, pentraxin, p-selectin, VEGF and sflt-1. Abnormal 1st trimester maternal biomarkers and PE of any onset were reported in 24 studies which included 61,745 women. Abnormal levels of the biomarkers PAPP-A (9 studies; OR 2.1, 95% CI 1.6, 2.6, I^2 45%), PP13 (4 studies; OR 4.4, 95% CI 2.9, 6.8, I^2 50%), sFlt-1 (4 studies; OR 1.3, 95% CI 2.9, 6.8, I^2 27%), pentraxin (1 study; OR 5.31, CI 1.9, 15.0) and inhibin-A (3 studies; OR 3.57, 95% CI 1.7, 7.6, I^2 21%) were significantly associated with development of any PE. Ten studies (15,760 women) evaluated six biomarkers with the development of PE before 34 weeks of gestation. PlGF (4 studies; OR 3.4, 95% CI 1.6, 7.2, I^2 87%), PAPP-A (5 studies; OR 4.8, 95% CI 2.5, 22.5, I^2 72%), PP13 (5 studies; OR 7.5, 95% CI 2.5, 22.5, I^2 69%), soluble endoglin (2 studies; OR 18.5, 95% CI 8.4, 41.0, 14%) and inhibin-A (1 study; OR 4.1, 95% CI 1.9, 8.8) were significantly associated with early onset PE. The relationship of late onset PE and abnormal biomarker levels was evaluated in seven studies (12,053 women), examining five biomarkers. A significant association was seen for soluble endoglin (3 studies; OR

					2.1, 95% CI 1.9, 2.4, I^2 0%) and inhibin-A (2 studies; OR 1.9, 95% CI 1.4, 2.8, I^2 0%).
	Pregnant women in the 1st and 2nd trimester	Measurement of UADs	Stillbirth	Systematic review and meta-analysis	340 citations. After screening, 13 2nd trimester studies (n = 85,846) including 508 stillbirths, and two 1st trimester studies (n=9935, 66 stillbirths), were included in the systematic review. The sensitivity of UADs in the 2 individual studies ranged from 14.5-100%. The specificity ranged from 64-91%. Due to the limited number of studies and participants and the high heterogeneity observed we could not obtain a pooled estimate of sensitivity and specificity. For 2nd trimester studies the bivariate pooled estimate for sensitivity was 65% (95% CI 38 –85%) and for specificity, it was 82% (95% CI 72–88%). The positive was 3.5 (95% CI 2.3 –5.5) and negative LR 0.43 (95% CI 0.22 – 0.85). The diagnostic odds ratio (DOR) was 8.3 (95% CI 3 – 22.4). Subgroup analysis of the high risk group (n=6) showed that the average sensitivity was 90% (95% CI 36 – 99 %) with a pooled specificity of 69% (95% CI 54 – 81%). The positive LR was 2.9 (95% CI 1.8 – 4.9), negative LR 0.14 (95% CI 0.1 – 1.8) and diagnostic OR 21.3 (95% CI 1.2 – 380). In the seven 2nd trimester studies that performed UAD in an unselected population Pooled sensitivity is 46%, (95% CI: 32 – 60%), pooled specificity is 88% (95% CI: 82 – 93%). The positive LR was 3.96 (95% CI: 3.1 – 5.0), negative LR 0.62 (95% CI: 0.50 – 0.75) and diagnostic OR 6.4 (95% CI: 5.2 – 8.0).

	All pregnant women	Diet and lifestyle interventions -Fibre -Essential fatty acids -Physical Activity -Dietary advice	PE	Systematic review and meta-analysis	18 randomised trials (8712 women) from a total of 202 citations included. . Six studies evaluated diet (2695 women), six studied mixed interventions with diet, physical activity and lifestyle (1438 women) and six assessed essential fatty acid supplementation (4579 women). The interventions overall reduced the risk of PE (RR 0.81, 95% CI 0.69, 0.94; p=0.006 I^2=0%) compared to the control group. Dietary interventions reduced the risk of PE by 33% (RR 0.67, 95% CI 0.53 to 0.85; p=0.001; I^2=0%). There was no reduction in the risk of PE with mixed interventions (RR 0.93, 95% CI 0.66 to 1.32, p=0.68, I^2=0%) or fatty acid supplementation (RR 0.92, 95% CI 0.71 to 1.18; p=0.49, I^2=15%). Meta-regression showed a borderline impact of gestational diabetes status (p=0.05) on the observed effect.

Section 8.2 Strengths and Limitations

Systematic reviews were performed with robust methodology. Extensive literature searches were performed without language restrictions to avoid missing any studies and reference lists of articles were checked to identify any relevant studies not captured by electronic searches. Authors were also contacted if the relevant data was not reported. Predefined data extraction forms were used and data was extracted in duplicate by two reviewers. Any disagreements were discussed with a third reviewer. Rigorous assessment of the quality of included studies was performed using the recommended assessment tools.

The biomarker review was the first review to provide summary estimates of the association between abnormal 1st trimester maternal blood biomarkers and PE. Previous reviews have not quantified the strength of association for all relevant biomarkers limiting direct comparison of the markers and have only assessed four biomarkers from any trimester of pregnancy. However, the review was limited by the amount of heterogeneity and the need to exclude several studies from analysis due the way the results were reported and suitable results not being provided by authors despite contacting them.

The stillbirth review was also limited by high heterogeneity and the large variation between the UAD parameters measured and the thresholds used. As our literature search only revealed two 1st trimester studies, our analysis was limited for this subgroup and more in depth for the 2nd trimester studies. This review was also hampered by difficulty obtaining relevant primary data from authors.

In our prevention review we explored for possible sources of heterogeneity such as type of intervention and BMI. We examined the impact of study quality on the estimation of effect according to individual quality items. Multivariable analysis was performed after univariable analysis to control for any confounders. Sensitivity analysis was performed excluding studies in women with GDM and publication bias explored. The use of insulin in two of the diet studies with women with GDM and the higher incidence of the use of insulin in the intervention group meant that insulin could have contributed to the beneficial effect observed.

The validation study was the first to evaluate several 1st trimester prediction models. Thorough statistical analysis was performed. Two validation cohorts, recruited by two different researchers, from the same patient population were compared and assessment of the models performance was the same supporting the overall findings. The low incidence of PE in the validation cohort leading to early and late cases being combined may have affected evaluation of the models performance.

A large number of unselected women were recruited in our primary study and previously unpublished variables were assessed e.g. AFP. A major limitation was the low incidence of early PE (one case) leading to all cases of PE being combined. As it is likely that there are two different pathophysiologies for early and late disease this is likely to have affected our assessment of the biomarkers performance in our study population.

Section 8.3 Implications for Clinical Practice

The finding of high 1st trimester AFP levels in association with stillbirth raises the possibility of combining it with other biomarkers e.g. PlGF, and using it as a screening tool. If 1st trimester levels were elevated these women could go on to have 2nd trimester UAD parameters measured and if positive serial growth scans and placental and fetal Dopper measurements performed. The significant differences in haemodynamic parameters in the population that developed GDM and hypertensive diseases of pregnancy demonstrate a role for the routine use of arteriography which could be performed at the booking appointment or the 1st trimester scan. It is an easy test to perform which only takes five minutes and is cheap following the initial purchase of the machine. Women at high risk of GDM could have early glucose tolerance tests performed and/or start performing capillary blood glucose monitoring early in order to prevent complications developing.

The low false positive rate and high positive LR of 2nd trimester UAD for the prediction of stillbirth make it a useful screening test that should be routinely performed at the anomaly scan. It is easy, cheap and quick to perform. Screen positive pregnancies should then be offered serial surveillance of fetal wellbeing with growth scans, placental and fetal Doppler measurements.

Section 8.4 Implications for Research

The variable quality of published studies and the difference in the reporting of results leads to high heterogeneity and difficulty in performing meta-analysis with many studies having to be excluded due to the way that results have been reported. Future studies

need to adhere to standardised methodology to enable easier comparison and meta-analysis, with study outcomes and population clearly differentiated. Future prediction models need to ensure the actual model is published allowing application and validation.

The stillbirth review has highlighted the need for an individual patient data meta-analysis to allow subgroup analysis based on timing of stillbirth and determine which UAD parameter and threshold value should be measured.

The low number of cases of early PE in the primary study population identifies the need for further studies measuring maternal serum biomarkers in women with early onset disease. A retrospective case control study might be better at ensuring appropriate numbers of cases are included for analysis. The high number of women of South Asian ethnicity in our study population coupled with the high incidence of GDM and low incidence of PE demonstrates a possible role for investigating whether there is any protective effect from ethnicity and GDM.

Further attempts to validate pre-existing PE models using other study group's data from other populations should be performed to see if our results are replicated. Current models should be implemented in clinical practice and compared to existing management to assess whether the models improve patient care and outcomes.

Future research on the effect of diet and lifestyle based metabolic risk modifying interventions on PE need to evaluate the effect of dietary interventions in women with pre-existing metabolic risk factors. Further research should also assess the effect of

interventions that modify metabolic risk factors such as lipid levels, blood pressure and glycaemic control on preeclampsia. Individual patient data meta-analysis of interventions of published trials will allow us to evaluate the differential effects of the intervention in various subgroups of women, and to undertake additional analysis by excluding women with added interventions such as insulin.

Appendices

Appendix 3.1: Details of the included studies in the biomarker review

Paper	Abdelaziz et al. 2012
Methods	Nested case controlled study
Participants	2120 singleton pregnancies without a history hypertension, renal disease, autoimmune disease or obesity were recruited. After exclusion for loss to follow up and fetal loss <24 weeks there was 1898 patients. There were 16 cases of PE<34 weeks and 60 cases of PE>34 weeks.
Interventions	Endoglin levels at 11-14 weeks
Outcomes	Gestational hypertension, PE requiring delivery <34 weeks, PE requiring delivery >34 weeks
Paper	Akolekar et al. 2009
Methods	Case controlled study
Participants	8234 singleton pregnancies were recruited. There was 26 cases of early PE and 95 cases of late PE. These were matched with 208 controls on the basis of length of sample storage.
Interventions	Inhibin-a levels at 11-13 weeks
Outcomes	Early PE, Late PE, gestational HTN
Paper	Akolekar et al. 2011
Methods	Case controlled study
Participants	36,743 singleton pregnancies were recruited. After exclusion for incomplete data, fetal loss or major fetal anomaly there were 33,602 patients. There were 112 cases of PE<34 weeks, 187 casesPE34-37 weeks and 453 cases of PE>37 weeks. The 32,850 controls were pregnancies with no complications and normal outcome matched for duration of sample storage.
Interventions	activin-a, beta HCG, endoglin, Inhibin-a, PAPP-A, pentraxin3, PLGF, PP13, P-selectin levels at 11-13 weeks
Outcomes	PE <34 weeks,PE34-37 weeks, PE>37 weeks
Paper	Bauman et al. 2008
Methods	Case controlled study

Participants	46 cases of PE>34 weeks were identified in singleton pregnancies with no history of hypertensive disorders, renal disorders, immunological disease or HELLP. These were matched with 92 controls on the basis of gestational age at blood sampling, maternal age, pre-pregnancy weight and duration of sample storage.
Interventions	Inhibin-a, PLGF, sEng, SFlt-1 levels at 11+2- 13+6 weeks
Outcomes	PE >34weeks
Paper	Bills et al. 2009
Methods	Cohort study
Participants	50 pregnancies were recruited at antenatal clinic, a further 20 pregnancies were recruited when a diagnosis of PE was made. 30 pregnancies were excluded for PIH, idiopathic fetal growth restriction, IUD, loss to follow up, preterm labour or no available 1st trimester serum sample. There were 25 cases of PE and 45 normotensive pregnancies.
Interventions	VEGF levels in the 1st trimester
Outcomes	PE
Paper	Bosio et al. 2011
Methods	Case controlled study
Participants	400 healthy primigravid women were recruited. The 20 cases of PE were matched with 26 controls on the basis of body mass index.
Interventions	P-selectin levels at 10-14 weeks
Outcomes	PE and gestational HTN
Paper	Cetin et al. 2009
Methods	Nested case controlled study
Participants	A cohort of singleton pregnancies without medical disorders or drug therapy were recruited. There were 16 cases of PE. The following two patients to be recruited after each case of PE or SGA acted as controls.
Interventions	Pentraxin3 levels at 11-14 weeks
Outcomes	PE and SGA requiring delivery <37 weeks
Paper	Chafetz et al. 2007
Methods	Nested case controlled study
Participants	Singleton pregnancies from the Massachusetts General Hospital

	Obstetric Maternal Study cohort were recruited. Pregnancies were excluded if they were complicated by AIDS, hepatitis, fetal anomaly, fetal death, delivery <26 weeks, placenta praevia, placenta accreta or placental abruption. The 47 cases of PE were matched to 290 controls on the basis of gestational age at time of sampling and storage time.
Interventions	PP13 levels at 9-12 weeks
Outcomes	PE, SGA, preterm labour
Paper	Chaiworapongsa et al. 2005
Methods	Case controlled study
Participants	Retrospective identification of 44 cases of PE. Patients with multiple pregnancies, major fetal anomaly or demise, active vaginal bleeding, serious medical illness, chronic hypertension, asthma and patients using non-steroidal anti-inflammatory drugs or anti-platelet agents were excluded. In the group of patients who had blood sampled between 7-16 weeks there were 4 cases of PE. The 37 controls were matched for gestational age at the time of sampling.
Interventions	sVEGFR-1 levels at 7-16 weeks
Outcomes	PE, PE<34wks
Paper	Dugoff et al, 2004
Methods	Prospective cohort study
Participants	33,395 singleton pregnancies without fetal anomalies or insulin dependent diabetes mellitus were recruited. There were 764 cases of PE.
Interventions	Beta HCG, PAPP-A levels at 10+3-13+6 weeks
Outcomes	PE, Gestational hypertension, IUD, NND, preterm birth <37 weeks, preterm birth <32 weeks, placental abruption
Paper	El-Gharib et al, 2011
Methods	Prospective cohort study
Participants	327 singleton pregnancies without hypertensive disorders, infection, diabetes mellitus or renal disease were recruited. 24 were lost to follow up leaving 303 patients. There were 26 cases of PE.
Interventions	Inhibin-A levels at 10-12 weeks
Outcomes	PE
Paper	El-Sherbiny et al. 2012

Methods	Case controlled study
Participants	Pregnancies not complicated by essential hypertension, diabetes mellitus, recurrent pregnancy loss, hepatic disease, renal disease ,severe infections, administration of antioxidants or smoking were recruited. 50 cases of PE were matched to 50 controls on the basis of gestational age.
Interventions	PP13 levels at 9-13 weeks
Outcomes	PE
Paper	Goetzinger et al. 2010
Methods	Retrospective cohort study
Participants	4,035 singleton pregnancies were recruited, after exclusion for incomplete data and aneuploidy there were 3716 patients. There were 293 cases of PE.
Interventions	beta HCG and PAPP-A levels at 11-13 weeks
Outcomes	PE
Paper	Lynch et al, 2010
Methods	Prospective cohort study
Participants	668 singleton pregnancies were recruited. Women were excluded if they had a repeat delivery within study period, PIH or IUD. There were 31 cases of PE.
Interventions	Bb, C3a, PlGF, SC5b-9,sEng, SFlt-1 levels at 10-15 weeks
Outcomes	PE
Paper	Madazili et al. 2013
Methods	Case controlled study
Participants	31 cases of PE and 30 controls were selected after exclusion for multi fetal pregnancies, obesity, medical complications, pre-labour rupture of membranes and chorioamnionitis.
Interventions	PlGF, metastin and chitotriosidase levels at 11-14 weeks
Outcomes	PE, SGA
Paper	Noori et al, 2010
Methods	Prospective cohort study
Participants	159 women with singleton pregnancies were recruited during 1st trimester ultrasound scan and the obstetric medicine clinic. There

	were 10 cases of PE<37 weeks and 11 cases of PE>37 weeks.
Interventions	PLGF, sENG, sFlt-1 levels at 10-17 weeks
Outcomes	PE <37weeks, PE>37weeks, gestational hypertension
Paper	Odibo et al. 2011
Methods	Prospective cohort study
Participants	477 singleton pregnancies were recruited. After exclusion for fetal loss<20 weeks, loss to follow up and fetal anomaly there were 452 patients. There were 42 cases of PE and of these 12 required delivery <34 weeks.
Interventions	PAPPA, PP13 and PAPPA/PP13 levels at 11-14 weeks
Outcomes	PE and PE<34 weeks
Paper	Poon et al. 2009
Methods	Prospective cohort study
Participants	8,679 singleton pregnancies were recruited. After exclusion for incomplete data, unclear diagnosis, fetal abnormality, miscarriage or termination there were 8,051 patients. There were 32 cases of PE<34 weeks and 124 after 34 wks.
Interventions	PAPP-A at 11+0-13+6 weeks
Outcomes	PE <34/40,PE>34/40
Paper	Radoi et al. 2009
Methods	Prospective cohort study
Participants	484 pregnancies were recruited. After exclusion for multiple pregnancy, missing values for birth weight or perinatal outcome, gestational age at delivery outside range of 24-43 weeks, abnormal or missing karyotype there were 456 pregnancies. There were 19 cases of PE.
Interventions	beta HCG and PAPP-A levels at 10-13+6 weeks
Outcomes	PE, birth weight <5th centile, preterm delivery and stillbirth
Paper	Rana et al. 2007.
Methods	Nested case controlled study
Participants	Singleton pregnancies without fetal anomalies were selected from the Massachusetts General Hospital Obstetric Maternal Study cohort. 39 cases of PE were matched with 147 controls on the basis of age and body mass index.

Interventions	sEng and sFLT-1 levels at 11-13 weeks.
Outcomes	PE
Paper	Saloman et al. 2003.
Methods	Nested case controlled study
Participants	4,870 singleton pregnancies were recruited. Patients were excluded in the presence of fetal abnormalities, glucose intolerance or gestational diabetes identified in the 2nd trimester. 30 cases of severe PE were matched to 60 controls on the basis of timing of sampling.
Interventions	Inhibin A and leptin levels at 7-13 weeks
Outcomes	Severe PE
Paper	Saruhan et al. 2011
Methods	Retrospective cohort study
Participants	663 pregnancies were recruited. After exclusion for incomplete data, multiple pregnancy, fetal anomaly or the pregnancy resulting in miscarriage there were 318 pregnancies including 4 cases of PE.
Interventions	PAPP-A levels at 11-14 weeks
Outcomes	PE, SGA, preterm delivery, gestational HTN, gestational diabetes mellitus
Paper	Schneuer et al. 2012.
Methods	Prospective cohort study
Participants	2,989 patients were recruited. After exclusion if sampling took place <10 or >14 weeks, termination of pregnancy, multi-fetal pregnancy or a major congenital anomaly there were 2,784 pregnancies including 71 cases of PE and 4 cases of PE<34 weeks.
Interventions	PP13 levels at 10-14 weeks
Outcomes	PE,PE<34 weeks, SGA
Paper	Sebire et al. 2000
Methods	Prospective cohort study
Participants	876 singleton pregnancies were recruited. After exclusion for incomplete data and chromosomally abnormal pregnancies there were 759 pregnancies including 9 cases of PE.
Interventions	Inhibin A and beta HCG levels at 10-14 weeks

Outcomes	PE, SGA
Paper	Spencer et al. 2005
Methods	Case controlled study
Participants	64 cases of PE were matched to 3,999 controls. Patients were excluded if there was a multiple pregnancy or a fetal anomaly was identified.
Interventions	beta HCG and PAPP-A levels at 11-13+6 weeks
Outcomes	PE, preterm delivery, SGA
Paper	Thadhani et al. 2004
Methods	Prospective nested case control study
Participants	Pregnancies without diabetes, thyroid, liver or renal disease were selected from the Massachusetts General Hospital Obstetrical Maternal Study cohort. 40 cases of PE were matched with 80 controls on basis of timing of sampling and gestational age at delivery.
Interventions	PlGF and sFlt1 levels <12 weeks
Outcomes	PE, SGA, gestational HTN
Paper	Vatten et al, 2007
Methods	Nested case control study
Participants	Cohort of 29,948 women from a prospective study of toxoplasma gondii infection in pregnancy. Patients were excluded if there was a multiple pregnancy or insufficient serum for sampling. 111 cases of preterm PE and 143 term PE were matched with 273 randomly selected controls in the PlGF group. 110 cases of preterm PE and 144 cases of term PE were matched with 276 randomly selected controls in the sflt-1 group.
Interventions	PlGF and sFlt-1 levels 4-12 weeks
Outcomes	PE <37 weeks, PE>37 weeks
Paper	Wortelboer et al. 2010
Methods	Case controlled study
Participants	88 cases of early PE were matched with 480 controls on the basis of gestational age at sample date and duration of sample storage.
Interventions	PP13, PlGF, ADAM-12, PAPP-A, beta HCG levels at 8-13+6 weeks
Outcomes	Severe PE requiring delivery <34 weeks

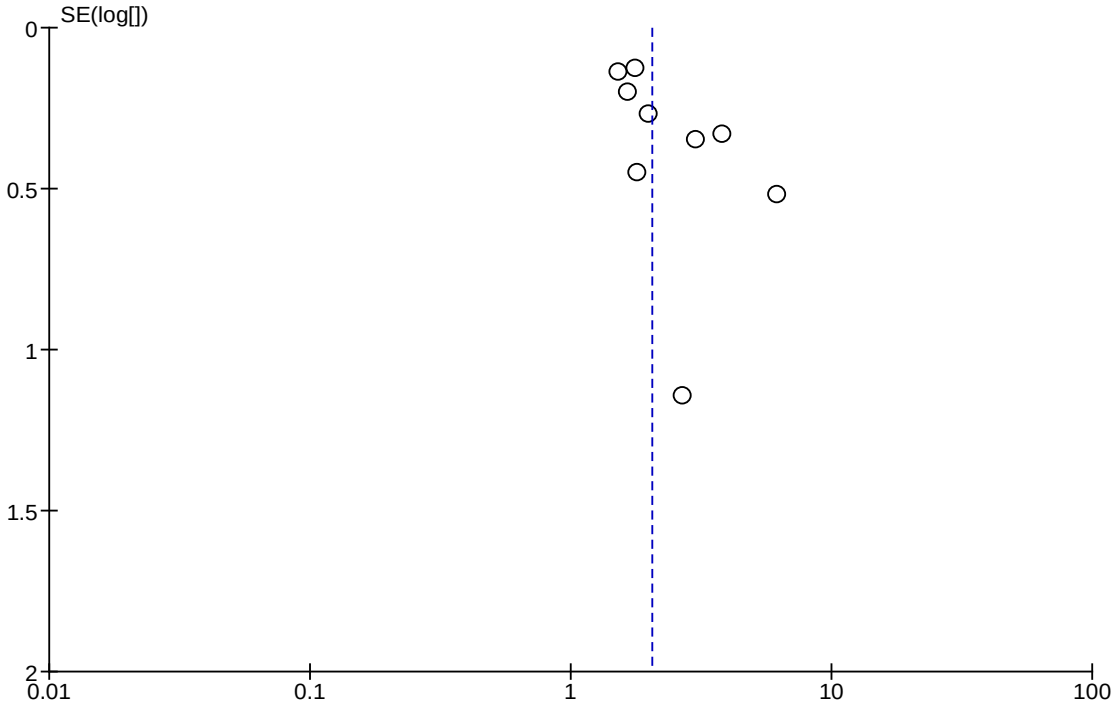
Paper	Yaron et al. 2002
Methods	Prospective cohort study
Participants	1772 patients were recruited. After exclusion for incomplete data, chromosomal abnormalities, and termination of pregnancy for fetal anomaly there were 1622 patients including 27 cases of PE.
Interventions	PAPP-A levels at 10-13 weeks
Outcomes	preterm labour, SGA, gestational hypertension, PE, intrauterine fetal death, oligohydramnios, miscarriage and placental abruption.
Paper	Zhong et al. 2011
Methods	Case controlled study
Participants	4000 singleton pregnancies were recruited. After exclusion for incomplete data there were 3475 pregnancies including 278 cases of PE.
Interventions	beta HCG and PAPP-A levels at 11-13+6 weeks
Outcomes	PE, SGA, pregnancy loss, preterm delivery

Appendix 3.2: Quality assessment of included studies in the biomarker review

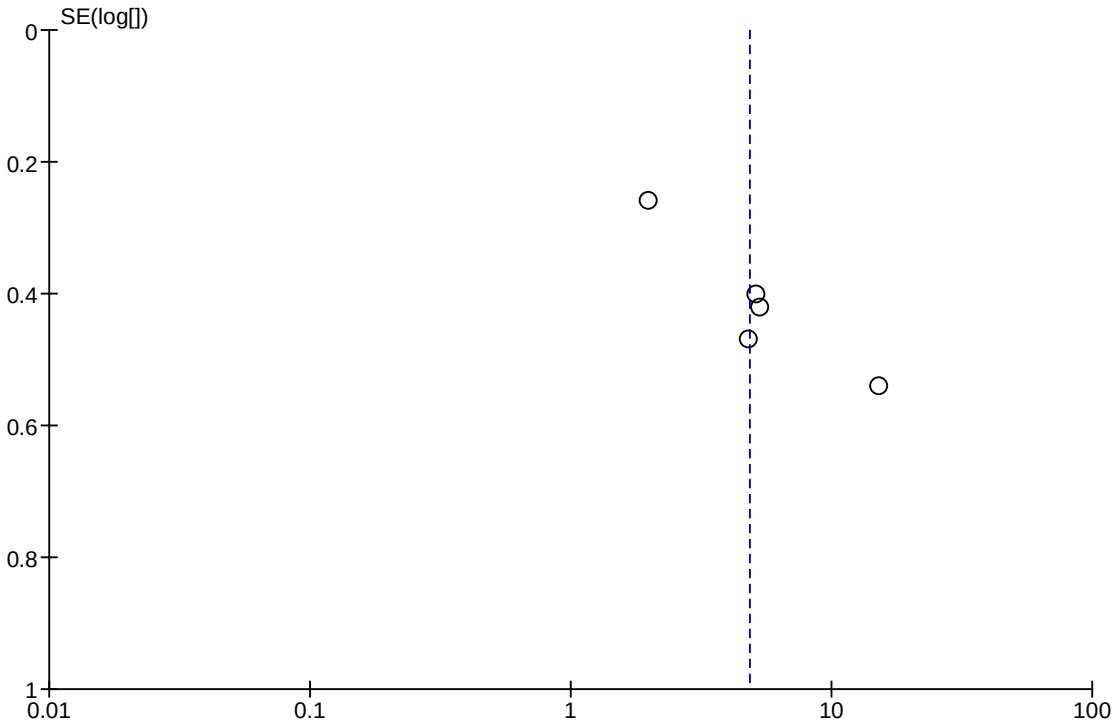
Study Year	Selection						Comparability			Outcome				
	Representativeness of exposed cohort	Selection of non- exposed cohort	Ascertainment of exposure	present at start	Total	Risk of bias	Comparability of cohorts on basis of design or analysis	Total	Risk of bias	Assessment of outcome	occur	Adequate follow up of cohorts	Total	Risk of bias
Bills 2009	–	★	★	–	2	Medium	-	0	High	★	-	-	1	Medium
Dugoff 2004	★	★	★	★	4	Low	★★	2	Low	★	-	-	1	Medium
El-Gharib 2011	★	★	★	★	4	Low	-	0	High	★	-	★	2	Medium
Goetzinger 2010	★	★	–	★	3	Medium	-	0	High	-	-	★	1	Medium
Lynch 2010	★	★	★	★	4	Low	★	1	Medium	★	-	★	2	Medium
Noori 2010	★	★	–	★	3	Medium	★★	2	Low	-	★	★	2	Medium
Odibo 2011	★	★	★	★	4	Low	★	1	Medium	-	-	★	1	Medium
Poon 2009	★	★	–	★	3	Medium	★	1	Medium	★	-	★	2	Medium
Radoi 2009	★	★	–	★	3	Medium	-	0	High	-	-	★	1	Medium
Saruhan 2011	★	★	–	–	2	Medium	-	2	High	-	-	-	0	High
Schneuer 2012	★	★	★	★	4	Low	-	2	High	★	-	★	2	Medium
Sebire 2000	★	★	–	★	3	Medium	-	2	High	-	-	★	1	Medium
Yaron 2002	★	★	–	★	3	Medium	-	1	High	-	-	★	1	Medium

Appendix 3.3 Funnel plots assessing publication bias

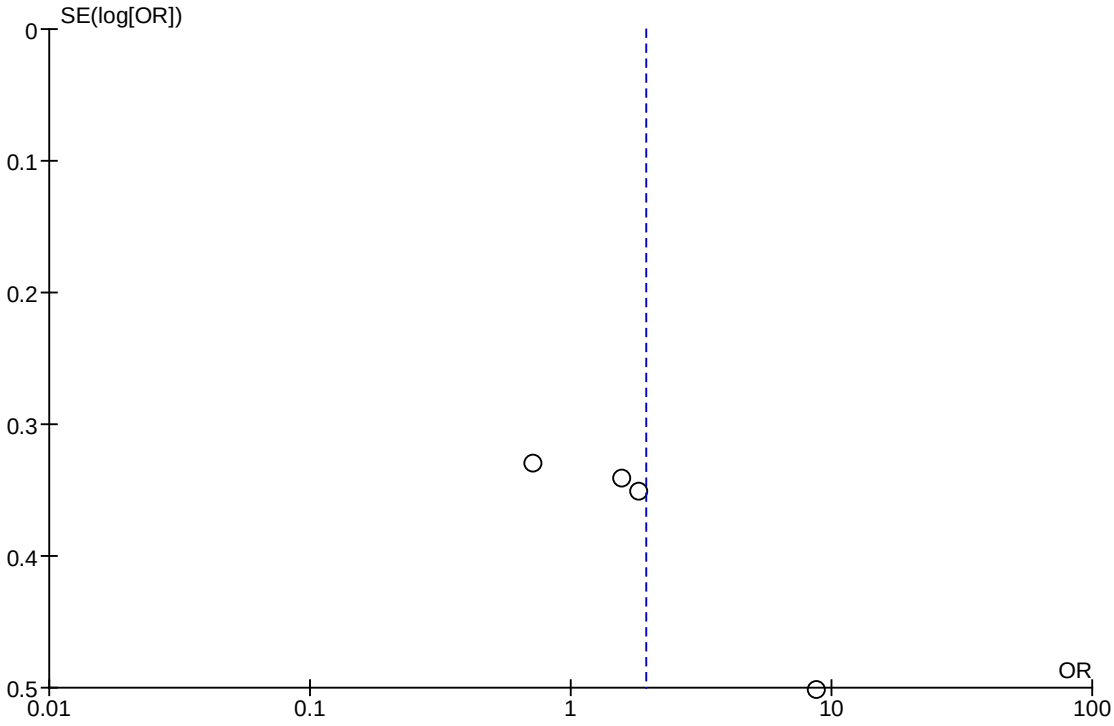
PAPP-A and any onset PE



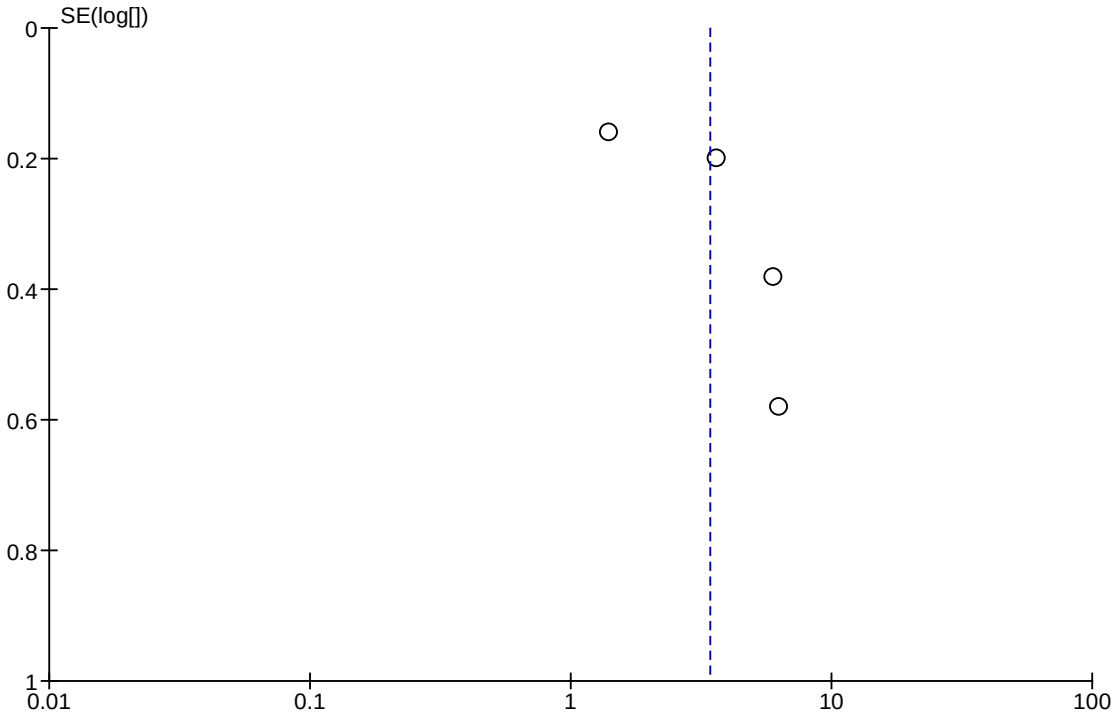
PAPP-A and early onset preeclampsia



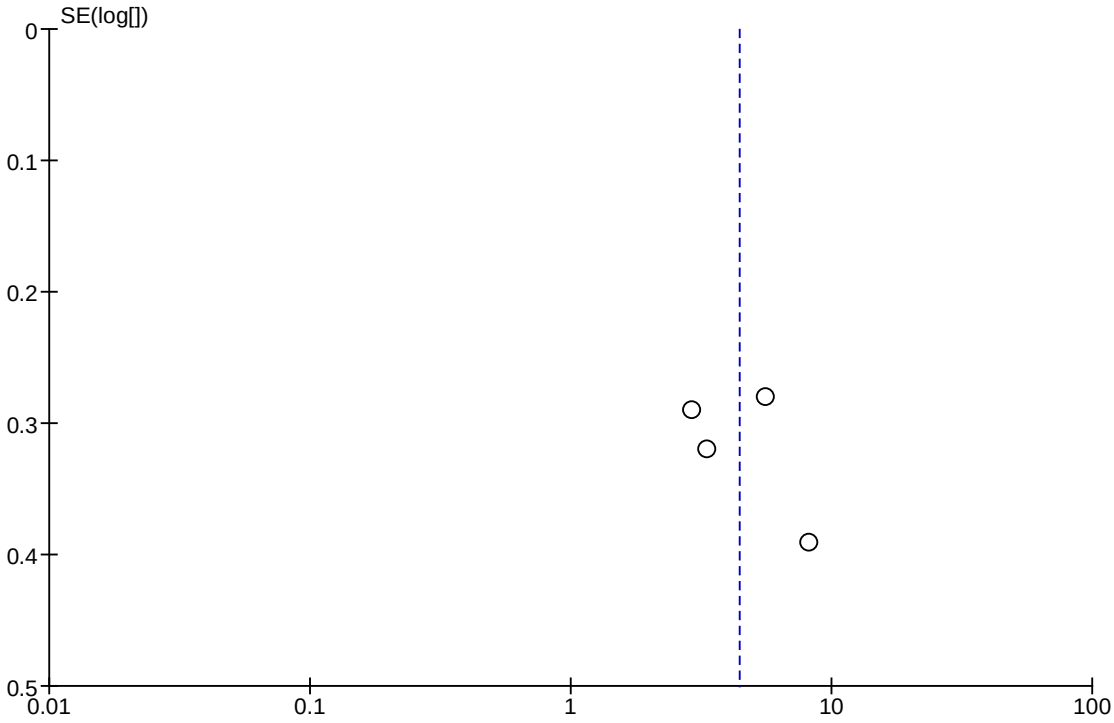
PlGF and any onset preeclampsia



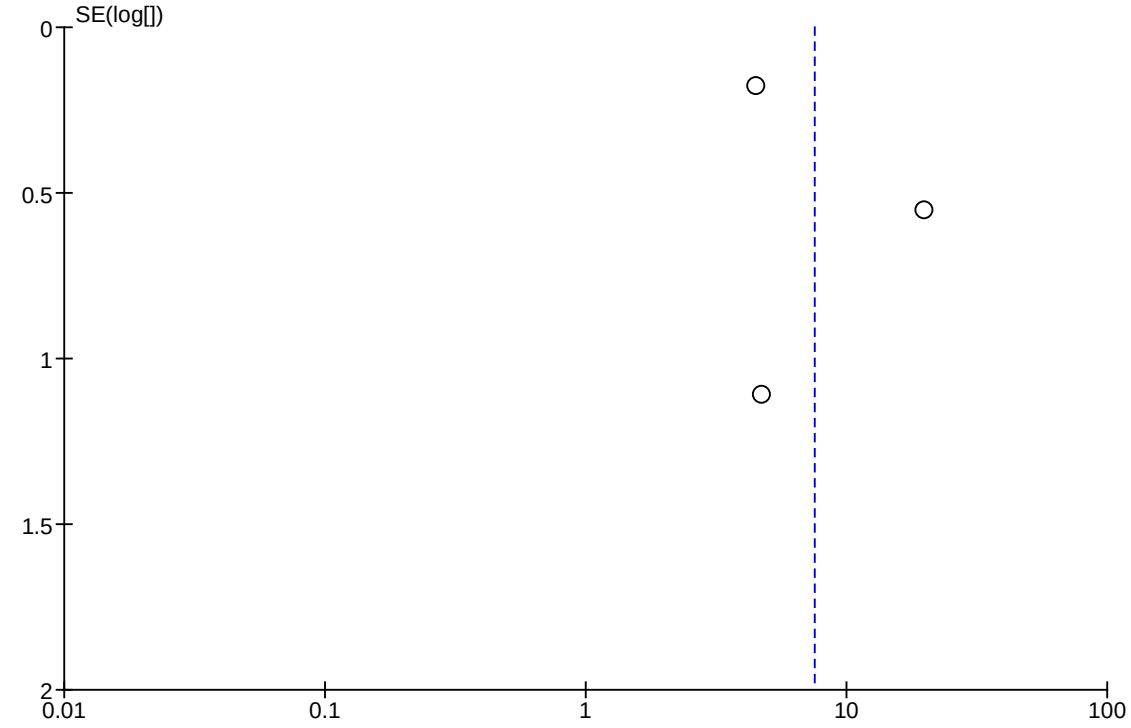
PLGF and early onset preeclampsia



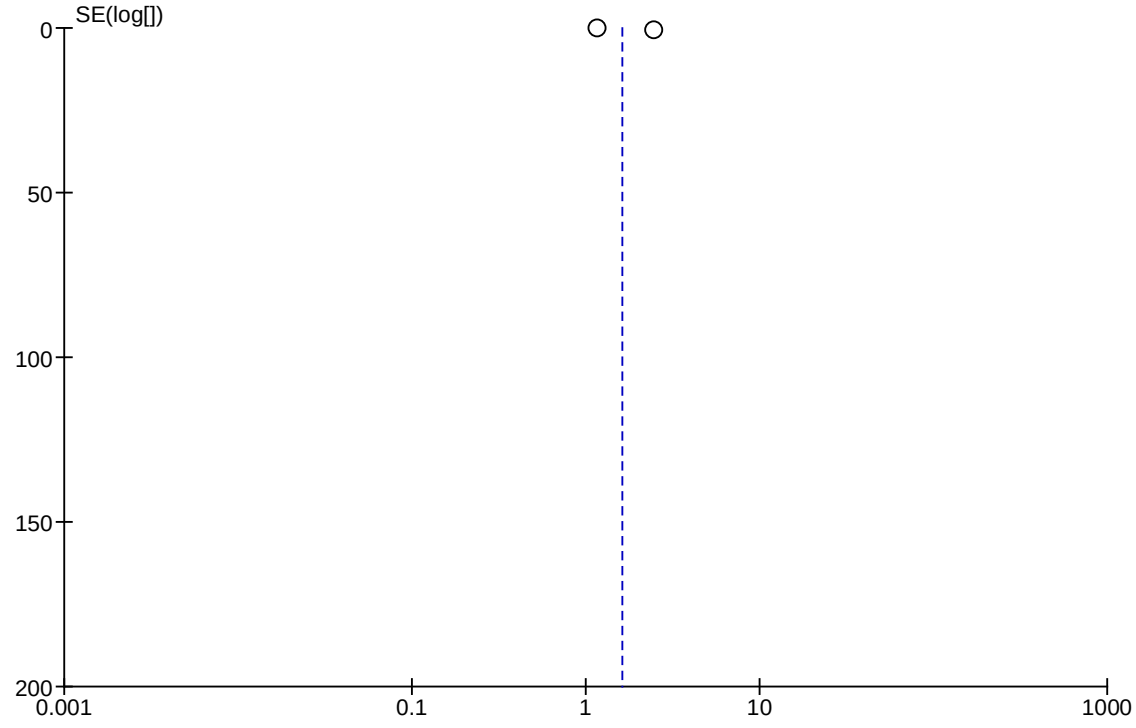
PP13 and any onset preeclampsia



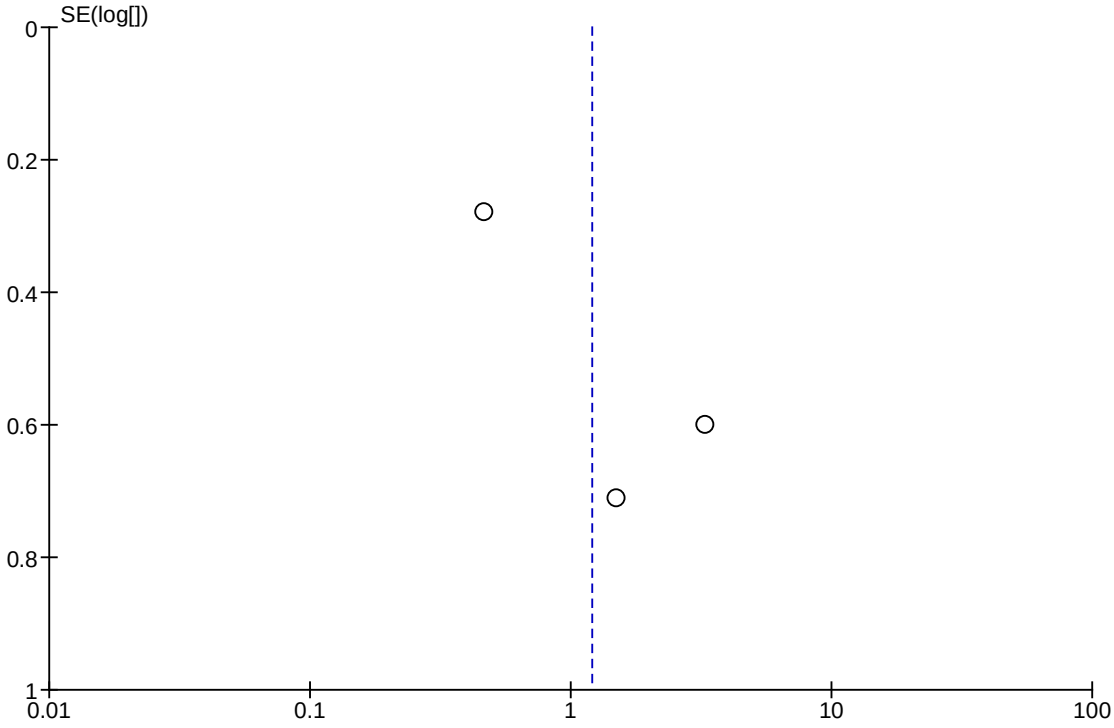
PP13 and early onset preeclampsia



SFLT-1 and any onset preeclampsia



sFlt-1 and early onset preeclampsia



Appendix 4.1: Data extraction form for stillbirth review

Reviewer: Paper No.: Language: 1st Author:

1. Population –pregnant women yes / no

2. Test - Transabdominal UADS Transvaginal UADS

3. Reference standard Stillbirth

2x2 table construction possible yes / no

Select this diagnostic test study (1-3 inclusive) yes / no if no reject & specify reason below

…… ……

…… ……

Population:

Study Design Cohort / Cross-sectional / Case control / Other……

Data Collection Prospective / Retrospective / Cannot tell / Other……

Patient Enrolment Consecutive / Arbitrary / cannot tell / Other……

Additional Description of Study Design

Blind comparison with reference standard	Yes	No	Can"t tell
Defined sample of patients	Yes	No	Can"t tell
Narrow population spectrum	Yes	No	Can"t tell
Differential use of reference standard	Yes	No	Can"t tell

Baseline prevalence of the disease: ..... (State figure if provided, otherwise circle below)

High Low Not mentioned

Please circle characteristic of sample population(s): [Assuming this is not a risk factor study]

Associated medical problem Can"t tell

If sample classified, state the definition:

.....

Inclusion criteria stated Yes No Description:

.....

No. patients recruited

A original population n=……

B Pre-enrolment exclusions n=…… (reasons eg pop characteristics)……

C actually recruited (A-B) n=……

D post-enrolment exclusions n=…… (reasons eg missing data etc)……

E analysable data (C-D) n=……

Parity stated: Yes No not sure

Intervention:

Type of intervention („the test"): ……

How many measurement(s): Single Multiple

Gestation at which „the test" was applied: .....weeks or ..... trimester

Method described: Yes No

If there"s a cut off level, this must be stated for the test to be considered adequate: Yes No

Cut off level of the test: ……

Test positive cases n=……

Test negative cases n=……

State fetal outcome: …

Blinding of test result yes / no

Completeness of Follow up (%) >90 / 81-90 / <81 (FU% = E/C x 100% =…… %)

Completeness of Follow up (%)

Positive cases %

Negative cases %

Regarding the outcome(s):

How was the result reported [ie the summary outcome measure(s)]: {please circle}

Receiver Operator Curve (summary) ROC LR (LR)

Sensitivity Specificity Negative predictive value Positive predictive value

Others (please state): .....

Space for free comment by reviewer:

Outcome (stillbirth): (2x2 table)

Population.....Outcome.....

	Outcome present	Outcome absent	Total
Positive			
Negative			
Total			

Appendix 4.2: Details of the included studies in the stillbirth review

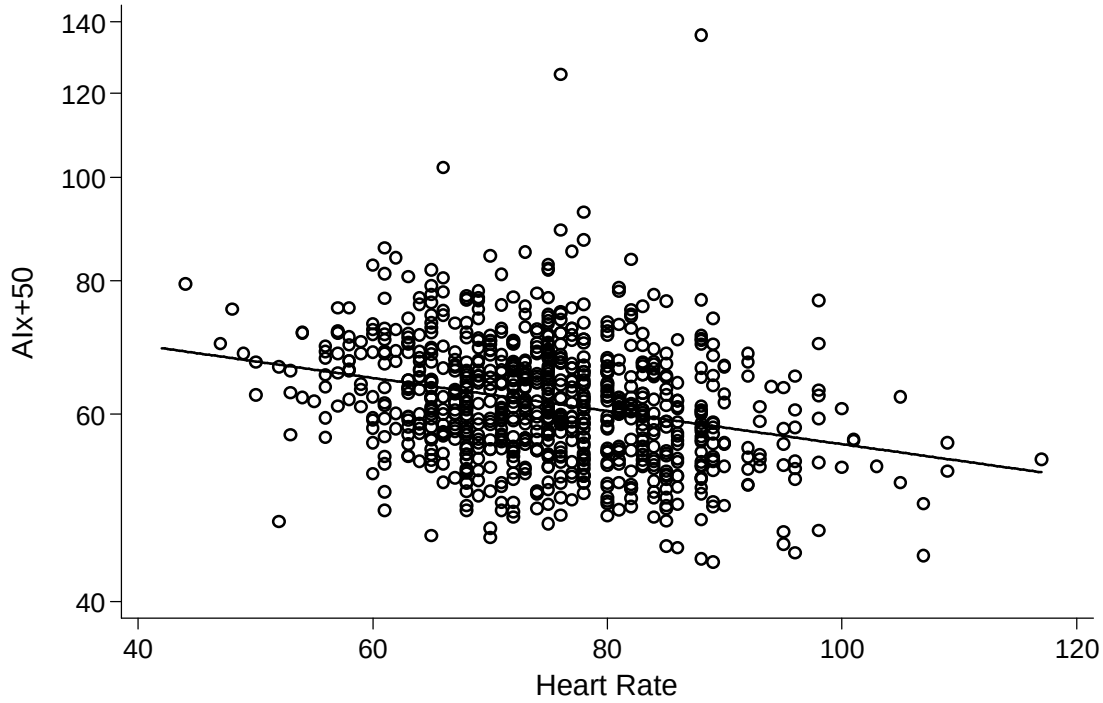
Paper	Madazali et al. 2014
Methods	Cohort study
Participants	65 pregnancies in women with SLE were examined between 2002-2011
Interventions	Average UAD SD ratio>2.6 and bilateral notching
Outcomes	Stillbirth, fetal growth restriction, PE, preterm birth
Paper	Jamal et al. 2013
Methods	Cohort study
Participants	435 consecutive singleton pregnancies attending for prenatal care at 18-24 weeks in 3 university hospitals. Inclusion criteria were signet pregnancies with normal foetuses, not taking aspirin, heparin, metformin or anti-hypertensive drugs
Interventions	UAD mean PI >95th percentile and/or bilateral notches
Outcomes	Stillbirth, PE, fetal growth restriction, preterm delivery, placental abruption
Paper	Poon et al 2013
Methods	Cohort study
Participants	65, 819 singleton pregnancies. 30, 566 pregnancies were examined between 1999 and 2002 at 7 hospitals that were collaborating in a trial using low dose aspirin for the prevention of PE. In this group gestational age was calculated by measurement of head circumference. 35, 253 pregnancies were examined between 2006 and 2011 at 3 hospitals , gestational age was calculated by measuring the crown rump length in this group. 306 women had stillbirths
Interventions	Uterine artery PI MoM >90th centile at 20-24 weeks gestation
Outcomes	Antepartum stillbirth, intrapartum stillbirth, placental abruption, SGA, PE
Paper	Singh et al. 2012
Methods	Cohort study
Participants	Nulliparous women or women with obstetric history of placental syndromes (fetal growth restriction, PE, or stillbirth) underwent second-trimester UAD assessment at 19-23 weeks' gestation. Outcomes were available for a final cohort of 15,796 women included 134 antepartum stillbirths.
Interventions	Mean uterine artery resistance index at 19-23 weeks.
Outcomes	Stillbirth.

Paper	Iacovella et al. 2012
Methods	Cohort study
Participants	9859 unselected women with singleton pregnancies attending for a routine 11-14 week ultrasound scan.
Interventions	UAD RI >90th centile
Outcomes	Stillbirth.
Paper	Filippi et al. 2011
Methods	Cohort study
Participants	273 women with one or more extreme levels of feto-placental proteins (PAPP-A ≤0.28 MoM, inhibin A ≥3.0 MoM, 2nd trimester β-hCG ≥4.0 MoM, AFP ≥2.5 MoM and estriol ≤0.5 MoM) were offered UAD at 24-26 weeks. UAD examination was performed in 159 women. Among these women there were two cases of stillbirth.
Interventions	Uterine artery notches or mean uterine artery pulsatile index ≥ 1.45 at 24-26 weeks.
Outcomes	SGA, low birthweight, preterm delivery, late miscarriage, stillbirth, placental abruption and gestational hypertension.
Paper	Proctor et al. 2009
Methods	Cohort study
Participants	90 women with singleton, chromosomally normal pregnancies and very low levels of PAPP-A (≤0.30 MoM) at 11–13 weeks' gestation. 15 stillbirths were observed in the cohort.
Interventions	Placental ultrasound assessment was performed at 18-24 weeks. The ultrasound examination included measurement of placental size and assessment of the UAD mean pulsatility index.
Outcomes	IUGR, preterm delivery before 32 weeks, intra-uterine death.
Paper	Fratelli et al. 2008
Methods	Prospective cohort study
Participants	76 women with singleton pregnancies between 11-14 weeks gestation classified as high risk due to a previous history of HELLP, eclampsia, early onset PE, chronic disease e.g. chronic hypertension, inherited thrombophilia, autoimmune disease, renal disease and a history of IUGR,

	stillbirth or placental abruption in a previous pregnancy
Interventions	Bilateral notches or mean UAD RI >0.8
Outcomes	IUGR, PE, intra-uterine death and placental abruption
Paper	Schwarze et al. 2005
Methods	Cohort study
Participants	346 women with singleton "low risk" pregnancies between 19 and 26 weeks' gestation. Women who fulfilled the following criteria were excluded from the study: essential hypertension, diabetes mellitus, autoimmune disorders, history of PE, IUGR, IUD and/or placental abruption in previous pregnancies. Two IUDs occurred in the study group.
Interventions	Uterine artery notches or mean uterine artery resistance index at 19-26 weeks.
Outcomes	PE, intra-uterine growth restriction, intra-uterine death and placental abruption.
Paper	Axt-Fliedner et al. 2005
Methods	Cohort study
Participants	52 women with singleton "risk" pregnancies defined by the presence of essential hypertension or an obstetric history of: PE, intrauterine growth retardation, IUD, placental abruption. Four IUDs occurred in the study group.
Interventions	Uterine artery notches or mean uterine artery resistance index at 19-26 weeks.
Outcomes	PE, intra-uterine growth restriction, intra-uterine death and placental abruption.
Paper	Albaiges et al. 2000
Methods	Cross-sectional study
Participants	1941 consecutive women with singleton pregnancies at 22 to 25 weeks' gestation. Complete demographic and outcome data were available for 1757 women. Six women had an IUD.
Interventions	Bilateral uterine artery notches or mean uterine artery pulsatile index of 1.45 or higher at 22-25 weeks.
Outcomes	PE, birth weight, fetal death and placental abruption.
Paper	Coleman et al, 2000

Methods	Prospective cohort study
Participants	116 pregnancies in women who had high risk for PE due to one of the following conditions: essential hypertension, secondary hypertension, pre-existing renal disease, systemic lupus erythematosus, antiphospholipid syndrome, previous recurrent PE, previous early-onset PE requiring delivery at ≤ 32 weeks, previous placental abruption. There were two IUDs in the cohort.
Interventions	Uterine artery notches or any resistance index > 0.58 or ≥ 0.7 at 22-24 weeks.
Outcomes	PE, SGA, intra-uterine death, placental abruption.
Paper	Bewley et al. 1991
Methods	Cross-sectional study
Participants	977 women at 16-24 weeks of gestation. Statistical analysis was performed in 925 women, the remainder being excluded for a variety of reasons. Twelve women had an IUD (nine at > 24 weeks).
Interventions	Average resistance index from four sites (left and right uterine and arcuate arteries) at 16-24 weeks.
Outcomes	IUD, birthweight, pregnancy-induced hypertension, antepartum haemorrhage.
Paper	Steel et al. 1990
Methods	Cohort study
Participants	1014 nulliparous women <25 weeks gestation
Interventions	Uterine artery >RI.0.58
Outcomes	Stillbirth, PIH, PE, SGA
Paper	Fleischer et al. 1986
Methods	Cohort study
Participants	71 women with hypertensive disorders in pregnancy (chronic hypertension, PE, chronic hypertension with superimposed PE) including 17 cases of stillbirth.
Interventions	Uterine artery notches or systolic/diastolic ratio >2.6 in the third trimester.
Outcomes	Stillbirth, premature birth, intrauterine growth retardation, PE.

Appendix 5.1: Univariate and multivariate odds ratios in prediction of each outcome. ROC curves based on multivariate model



Log_{10}(AIX+50)=1.905185-0.0015535×heart rate

To calculate AIX-5075/(10^(2.12215+0.0036873×(age-30) -0.189846×height in meters – 0.000464×weight in kg + 0.0159757 if ethnicity=2 +0.0209559 if ethnicity=5 – 0.0085961 if parity=1))

laix5075	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
age	.0036873	.0003851	9.57	0.000	.0029315	.004443
heightmeters	-.189846	.0310765	-6.11	0.000	-.2508264	-.1288656
weight	-.000464	.0001542	-3.01	0.003	-.0007666	-.0001614
_Iethnicity_2	.0159757	.0046511	3.43	0.001	.006849	.0251025
_Iethnicity_5	.0209559	.0095659	2.19	0.029	.002185	.0397267
_Iparity_1	-.0085961	.0039958	-2.15	0.032	-.016437	-.0007553
_cons	2.12215	.0489331	43.37	0.000	2.026131	2.21817

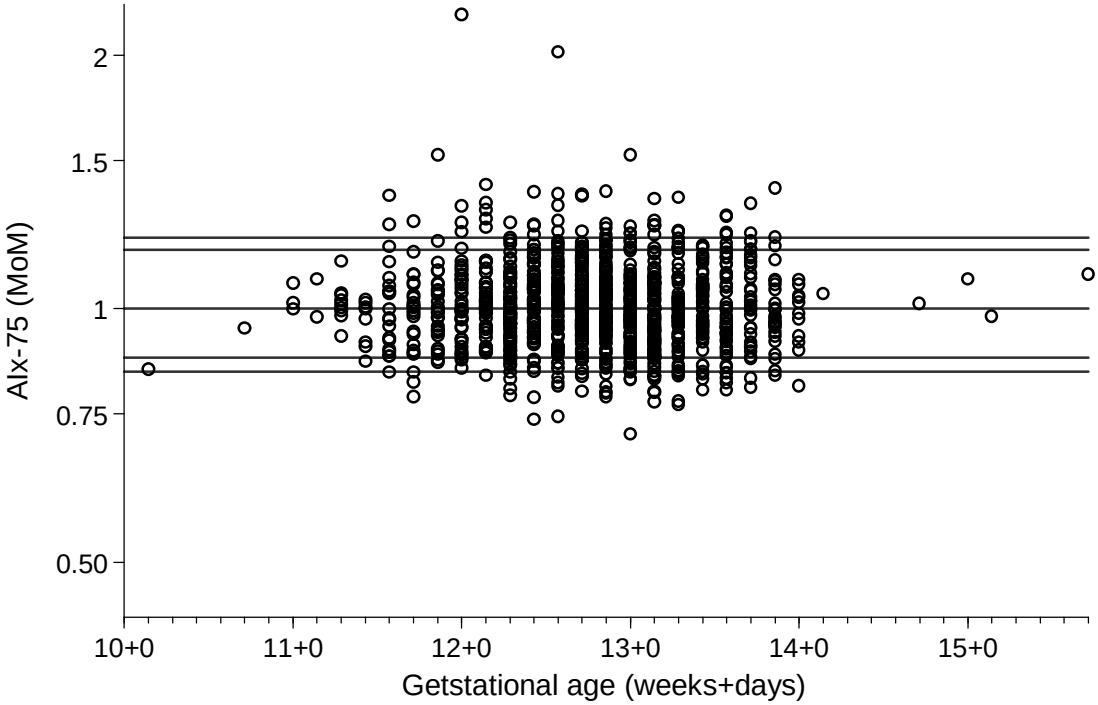
To calculate PWV MoMs: PWV/(10^((0.5984581 +0.0032231×(age-30)+ 0.0120506 if parity=1 + 0.00711×weight in kg + 0.0021199×MAP))

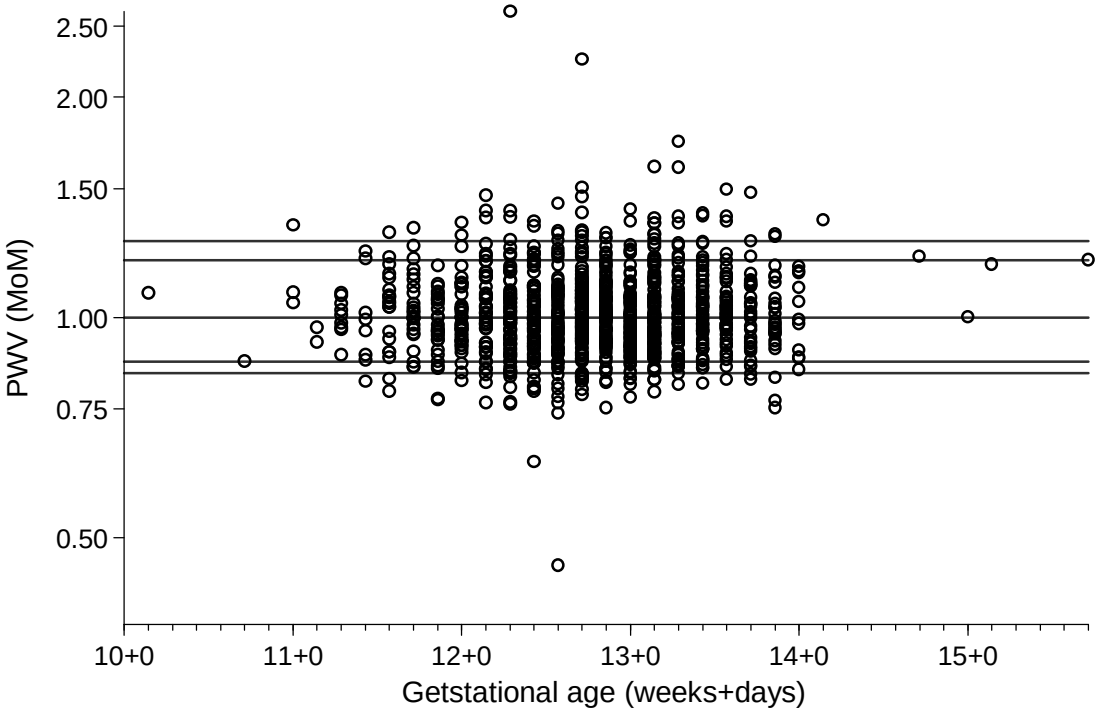
lpwv	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
age	.0032231	.0004008	8.04	0.000	.0024365	.0040096
_Iparity_1	.0120506	.0041692	2.89	0.004	.0038696	.0202316
weight	.000711	.0001567	4.54	0.000	.0004034	.0010185
map	.0021199	.0002175	9.75	0.000	.0016931	.0025466
_cons	.5984581	.0195684	30.58	0.000	.5600598	.6368565

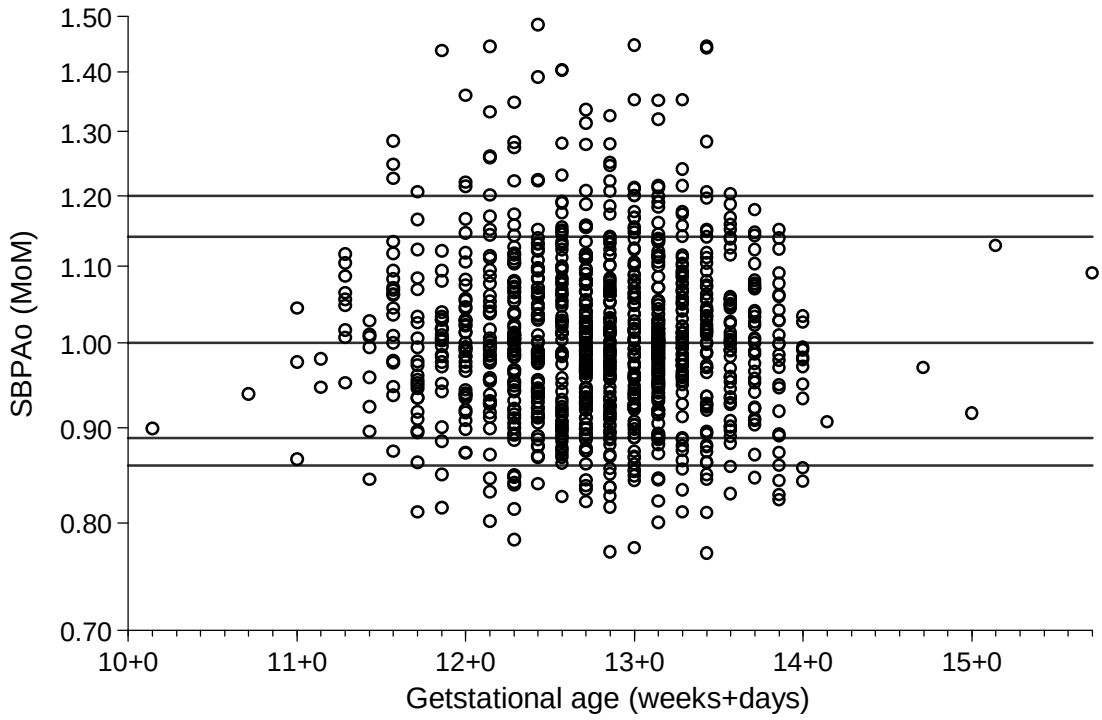
To calculate SBPAO MoMs: SBPAO/(10^(1.987581+.0012261×(age-30) -0.0133686 if parous+0.000805×weight + 0.0104739 if ethnicity=South Asian+0.0197938 if ethnicity=unknown/other/mixed)

lsbpao	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
age	.0012261	.0002847	4.31	0.000	.0006673	.0017848
_Iparity_1	-.0133686	.002942	-4.54	0.000	-.0191415	-.0075957
weight	.000805	.000108	7.45	0.000	.0005931	.001017
_Iethnicity_2	.0104739	.0031164	3.36	0.001	.0043588	.0165891
_Iethnicity_5	.0197938	.007065	2.80	0.005	.0059303	.0336573
_cons	1.987581	.0072693	273.42	0.000	1.973316	2.001845

MoMs


AIx-75 (MoM)
2
1.5
1
0.75
0.50
10+0
11+0
12+0
13+0
14+0
15+0
Getstational age (weeks+days)


PWV (MoM)
2.50
2.00
1.50
1.00
0.75
0.50
10+0
11+0
12+0
13+0
14+0
15+0
Getstational age (weeks+days)



PAPP-A MoM : log linear AxB $^{GA(days)}$

free beta hCG: log quadratic AxB $^{GA\ (days)}$xC $^{(GAxGA)}$

PIGF MoM = PIGF/(10^(0.3715965 +0.0168119*gestational age – 0.0023571*weight + 0.127651 if smoke + 0.0479465 if Black + 0.1090515 if Mixed/other))

AFP MoM= AFP/(10^(-0.1200957+0.017231*gestational age – 0.0042275*weight + 0.0837082 if smoke + 0.0780421 if Indian/Pakistani + 0.0674801 if Black + 0.1537812 if Mixed/other))

PIGF

lplgf	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
ga	.0130329	.0015017	8.68	0.000	.0100863	.0159796
weight	-.0015144	.0005067	-2.99	0.003	-.0025086	-.0005202
_Ismoker_1	.1281146	.0350467	3.66	0.000	.0593442	.1968851
_Iethnicity_2	.0937059	.0150135	6.24	0.000	.0642456	.1231663
_Iethnicity_3	.0667281	.0226997	2.94	0.003	.0221856	.1112706
_Iethnicity_4	.1622237	.0226803	7.15	0.000	.1177192	.2067283
_cons	.60298	.1406872	4.29	0.000	.3269159	.8790441

AFP

lafp	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
ga	.0188195	.0017715	10.62	0.000	.0153433	.0222956
weight	-.0028302	.0005904	-4.79	0.000	-.0039887	-.0016717
_Iethnicity_4	.0808776	.0262722	3.08	0.002	.029325	.1324303
_Iethnicity_2	-.0472127	.0167032	-2.83	0.005	-.0799885	-.0144369
_cons	-.342283	.1659126	-2.06	0.039	-.667845	-.016721

MAP

map	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
_Iethnicity_3	-2.42	1.10586	-2.19	0.029	-4.589978	-.250022
weight	.14	.0253828	5.52	0.000	.0901926	.1898074
_cons	78.44	1.66716	47.05	0.000	75.16861	81.71139

Mean PI

meanpi	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
crl	-.0047717	.0020781	-2.30	0.022	-.0088494	-.000694
heightmeters	-.7249249	.2280844	-3.18	0.002	-1.172485	-.2773653
_cons	2.935801	.3979804	7.38	0.000	2.154862	3.716739

Univariate and multivariate odds ratios in prediction of each outcome. ROC curves based on multivariate model

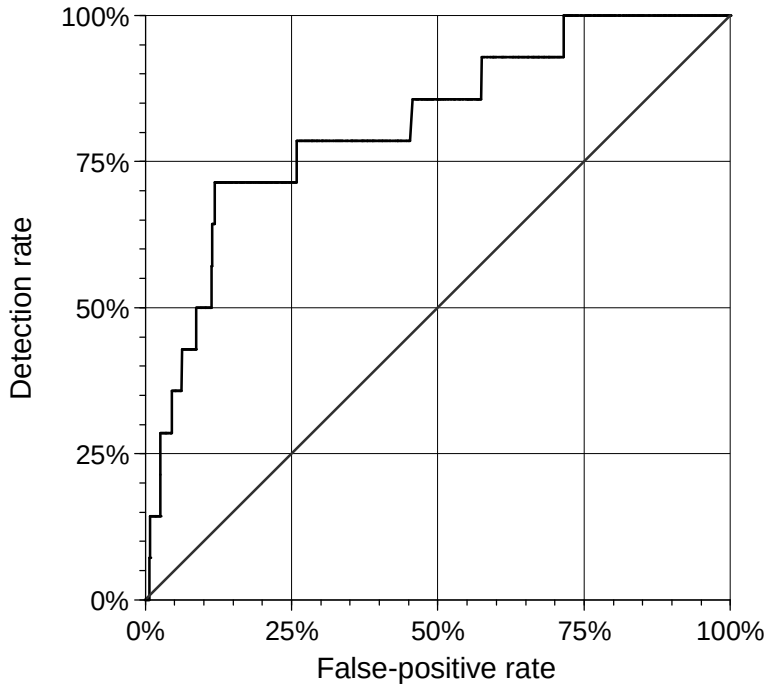
PE

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	0.62	(0.15 to 2.59)	0.509	-		
Oriental	-			-		
Afro-Caribbean	4.61	(1.38 to 15.41)	0.013	6.22	(2.07 to 18.67)	0.001
Other/Mixed/Unknown	-			-		
Previous PE	-			-		
FHx Mother	6.02	(0.73 to 49.45)	0.095			
FHx Sister	-					
FHx Mother/sister	2.75	(0.35 to 21.78)	0.337			
Hypertension	8.73	(1.03 to 73.96)	0.047	-		
Diabetes	-			-		
Smoker	2.06	(0.26 to 16.20)	0.49	-		
Parity	0.95	(0.33 to 2.76)	0.927	-		
Conception	3.88	(0.48 to 31.14)	0.201	-		
Systolic BP	1.05	(1.02 to 1.09)	0.001	-		
Diastolic BP	1.08	(1.03 to 1.13)	0.001	-		
Height (m)	0.94	(0.00 to 1233.71)	0.987	-		
Weight (kg)	1.01	(0.98 to 1.05)	0.415	-		
log10 AIX-75 MoM	8297.71	(3.46 to 2.0e+07)	0.023	-		
log10 PWV MoM	0.77	(0.00 to 7337.85)	0.956	-		
log10 SBPAO MoM	5.40E+07	(3181.71 to 9.3e+11)	<0.001	-		
MAP MoM	693.63	(15.58 to 30877.72)	0.001	491.46	(10.28 to 23487.85)	0.002
Mean PI MoM	3.69	(0.86 to 15.81)	0.078	-		
log10 AFP MoM	0.65	(0.05 to 8.90)	0.746	-		
log10 free β-hCG MoM	0.55	(0.07 to 4.42)	0.575	-		
log10 PAPP-A MoM	0.54	(0.06 to 5.11)	0.587	-		
log10 PlGF MoM	0.66	(0.04 to 11.35)	0.773	-		

Probability=exp(-11.23373+6.197378* MAP MoM)/(1+exp(-11.23373+6.197378*MAP MoM))

OR Probability=exp(-11.23373+6.197378* MAP MoM +1.827809)/(1+exp(-

11.23373+6.197378* MAP MoM +1.827809)) if ethnicity group=4



Area under ROC curve = 0.8134

95% CI for AUC 0.6928-0.9340

PIH

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	0.77	(0.26 to 2.24)	0.631	-		
Oriental	1.37	(0.36 to 5.23)	0.651	-		
Afro-Caribbean	2.36	(0.76 to 7.37)	0.139	-		
Other/Mixed/Unknown	1.13	(0.14 to 9.26)	0.909	-		
Previous PE	-			-		

FHx Mother	3.52	(0.44 to 28.14)	0.235	-		
FHx Sister	-			-		
FHx Mother/sister	1.61	(0.21 to 12.38)	0.646	-		
Hypertension	5.11	(0.62 to 42.10)	0.129	-		
Diabetes	-			-		
Smoker	1.21	(0.16 to 9.21)	0.855	-		
Parity	0.44	(0.17 to 1.13)	0.087	-		
Conception	-			-		
Systolic BP	1.05	(1.02 to 1.08)	<0.001	-		
Diastolic BP	1.09	(1.05 to 1.13)	<0.001	-		
Height (m)	78.88	(0.32 to 19316.29)	0.120	-		
Weight (kg)	1.04	(1.01 to 1.06)	0.001	1.04	(1.01 to 1.06)	0.002
log10 AIX-75 MoM	1696.54	(2.17 to 1.3e+06)	0.029	-		
log10 PWV MoM	28.66	(0.05 to 18164.26)	0.308	-		
log10 SBPAO MoM	4.90E+05	(153.27 to 1.5e+09)	0.001	-		
MAP MoM	633.52	(29.12 to 13781.10)	<0.001	457.66	(21.71 to 9647.42)	<0.001
Mean PI MoM	1.01	(0.25 to 3.99)	0.992	-		
log10 AFP MoM	1.1	(0.14 to 8.43)	0.926	-		
log10 free β-hCG MoM	0.42	(0.07 to 2.35)	0.323	-		
log10 PAPP-A MoM	0.66	(0.10 to 4.27)	0.665	-		
log10 PlGF MoM	0.16	(0.02 to 1.26)	0.081	-		

```
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         pih |      Coef.   Std. Err.      z    P>|z|     [95%
Conf. Interval]
-------------+----------------------------------------------------
--------------
        mmap |    6.12613   1.555292     3.94   0.000
3.077813    9.174446
```
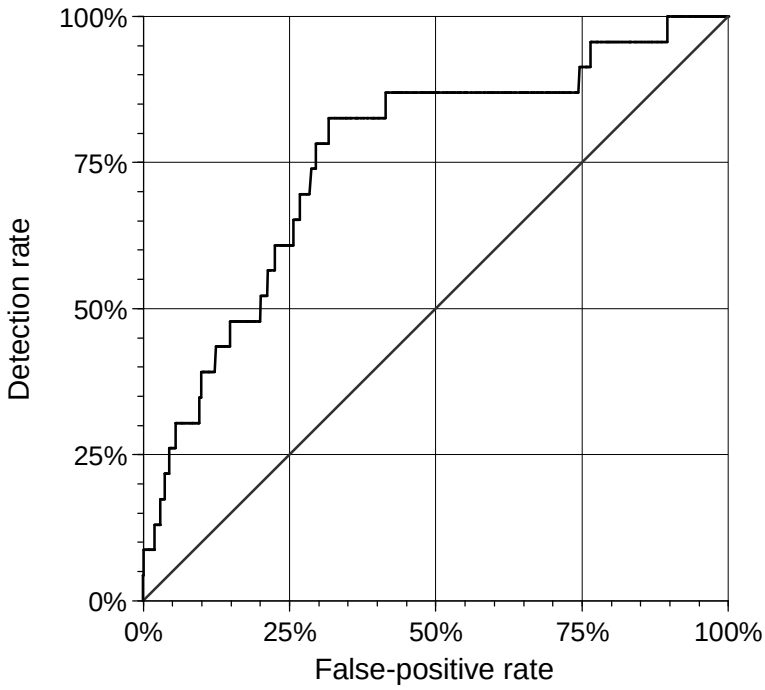

```
      weight |   .0367303   .0119601     3.07   0.002
.0132889    .0601716
       _cons |  -12.68619   1.904232    -6.66   0.000    -
16.41842   -8.953964
------------------------------------------------------------------------------
```



Area under ROC curve = 0.7597

95% CI for AUC 0.6560-0.8633

GDM

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	5.91	(2.44 to 14.30)	<0.001	-		

Oriental	3.76	(1.19 to 11.90)	0.024	-		
Afro-Caribbean	1.86	(0.46 to 7.57)	0.385	-		
Other/Mixed/Unknown	1.52	(0.18 to 12.88)	0.703	3.77	(2.03 to 6.98)	<0.001
Previous PE	-			-		
FHx Mother	-			-		
FHx Sister	-			-		
FHx Mother/sister	-			-		
Hypertension	-			-		
Diabetes	-			-		
Smoker	1.16	(0.27 to 4.96)	0.842	-		
Parity	1.4	(0.78 to 2.50)	0.254	-		
Conception	1.04	(0.14 to 7.90)	0.971	-		
Systolic BP	1.02	(1.00 to 1.04)	0.091	-		
Diastolic BP	1.03	(1.00 to 1.06)	0.036	-		
Heignt (m)	0	(0.00 to 0.10)	0.002	-		
Weight (kg)	1	(0.98 to 1.03)	0.705	-		
log10 AIX-75 MoM	228.73	(1.38 to 37818.54)	0.037	-		
log10 PWV MoM	38.36	(0.41 to 3612.02)	0.116	-		
log10 SBPAO MoM	888.85	(1.98 to 4.0e+05)	0.029	-		
MAP MoM	16.58	(1.39 to 197.33)	0.026	21.78	(1.76 to 270.24)	0.016
Mean PI MoM	0.72	(0.26 to 1.93)	0.510	-		
log10 AFP MoM	0.83	(0.20 to 3.48)	0.801	-		
log10 free β-hCG MoM	0.28	(0.07 to 1.07)	0.062	-		
log10 PAPP-A MoM	1.88	(0.44 to 8.15)	0.396	-		
log10 PlGF MoM	0.42	(0.09 to 1.96)	0.270	-		

```
------------------------------------------------------------------------------
---------------
          gdm |      Coef.   Std. Err.      z    P>|z|     [95%
Conf. Interval]
--------------+---------------------------------------------------------------
---------------
_Iethnicity_5 |   1.326385   .3147693     4.21   0.000
.7094489    1.943322
```

```
        mmap |   3.080947   1.284895     2.40   0.016
.5625987    5.599296
       _cons |  -6.861906   1.369835    -5.01   0.000    -
9.546734   -4.177077
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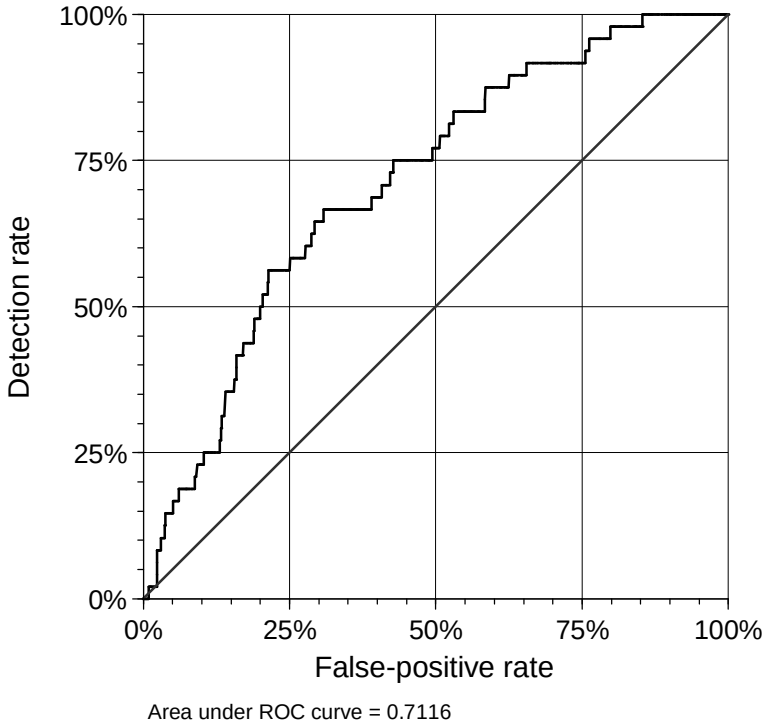


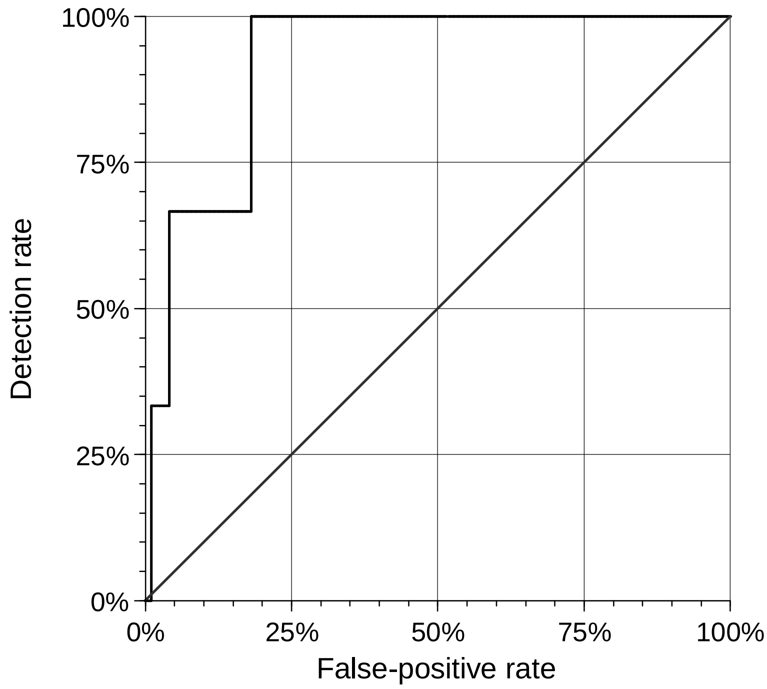
95% CI for AUC 0.6424-0.7809

IUD

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	2.07	(0.19 to 22.90)	0.554	-		
Oriental	-			-		
Afro-Caribbean	-			-		
Other/Mixed/Unknown	-			-		
Previous PE	-			-		
FHx Mother	-			-		
FHx Sister	-			-		

FHx Mother/sister	-			-		
Hypertension	-			-		
Diabetes	-			-		
Smoker	13.57	(1.20 to 153.00)	0.035	17.88	(1.43 to 224.28)	0.025
Parity	2.55	(0.23 to 28.16)	0.446	-		
Conception	-			-		
Systolic BP	1.04	(0.97 to 1.12)	0.248	-		
Diastolic BP	1.08	(0.98 to 1.18)	0.104	-		
Heignt (m)	174.81	(0.00 to 5.5e+08)	0.499	-		
Weight (kg)	1.02	(0.96 to 1.09)	0.463	-		
log10 AIX-75 MoM	0	(0.00 to 5.8e+06)	0.473	-		
log10 PWV MoM	2251.22	(0.00 to 1.1e+09)	0.248	-		
log10 SBPAO MoM	2065.3	(0.00 to 2.4e+13)	0.519	-		
MAP MoM	425.57	(0.15 to 1.2e+06)	0.137	-		
Mean PI MoM	1.81	(0.05 to 60.35)	0.739	-		
log10 AFP MoM	1197.4	(4.41 to 3.3e+05)	0.013	-		
log10 free β-hCG MoM	0.04	(0.00 to 15.53)	0.292	-		
log10 PAPP-A MoM	6.7	(0.01 to 4021.93)	0.56	-		
log10 PlGF MoM	0.01	(0.00 to 0.50)	0.022	0.01	(0.00 to 0.37)	0.016

```
------------------------------------------------------------------------------
         iud |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      lmplgf |  -5.262748   2.187921    -2.41   0.016    -9.550995   -.9745017
      smoker |   2.883772   1.290401     2.23   0.025     .3546324    5.412911
       _cons |  -6.843647   .9315489    -7.35   0.000    -8.669449   -5.017845
```

Area under ROC curve = 0.9225

95% CI for AUC 0.8194-1.0000

SGA5

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	1.75	(0.94 to 3.26)	0.076	1.83	(1.01 to 3.33)	0.047
Oriental	1.06	(0.38 to 2.95)	0.905	-		

Afro-Caribbean	2.03	(0.88 to 4.70)	0.097	-		
Other/Mixed/Unknown	2.87	(1.00 to 8.19)	0.049	3.1	(1.04 to 9.28)	0.043
Previous PE	-			-		
FHx Mother	1.18	(0.15 to 9.17)	0.874	-		
FHx Sister	1.1	(0.14 to 8.46)	0.931	-		
FHx Mother/sister	1.14	(0.26 to 4.90)	0.862	-		
Hypertension	6.84	(1.72 to 27.09)	0.006	-		
Diabetes	1.54	(0.19 to 12.22)	0.683	-		
Smoker	3.06	(1.23 to 7.62)	0.016	3.36	(1.23 to 9.15)	0.018
Parity	1.47	(0.89 to 2.45)	0.134	-		
Conception	1.63	(0.37 to 7.16)	0.517	-		
Systolic BP	1.01	(0.99 to 1.03)	0.242	-		
Diastolic BP	0.99	(0.97 to 1.02)	0.601	-		
Heignt (m)	0.79	(0.03 to 24.89)	0.895	-		
Weight (kg)	1.01	(0.99 to 1.03)	0.238	-		
log10 AIX-75 MoM	12.42	(0.11 to 1463.16)	0.300	-		
log10 PWV MoM	0.43	(0.01 to 36.10)	0.708	-		
log10 SBPAO MoM	13.76	(0.05 to 3607.49)	0.356	1.50E+08	(1246.77 to 1.8e+13)	0.002
MAP MoM	0.49	(0.04 to 5.54)	0.567	0.00	(0.00 to 0.17)	0.008
Mean PI MoM	2.52	(1.16 to 5.46)	0.019	-		
log10 AFP MoM	4.02	(1.17 to 13.80)	0.027	-		
log10 free β-hCG MoM	1.7	(0.61 to 4.76)	0.313	3.55	(1.20 to 10.52)	0.022
log10 PAPP-A MoM	0.13	(0.04 to 0.41)	<0.001	0.26	(0.07 to 0.90)	0.034
log10 PlGF MoM	0.05	(0.01 to 0.17)	<0.001	0.06	(0.01 to 0.26)	<0.001

```
------------------------------------------------------------------------------
        sga5 |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  ethnicity2 |   .6061936   .3050174     1.99   0.047     .0083705    1.204017
  ethnicity5 |   1.131371   .5592371     2.02   0.043     .0352868    2.227456
     lmsbpao |   18.83594   5.973393     3.15   0.002     7.128309    30.54358
```

```
        mmap |  -6.720448   2.513498    -2.67   0.008    -
11.64681   -1.794083

      lmplgf |  -2.886241   .7754534    -3.72   0.000    -
4.406102    -1.36638

     lmfbeta |   1.266588   .5543333     2.28   0.022
.1801152    2.353062

     lmpappa |  -1.354924   .6383569    -2.12   0.034    -
2.606081   -.1037678

      smoker |   1.210795   .5115088     2.37   0.018
.2082565    2.213334

       _cons |   3.303709   2.484663     1.33   0.184    -
1.566142     8.17356

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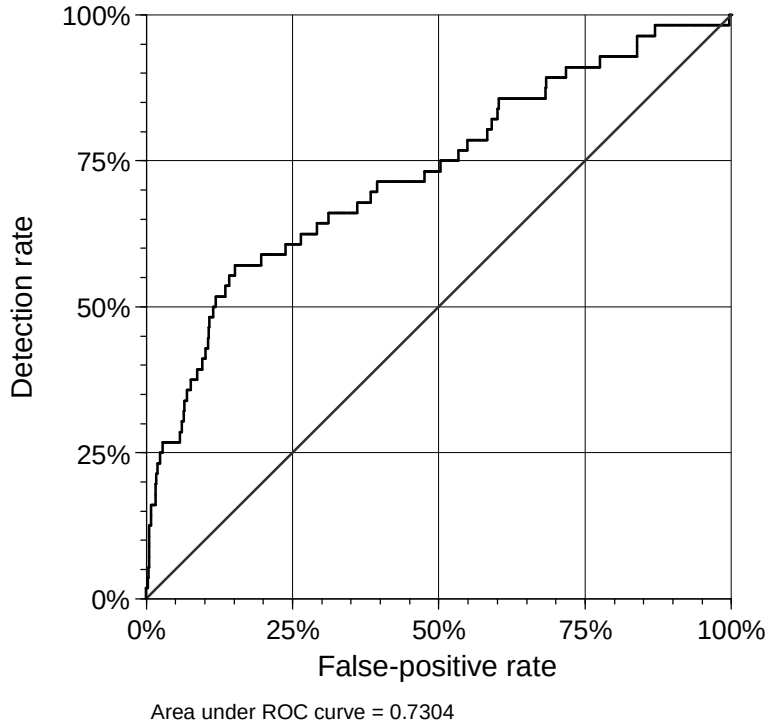

95% CI for AUC 0.6536-0.8071

SGA10

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Ethnicity (vs Caucasian)						
South Asian	1.32	(0.84 to 2.09)	0.234	-		
Oriental	1.30	(0.67 to 2.55)	0.438	-		
Afro-Caribbean	1.57	(0.83 to 2.98)	0.169	-		
Other/Mixed/Unknown	1.84	(0.77 to 4.42)	0.172	-		
Previous PE	-			-		
FHx Mother	2.17	(0.60 to 7.89)	0.24	-		
FHx Sister	1.21	(0.27 to 5.43)	0.803	-		
FHx Mother/sister	1.66	(0.62 to 4.44)	0.311	-		
Hypertension	5.38	(1.50 to 19.35)	0.01	4.40	(1.16 to 16.78)	0.030
Diabetes	1.76	(0.37 to 8.23)	0.475	-		
Smoker	1.20	(0.46 to 3.13)	0.713	-		
Parity	1.31	(0.89 to 1.92)	0.169	-		
Conception	0.82	(0.19 to 3.58)	0.795	-		
Systolic BP	1.00	(0.99 to 1.02)	0.686	-		
Diastolic BP	0.99	(0.97 to 1.01)	0.452	-		
Heignt (m)	0.54	(0.04 to 7.44)	0.648	-		
Weight (kg)	1.01	(1.00 to 1.03)	0.082	-		
log10 AIX-75 MoM	3.46	(0.08 to 141.89)	0.512	-		
log10 PWV MoM	1.65	(0.06 to 43.99)	0.765	-		
log10 SBPAO MoM	1.06	(0.01 to 80.11)	0.98	-		
MAP MoM	0.52	(0.08 to 3.20)	0.477	-		
Mean PI MoM	1.69	(0.92 to 3.13)	0.093	-		
log10 AFP MoM	1.41	(0.55 to 3.62)	0.474	-		
log10 free β-hCG MoM	1.08	(0.49 to 2.37)	0.856	-		
log10 PAPP-A MoM	0.21	(0.09 to 0.50)	<0.001	0.38	(0.15 to 0.97)	0.042
log10 PlGF MoM	0.12	(0.04 to 0.32)	<0.001	0.15	(0.05 to 0.48)	0.001

```
------------------------------------------------------------------------------
       sga10 |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
```

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-------------+------------------------------------------------
--------------

      lmplgf |  -1.906537   .5976591    -3.19   0.001    -
3.077927   -.7351466

     lmpappa |  -.9722679   .4782688    -2.03   0.042    -
1.909657   -.0348783

hypertension |   1.482275   .6825878     2.17   0.030
.1444273    2.820122

       _cons |  -2.206498   .1142247   -19.32   0.000    -
2.430375   -1.982622

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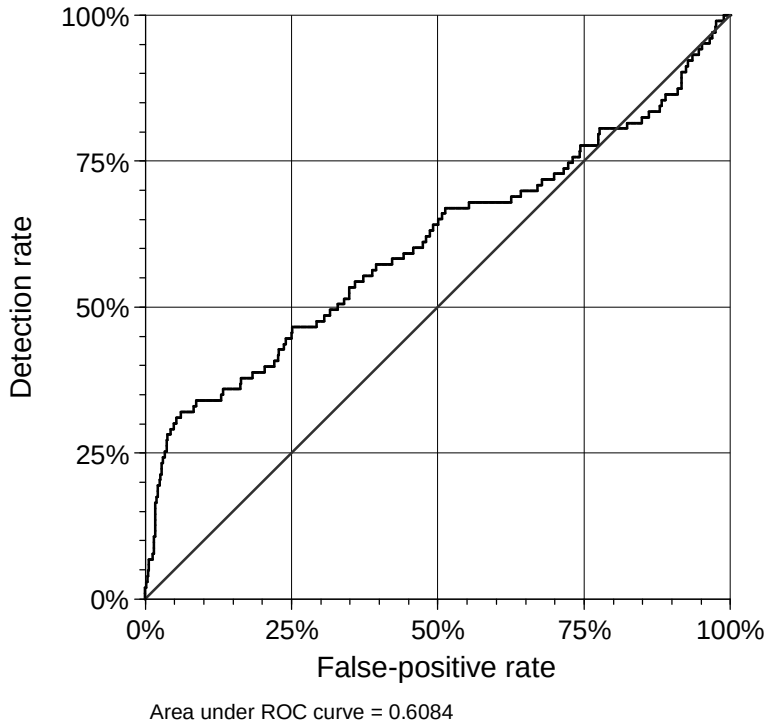

95% CI for AUC 0.5397-0.6770

Appendix 5.2: Summary table of results for all outcomes

Outcome	**PET**				**PIH**				**SGA<10th centile**				**SGA<5th centile**				**Stillbirth**				**GDM**			
FPR	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Sensitivity	28	50	72	72	30	40	48	72	27	30	30	40	27	50	57	57	62	65	67	100	17	25	45	55
AUC	0.8134				0.7597				0.6084				0.7304				0.9225				0.7116			
95% CI	0.69-0.93				0.66-0.86				0.54-0.67				0.65-0.81				0.82-1.00				0.64-0.78			

Appendix 5.3: Serial results for AFP and PlGF

Outcome	AFP			PlGF		
	OR	95% CI	p-value	OR	95% CI	p-value
PE	1.62	(0.00 to 7518.95)	0.911	0.72	(0.00 to 123.13)	0.900
PIH	0.74	(0.00 to 128.16)	0.91	0.00	(0.00 to 1.07)	0.053
GDM	1.76	(0.01 to 474.14)	0.843	0.86	(0.01 to 72.52)	0.947
SGA5	0.95	(0.00 to 509.06)	0.988	0.00	(0.00 to 0.56)	0.037
SGA10	0.12	(0.00 to 8.7e+14)	0.909	0.00	(0.00 to 0.25)	0.014

Appendix 5.4: Power calculation

When comparing two screening tests for prediction of preeclampsia in the same group of women, comparison is not made between the number of preeclamptics correctly predicted by the two tests, but instead between the number correctly predicted by test 1, but not by test 2, and the number detected by test 2 and not by test 1. To obtain 70 cases of PE amongst pregnant women in whom the prevalence of the disease is 5%, 1250 women would have to be recruited. Assuming the sensitivity of a test based on UADs alone in the first trimester is 60%, it would be expected that 42 of the 70 cases would be detected by Test 1. A test based on UADs and biochemistry would be considered superior if the sensitivity could be increased to at least 80%, while retaining the specificity of Test 1 (which can be fixed in the analysis). Therefore Test 2 is considered superior if it can detect at least 56 of the 70 cases. A table comparing the two tests can be constructed as shown below:

		Test 2		
		Positive	Negative	
Test 1	Positive	*a*	*b*	42
	Negative	*c*	*d*	28
		56	14	70

It is necessary to estimate the numbers in the body of the table (*a, b, c*, and *d*) to assess formally whether Test 2 is superior to Test 1. Statistical significance is assessed by comparing *b* and *c*. It seems reasonable to suppose that around 15% of cases would not be detected by either test (when the sensitivity of Test 2 is 80%), giving *d*=12 (then *a*=40, *b*=2 and *c*=16). With these figures, the study has 80% power to detect a sensitivity of at least 80% using Test 2, significant at the 5% level (2-sided test).

Appendix 6.1: AUC-ROC comparison between validation's cohorts

		Velauthar			Allen			
Study	PE Screening	PE events (%)	Analyzed women	AUC-ROC (95% CI)	PE events (%)	Analyzed women	AUC-ROC (95% CI)	P-Value (AUC-ROC Comparison)
Akolekar, 2008	Early	30 (3.1%)	961	0.714 (0.618; 0.810)	14 (1.5%)	927	0.717 (0.622; 0.813)	0.965
Akolekar, 2008	Late	34 (3.3%)	1,038	0.744 (0.657; 0.832)	14 (1.3%)	1,038	0.718 (0.590; 0.846)	0.742
DiLorenzo, 2012	Early	1 (11.1%)	9	-	13 (1.4%)	920	0.542 (0.353; 0.730)	-
DiLorenzo, 2012	Late	38 (3.3%)	1,143	0.716 (0.636; 0.796)	14 (1.3%)	1,043	0.675 (0.584; 0.766)	0.507
Goetzinger, 2010	Overall	38 (3.3%)	1,143	0.627 (0.535;	14 (1.3%)	1,043	0.683 (0.531;	0.537

				0.720)			0.835)	
Plasencia, 2008	Early	38 (3.3%)	1,136	0.703 (0.619; 0.787)	14 (1.3%)	1,038	0.700 (0.606; 0.794)	0.963
Plasencia, 2008	Late	38 (3.4%)	1,130	0.625 (0.538; 0.713)	14 (1.3%)	1,038	0.640 (0.504; 0.776)	0.856
Poon, 2008	Overall	38 (3.3%)	1,137	0.687 (0.606; 0.768)	14 (1.3%)	1,043	0.693 (0.549; 0.837)	0.943
Poon, 2009a	Early	30 (3.0%)	994	0.761 (0.671; 0.850)	14 (1.5%)	930	0.792 (0.691; 0.892)	0.652
Poon, 2009a	Late	30 (3.0%)	994	0.679 (0.581; 0.776)	14 (1.5%)	932	0.742 (0.624; 0.861)	0.421
Poon, 2009b	Early	38 (3.4%)	1,130	0.831 (0.764; 0.898)	14 (1.3%)	1,038	0.852 (0.783; 0.920)	0.668
Poon, 2009b	Late	38 (3.4%)	1,125	0.816 (0.753;	14 (1.4%)	1,037	0.830 (0.726;	0.821

				0.879)			0.934)	
Poon, 2009c	Early	30 (3.1%)	958	0.828 (0.758; 0.898)	14 (1.5%)	930	0.816 (0.736; 0.895)	0.824
Poon, 2009c	Late	30 (3.1%)	958	0.788 (0.712; 0.865)	14 (1.5%)	930	0.825 (0.723; 0.926)	0.568
Parra-Cordero, 2012	Early	34 (3.3%)	1038	0.713 (0.637; 0.788)	14 (1.3%)	1038	0.652 (0.517; 0.786)	0.438
Parra-Cordero, 2012	Late	34 (3.3%)	1038	0.667 (0.572; 0.763)	14 (1.3%)	1038	0.563 (0.407; 0.720)	0.266
Scazzocchio, 2013	Early	38 (3.4%)	1130	0.839 (0.780; 0.899)	14 (1.3%)	1038	0.821 (0.753; 0.888)	0.695
Scazzocchio, 2013	Late	30 (3.0%)	993	0.698 (0.596; 0.800)	14 (1.5%)	932	0.700 (0.533; 0.868)	0.984
Baschat, 2014	Early	38 (3.3%)	1143	0.735 (0.657; 0.812)	14 (1.3%)	1043	0.489 (0.305; 0.672)	0.015
Baschat,	Late	30 (3.0%)	1000	0.734 (0.643;	14 (1.5%)	932	0.518 (0.352;	0.025

2014				0.824)			0.683)	

Appendix 6.2 - Hosmer-Lemeshow calibration tables

Study	Risk group	Women	PE		No PE		Hosmer-Lemeshow P-Value
			Expected	Observed	Expected	Observed	
Akolekar Early	1	472	0.1	2	471.9	470	<0.001
	2	472	0.7	5	471.3	467	
	3	472	2.7	14	469.3	458	
	4	472	40.5	23	431.5	449	
Akolekar Late	1	519	1.7	3	517.3	516	0.167
	2	519	4.4	7	514.6	512	
	3	519	9	10	510.0	509	
	4	519	33	28	486.0	491	
DiLorenzo Early	1	233	0.4	5	232.6	228	<0.001
	2	232	1	1	231.0	231	
	3	232	2	4	230.0	228	
	4	232	10.6	4	221.4	228	
DiLorenzo Late	1	550	10.3	3	539.7	547	0.001
	2	550	12.3	7	537.7	543	
	3	546	13.6	18	532.4	528	

	4	540	15.7	24	524.3	516	
Goetzinger 2010	1	1145	14.6	16	1130.4	1129	0.342
	2	203	4.5	6	198.5	197	
	3	623	19.8	15	603.2	608	
	4	215	13.1	15	201.9	200	
Plasencia Early	1	544	0.4	2	543.6	542	<0.001
	2	543	1.8	10	541.2	533	
	3	544	5.6	15	538.4	529	
	4	543	44.2	25	498.8	518	
Plasencia Late	1	542	2.4	3	539.6	539	<0.001
	2	542	5.3	14	536.7	528	
	3	542	10.8	11	531.2	531	
	4	542	33.5	24	508.5	518	
Poon 2008	1	545	2.7	3	542.3	542	0.467
	2	545	7	7	538.0	538	
	3	545	11.6	15	533.4	530	
	4	545	30.7	27	514.3	518	

Poon 2009a Early	1	481	0.5	1	480.5	480	0.001
	2	481	1.9	6	479.1	475	
	3	481	5.4	9	475.6	472	
	4	481	36.2	28	444.8	453	
Poon 2009a Late	1	482	2.6	5	479.4	477	0.241
	2	481	5.8	7	475.2	474	
	3	482	9.7	9	472.3	473	
	4	481	25.9	23	455.1	458	
Poon 2009b Early	1	542	0.2	1	541.8	541	0.023
	2	542	1.2	3	540.8	539	
	3	542	4.5	6	537.5	536	
	4	542	46.2	42	495.8	500	
Poon 2009b Late	1	541	1.3	2	539.7	539	0.738
	2	540	4	3	536.0	537	
	3	541	9.2	9	531.8	532	
	4	540	37.5	38	502.5	502	
Poon 2009c Early	1	472	0	1	472.0	471	<0.001
	2	472	0.2	3	471.8	469	

	3	472	1.5	9	470.5	463	
	4	472	42.2	31	429.8	441	
Poon 2009c Late	1	472	0.5	1	471.5	471	0.075
	2	472	1.9	4	470.1	468	
	3	472	5.3	8	466.7	464	
	4	472	36.3	31	435.7	441	

Appendix 7.1: Complete search criteria

pregnancy, pregnant women, pregnant, gravidity AND preeclampsia, pre-eclampsia, PIH, gestational hypertension, hypertension AND statins, simvastatin, atorvastatin, pravastatin, pilavastatin, ifluvastatin, rosuvastatin, hydroxymethylglutaryl-Co-A reductase inhibitors OR fibre, dietary fibre, bran, isaphagula, methylcellulose, psyllium, fiber OR omega-3, fatty acids, fish oil, cod liver oil, flax seed oil, krill oil, nicotinic acid, OR fibrates, fibric acid, ciprofibrate, clofibrate, benzafibrate, gemfibrozil, fenofibrate

Appendix 7.2: Clinical characteristics of the studies evaluating the effectiveness of diet and lifestyle metabolic risk modifying interventions in pregnancy on PE

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
Bogaerts 2012 English	Selection bias: low Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: BMI ≥29 pre-pregnancy, 1st trimester of pregnancy Exclusion criteria: >15 weeks gestation, pre-existing type I diabetes mellitus, multiple pregnancy, primary need for nutritional advice and insufficient knowledge of the Dutch language Number of participants: 141	Four small group sessions with a midwife focussing on relation between energy intake and energy expenditure. Food diaries. Exercises in reading food labels and shopping methods performed. Methods for increasing level of physical activity discussed. Motivational interviewing.	PIH defined as blood pressure of at least 140/90mmHg on 2 occasions 6 or more hours apart after 20 weeks of pregnancy in an otherwise normotensive woman. PE defined as PIH in combination with significant proteinuria (>300mg/24hrs)
Crowther 2005 English	Selection bias: Low Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women with singleton or twin pregnancy, between 16-30 weeks' gestation, with a 75-g oral glucose-tolerance test at 24 to 34 weeks' gestation in which the venous plasma glucose level was less than 7.8 mmol /l after an overnight fast and was 7.8 to 11.0	Interventions included individualised dietary advice from a qualified dietician and instructions on how to self-monitor glucose levels. Introduction of insulin therapy if required. Interventions were commenced in the 2nd trimester.	Antenatal PE. PIH defined as blood pressure of at least 140/90mmHg on 2 occasions 4 or more hours apart.

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		mmol /l (198 mg /dl) at two hours. Exclusion criteria: Previously treated GDM, severe glucose impairment or active chronic systemic disease (except essential hypertension). Number of participants: 1000		
D'Almeida 1992 English	Selection bias: Low risk Performance bias: Low risk Detection bias: Unclear Attrition bias: Low risk Reporting bias: Low risk Other bias: Low risk	Inclusion criteria: Primiparous and multiparous women aged 14-40, </=16 weeks gestation Exclusion criteria: not documented Number of participants: 100	Treatment: 8 capsules/day providing a total 296mg Gammalinoleic acid, 80mg Docoshexanoiec acid and 144mg Eicospentanoeic acid/day Control: 8 capsules olive oil/day Interventions commenced in the 1st trimester	PIH, oedema, PE, eclampsia. PE defined as clinical triad of oedema, hypertension and proteinuria at any time during pregnancy. Hypertension defined as a rise in systolic BP greater than30mmHg and/or rise in diastolic BP greater than 15mmHg; either one or both during the course of the pregnancy constitutes PIH. Proteinuria-protein greater than 1 on urine dipstick
Guelinckx 2010 English	Selection bias: Unclear Performance bias: Unclear Detection bias: Unclear	Inclusion criteria: Obese (BMI >29.0), white Dutch speaking women	Lifestyle intervention based on a brochure or on active education.	Pregnancy-induced hypertension defined as BP ≥140/90mmHg appearing after 20

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
	Attrition bias: Low Reporting bias: High Other bias: Unclear	attending antenatal clinic before 15 wks of gestation. Exclusion criteria; Pre-existing diabetes/ GDM, multiple pregnancy, preterm delivery. Number of participants: 195	Passive group, was given a brochure during the first prenatal consultation. Active group, received brochure and was actively counselled about a healthy diet and limitation of energy dense foods by a trained nutritionist in 3 group 1 hour duration sessions. Behavioural modification techniques were used. Brochure provided advice on nutrition and on PA and tips to limit pregnancy-related weight gain. Interventions commenced in the 1st trimester	weeks of gestation. PE defined as the presence of PIH or chronic hypertension in combination with proteinuria.
Jeffries 2009 English	Selection bias: Low Performance bias: Unclear Detection bias: High risk Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: English speaking women 18-45, with singleton pregnancy, <14 weeks' gestation without diabetes mellitus. Exclusion criteria: Age	Advised of optimal gestational weight gain; given personalized weight measured card and instructed to record their weight at 16, 20, 24, 28, 30. 32,	PE, Pregnancy-induced hypertension -no definition given

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		<18, >45, type 1 or 2 diabetes mellitus, multiple pregnancy, non-English speaking Number of participants: 286	and 34 weeks' gestation. Interventions commenced in the 1st trimester	
Khoury 2005 English	Selection bias: Low Performance bias: Low Detection bias: Low Attrition bias: Unclear Reporting bias: Unclear Other bias: Unclear	Inclusion criteria: Non-smoking, non-vegetarian, white, women aged 21-38 with singleton low risk pregnancy, BMI 19-32 kg/m2 Exclusion criteria: high risk pregnancies, bleeding or hyperemesis beyond 12 weeks gestation Number of participants: 290	Cholesterol-lowering diet from gestational week 17-20 to birth. Cholesterol intake limited to 150mg/day and saturated fat intake reduced to 8% of dietary energy. Interventions commenced in the 2nd trimester	Hypertensive complications including PIH (BP >140/90mmHg after gestational week 20 measured at 2 occasions at least 6 hours apart) with 1+ proteinuria on a dipstick assay (PE) or without proteinuria
Landon 2009 English	Selection bias: Low Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women with new diagnosis of mild gestational diabetes between 24 and 30 weeks gestation. Exclusion criteria: Women with high risk pregnancy or pre-existing diabetes	Formal nutritional counselling and diet therapy, along with insulin if required. Interventions commenced in the 2nd trimester	Gestational hypertension was defined as a systolic pressure of 140 mm Hg or more or a diastolic pressure of 90 mm Hg or more on two occasions at least 4 hours apart, or one elevated blood-pressure value subsequently treated with medication.

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		Number of participants: 958		PE was defined as an elevation in blood pressure (according to the definition of gestational hypertension) together with proteinuria (300 mg of protein or more in a 24-hour urine collection or a result of 2+ or greater on a dipstick test when a 24-hour collection was not available). Elevated blood pressure with either elevated liver enzyme levels (aspartate aminotransferase level ≥70 U per liter) or thrombocytopenia (platelet count <100,000 per cubic millimeter) was also diagnosed as PE
Olsen 2000 English	Random sequence generation: Low Allocation concealment: Low Performance bias: Low Detection bias: Low Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Multips, recruited from 16 weeks gestation, previous preterm delivery, IUGR or PIH Exclusion criteria: pre-existing or gestational diabetes, severe fetal malformation or hydrops,	Fish oil capsules giving 1.3g Eicospentanoiec acid and 0.9g Docoshexanoiec acid/day given as 4 capsules/day. Controls were given matching olive oil capsules, 4/day Interventions commenced in the 2nd trimester	PIH defined as one or more recorded measurements of a diastolic bp >90mmHg at rest. PE defined as a combination of PIH and proteinuria, which was defined as a urinary measurement of >1+in albustix, 0.3g protein/L, 0.3g/24hrs or 300mmol protein/L

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		previous history of placental abruption or suspected placental abruption in index pregnancy, drug or alcohol abuse, fish allergy, regular intake of fish oil or NSAIDs or other drugs with an effect on thrombocyte function or eicosanoid metabolism Number of participants: 818		
Onuwude 1995 English	Random sequence generation: Low Allocation concealment: Low Performance bias: Low Detection bias: Low Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women between 19-26 weeks gestation, high risk pregnancies, singleton, previous history low birth weight (<3rd centile, stillbirth), previous pregnancy hypertension, primigravida with abnormal UADs at 24 weeks Exclusion criteria: multiple pregnancy, anticoagulant use, chronic hypertension, asthma, diabetes mellitus	Fish oil given as 9 capsules/day giving total dose 1.62g Eicospentanoiec /day and 1.08g Docoshexanoiec acid//day. Control group received matching air filled capsules. Treatment stopped at 38 weeks Intervention commenced in any trimester	PIH, PE. Raised blood pressure was defined by a diastolic blood pressure of at least 90 mmHg on two consecutive occasions at least 4 h apart Proteinuria was defined as two clean catch midstream specimens **of** urine collected > 4 h apart with 1 g albumin per litre or 2+more on reagent strip

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		Number of participants: 232		
Phelan 2011 English	Random sequence generation: Low Allocation concealment: Low Performance bias: Low Detection bias: Unclear Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women between 10 and 16 wk of gestation, BMI between 19.8 and 40, non-smoking, older than 18 years of age with singleton pregnancy Number of participants: 401 Completed treatment: 349	Behavioural lifestyle intervention designed to prevent excessive weight gain during pregnancy. The Fit for Delivery intervention was developed out of a preliminary study done by Polley et al. Use of social learning theory to promote changes in eating and physical activity. Interventions commenced in the 1st trimester	PE, maternal hypertension -no definition given
Polley 2002 English	Random sequence generation: Unclear Allocation concealment: Unclear Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women ≥18 years with uncomplicated pregnancy, BMI ≥20kg/m^2; who booked for antenatal care before 12 weeks gestation. Exclusion criteria: BMI<19.8, <18years old, 1st prenatal visit >12 weeks gestation, high risk pregnancies Number of	Intervention included education and behavioural strategies to promote healthy, low-fat eating, modest exercise and appropriate weight gain during pregnancy. Intervention commenced in the 1st and 2nd trimesters	PE, maternal hypertension -no definition given

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
		participants: 120		
Rae 2000 English	Random sequence generation: Low Allocation concealment: Unclear Performance bias: Low Detection bias: Unclear Attrition bias: High Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women with GDM, gestation <36 weeks; >110% of ideal body weight for height. Number of participants: 125	Energy restricted diet monitored by three-day food diaries. Intervention commenced in any trimester	PE -no definition given
Salvig 1992 English	Random sequence generation: Unclear Allocation concealment: Low Performance bias: Low Detection bias: Low Attrition bias: Low Reporting bias: Low Other bias: Unclear	Inclusion criteria: Women at approximately 30 weeks gestation, aged 18-44. Exclusion criteria: history of placental abruption, fish allergy, on prostaglandin inhibitors, multiple pregnancy, serious bleed in current pregnancy, regular intake of fish oil Number of participants: 533	Fish oil (2.7g omega 3 fatty acids/day) given as 4x1g capsules/day Control group given 4 olive oil capsules/day or no supplements Intervention commenced in the 3rd trimester	Systolic and diastolic BP, PIH and PE. Pregnancy-induced hypertension was considered to be present if arterial blood pressure was greater than 140/90 mmHg after rest at two subsequent measurements with a ***6*** h interval. PE was considered to be present if pregnancy-induced hypertension was accompanied by proteinuria (> 0.3 g/l).
Smuts 2003 English	Random sequence generation: Low Allocation	Inclusion criteria: Singleton pregnancies, women aged	Docoshexanoiec acid//day enriched eggs. Each egg contained	PE/eclampsia-no definition given

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
	concealment: High Performance bias: Low Detection bias: Low Attrition bias: Low Reporting bias: Low Other bias: Unclear	16-36, 24-28 weeks gestation at recruitment. Exclusion criteria: diabetes Number of participants: 350	133mg Docoshexanoiec acid. Women asked to eat 12/ week but reported eating 5.5/week. Control group ate normal eggs containing 33mg docoshexanoiec acid//day Asked to eat 12/week but reported eating 5.4 Interventions commenced in the 2nd trimester	
Thornton 2009 English	Random sequence generation: Low Allocation concealment: Unclear Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Unclear Other bias: Unclear	Inclusion criteria: Obese women (BMI ≥30 kg/m2) with singleton pregnancy, between 12 and 28 weeks gestation; Exclusion criteria: Pre-existing diabetes, hypertension, renal disease Number of participants: 257	Diet intervention based on a balanced nutritional regime; 18 to 24 KJ/kg. Food diary and encouragement to walk for 30 minutes per day. Interventions commenced in the 2nd trimester	PE, gestational hypertension no definition given. Outcome gathered from review of case notes
Vinter 2011 English	Random sequence generation: Low Allocation concealment: Unclear Performance bias: Unclear Detection bias: Unclear	Inclusion criteria: Women aged 18-40 years old between 10-14 weeks' gestation, BMI of 30-45 kgm^2. Exclusion criteria: Prior serious	Dietary counselling and physical activity. Women were encouraged to be moderately physically active 30-60 min daily. Interventions commenced in any trimester	PE defined as proteinuria and persistently elevated blood pressure (140/90mmHg) on more than one occasion. PIH was diagnosed if the blood pressure met the previously

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
	Attrition bias: Low Reporting bias: High Other bias: Unclear	obstetric complications, chronic diseases, positive glucose tolerance test early in pregnancy, alcohol or drug abuse, non-Danish speaking, multiple pregnancy Number of participants: 360 Completed treatment: 304		mentioned criteria but without the presence of proteinuria
Wolff 2008 English	Random sequence generation: Low Allocation concealment: Unclear Performance bias: Unclear Detection bias: Unclear Attrition bias: Low Reporting bias: Unclear Other bias: Unclear	Inclusion criteria: Caucasian women with uncomplicated pregnancy, aged 18-45 years, 12-18 weeks gestation, BMI ≥30 kg/m 2 Number of participants: 66	Dietary consultations (healthy diet, restriction of energy intake);10 consultations of 1 hr each with a dietician Intervention commenced in the 2nd trimester	PIH, PE-no definition given. Outcome gathered from review of case notes
Zhou 2012 English	Random sequence generation: Low Allocation concealment: Unclear Performance bias: Unclear Detection bias: Low Attrition bias: Low	Inclusion criteria: Pregnant women <20 weeks gestation, singleton pregnancies. Exclusion criteria: Already taking a dietary supplement containing	Treatment group received 3x Docoshexanoiec acid 500mg capsules providing 800mg daily Control group received 3 capsules of vegetable oil Intervention commenced in	PE defined as PIH and proteinuria or clinically diagnosed PE diagnosis made in the clinical record, PIH was defined as either 1) one systolic blood pressure reading of ≥160 mm Hg or diastolic blood pressure reading

Study Year Language	Study characteristics	Participants	Interventions	Outcomes
	Reporting bias: Low Other bias: Unclear	docoshexanoiec acid, their fetus had a known major abnormality, they had a bleeding disorder for which fish oil was contraindicated, they were receiving anticoagulant therapy, they had a documented history of drug or alcohol abuse, they were participating in another fatty acid trial, or English was not the main language spoken at home. Number of participants: 2399	1st and 2nd trimester	of ≥110 mm Hg or 2) 2 consecutive systolic blood pressure readings of ≥140 mm Hg and/or diastolic blood pressure readings of ≥90 mm Hg ≥4 h apart after 20 wk gestation. Proteinuria was defined as 1) a spot urine protein to creatinine ratio of ≥30 mg/mmol, 2) one 24-h urine specimen with a total protein content of ≥0.3 g/L on a dipstick test, or 3) a total protein content of ≥1 g/L from 2 random urine samples

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