ASSOCIATION OF MATERNAL SERUM PAPP-A LEVELS IN FIRST TRIMESTER AND ADVERSE PREGNANCY OUTCOMES: SYSTEMATIC REVIEW AND RETROSPECTIVE COHORT STUDY

By Ashwini Bilagi

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SYNOPSIS

THIS THESIS INCLUDES SYSTEMATIC REVIEW OF THE CURRENT LITERATURE AND A RETROSPECTIVE COHORT STUDY ON ASSOCIATION BETWEEN FIRST TRIMESTER MATERNAL SERUM PAPP-A AND ADVERSE PREGNANCY OUTCOMES.

Abstract

Adverse pregnancy outcomes such as preterm delivery, hypertensive disorders of pregnancy, small for age neonates and stillbirths are major determinants of perinatal mortality and morbidity. As the pathology of these complications of late pregnancy may be established in the first half of pregnancy, it seems reasonable to focus on this period to identify women at increased risk of poor outcomes.

This thesis focuses on one of the placenta-derived serum markers, pregnancy associated plasma protein -A (PAPP-A). It is a well-established biomarker for Down's syndrome screening. The main purpose of this thesis is to conduct a systematic review of the literature regarding the association between first trimester serum PAPP-A levels and adverse pregnancy outcomes and to investigate the same in a retrospective cohort study in a tertiary hospital.

The systematic review included 32 studies involving a total of 175,240 pregnancies. It showed that low maternal serum PAPP-A in the first trimester has an association with adverse pregnancy outcome but predictive values are poor. The retrospective cohort study of 12,592 pregnancies identified statistically significant lower odds of SGA, PTD, PE and stillbirth as PAPP-A increases. There was no statistically significant association between miscarriage, perinatal or neonatal death with PAPP-A.

Further work should address PAPP-A in combination with other predictors as a prediction model to foresee adverse pregnancy outcomes.

EXECUTIVE SUMMARY

Introduction

With the increasing understanding of the natural history of diseases down to the molecular level, the focus of medicine is gradually shifting from treatment of the disease to the prevention of the disease process before it can cause any adverse effects. There is emerging evidence that prematurity hinders normal developmental maturation of the multiple organ systems causing long term dysfunctions like cardiovascular disorders, lung disorders and mental health problems (1). Small for gestation age neonate is at higher risk of neurodevelopmental delays (2). Hypertensive disorders of pregnancy is a leading cause of maternal and fetal mortality and morbidity (3). Hence in obstetrics, this means predicting above mentioned adverse pregnancy outcomes (preterm delivery, hypertensive disorders of pregnancy, small for gestational age neonates and stillbirths) as these are major determinants of perinatal mortality and morbidity. Such prediction would enable us to target appropriate surveillance, intervention and possibly treatment at these high-risk pregnancies, hopefully before irreversible damage occurs, thereby improving the outcome.

Objectives

This thesis focuses on examining one of the placenta-derived serum markers, pregnancy associated plasma protein -A (PAPP-A) could predict adverse pregnancy outcomes mentioned above. PAPP-A is already a well-established marker for Down's

syndrome screening which is universally offered to all pregnant women in UK. It could prove to be a convenient and cost effective biomarker if it could predict adverse pregnancy outcomes along with fetal aneuploidy. It could be used in early identification of pregnancies at increased risk of subsequent poor outcomes.

This thesis also examines CRL and NT as prognostic indicators for adverse pregnancy outcomes. The clinical utilisation of CRL as an individual prognostic factor (i.e. outside of a model using it as a continuous factor) is less clear as standard care in the UK is for a single first trimester ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk.

The aim of this thesis was: 1) To perform a systematic review to assess the predictive accuracy of first trimester serum PAPP-A for adverse pregnancy outcomes measured in terms of pregnancy loss, preterm birth, intra-uterine death (IUD) and small for gestational age (SGA), and hypertensive disorders of pregnancy and 2) To perform a retrospective cohort study to assess the predictive accuracy of maternal serum PAPP-A along with CRL and NT in first trimester for adverse pregnancy outcome in the population at Birmingham Woman's Hospital NHS foundation Trust (BWNFT).

Methods

1) Systematic review

a) Search strategy

Medline, Embase and CINAHL (From inception to May 2015)

b) Selection criteria

Studies including pregnant women with PAPP-A in the first trimester and assessment of pregnancy outcome.

c) Data collection and analysis

Data were extracted on study characteristics, quality and results to construct 2x2 tables. Meta-analysis of odds ratios (OR), sensitivity, specificity and likelihood ratios and 95% confidence intervals (CI).

2) Retrospective cohort study

We investigated association using data on the serum PAPP-A along with CRL and NT and pregnancy outcome data Birmingham Women's NHS Foundation Trust from August 2011 to 31st March 2015.

Results:

1) **Systematic review**

Thirty-two studies including 175,240 pregnancies. PAPP-A <5th centile had a moderate association with: Birthweight <10th centile OR 2.08 (95% CI 1.89 – 2.29), <5th centile OR 2.83 (95% CI 2.52 – 3.18) and <3rd centile OR 2.76 (95% CI 1.78 – 4.28); pre-eclampsia OR 1.94 (95% CI 1.63 – 2.30), preterm birth <37 weeks OR 2.09 (95% CI 1.87 – 2.33), pregnancy loss ≤ 24 weeks OR 2.50 (95% CI 1.81 – 3.47) and stillbirth > 24 weeks gestation OR 2.40 (95% CI 1.45 – 3.99). For a composite adverse outcome OR 3.31 (95% CI 1.80 – 5.11). Where data was available, to look at odds of an adverse outcome with PAPP-A<1st centile this demonstrated increasing odds with decreasing PAPP-A.

2) Retrospective cohort study

12,592 pregnancies: 852 (6.8%) pre term birth (PTB), 352 (2.8%) pre-eclampsia (PE), 1824 (14.5%) Small for gestational age (SGA), 73 (0.6%) miscarriages, 37(0.3%) stillbirths, 73 perinatal deaths (0.6%) and 38 (0.30%) neonatal death (NND). For individual prognostic markers in adjusted analyses there were statistically significant lower odds of SGA [odds ratio (OR) 0.87 (95% CI 0.85, 0.90)], PTB [OR 0.92 (95%CI 0.90, 0.96)], PE [0.91 (95% CI 0.85, 0.97)] and stillbirth [OR 0.72 (95% CI 0.53, 0.99)] as PAPP-A increases. There were statistically significant lower odds of SGA [OR 0.80 (95% CI 0.71, 0.90)], but higher odds of miscarriage [OR 1.75 95% CI (1.12, 2.72)] as Nuchal Thickness (NT) increases, and statistically significant lower odds of stillbirth as crown rump length (CRL) increases [OR 0.94 95% CI (0.89, 0.99)]. Combining three first trimester potential prognostic factors there remains statistically significant associations between: a) PAPP-A, NT, CRL and SGA, b) PAPP-A and PTB, c) PAPP-A, CRL and PE, d) NT and miscarriage.

Conclusions:

Systematic review performed in our study showed that low maternal serum PAPP-A in the first trimester has an association with adverse pregnancy outcomes but predictive values are poor. The cohort study showed that low PAPP-A is a risk factor for adverse pregnancy outcomes, especially SGA. NT and CRL are potential prognostic factors. National guidelines have identified this evidence and have recommended increased surveillance for small for gestational age (4).

Perhaps an exclusive national guideline for managing pregnancies with low PAPP-A outlining surveillance for adverse outcomes like pre-eclampsia, pre term birth and SGA would be useful. Further work is needed to address PAPP-A as a continuous variable and in combination with other prognostic markers as a prediction model for adverse pregnancy outcomes.

Dedication page

This thesis is dedicated to my husband Vijay, my daughters Tanisha and Sasha, my parents and parents-in-law.

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LIST OF ABBREVIATIONS

AC	Abdominal circumference
AFP	Alpha feto-protein
APO	Adverse perinatal outcome
AUC	Area under curve
BMI	Body mass index
BNF	British National Formulary
BW	Birth weight
BWNFT	Birmingham Women's Hospital NHS Foundation Trust
CI	Confidence interval
CRL	Crown Rump Length
CT	Cytotrophoblast
DOR	Diagnostic odds ratio
DQASS	Downs syndrome screening quality assurance and support service
EFW	Estimated fetal weight
EVT	Extravillous trophoblast
FGR	Fetal growth restriction
HCG	Human chorionic gonadotrophin

HSROC	Hierarchical summary receiver operating characteristic
IUD	Intrauterine Death
IUGR	Intrauterine Growth Retardation
IPD	Individual patient data
LR	Likelihood ratio
MeSH	Medical subject heading
MoM	Multiples of the median
NA	Not applicable
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NICU	Neonatal intensive care unit
NPV	Negative predictive value
NRES	National research ethics service
OR	Odds ratio
PAPP-A	Pregnancy associated plasma protein A
PE	Pre-eclampsia
PI	Pulsatility index

PPV	Positive predictive value
QUADAS- 2	Quality assessment of diagnostic accuracy studies -2
QUOROM	Quality of reporting of meta-analyses
R&D	Research and development
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	Randomised controlled trial
RDOR	Ratio of diagnostic odds ratio
ROC	Receiver operating characteristic
RR	Relative risk
SD	Standard deviation
ST	Syncitiotrophoblast
SGA	Small for gestational age
STARD	Standards for reporting of diagnostic accuracy
UK	United Kingdom
UKNEQAS	United Kingdom National external quality assurance scheme (), Edinburgh Royal Infirmary, UK

CHAPTER 1: INTRODUCTION

Adverse pregnancy outcomes such as stillbirth, preterm birth, intrauterine death (IUD), small for gestational age neonate (SGA) and preeclampsia are the major determinants of perinatal mortality and morbidity of pregnancy and thus have a major psychological impact for the family as well as an increased cost to the health care system. However, the conditions often are not detected until the pathological processes have become too advanced to facilitate optimal management. Early identification of high-risk pregnancies would make it possible to target appropriate surveillance, intervention and possibly early treatment at these high-risk pregnancies, hopefully before irreversible damage occurs, thereby improving the outcome. At present this increased surveillance is offered in pregnancies deemed to be high risk mainly on risk factors identified in the past medical or obstetric history rather than the current pregnancy.

The use of first trimester screening including nuchal translucency (NT), pregnancy-associated plasma protein-A (PAPP-A), free-beta subunit human chorionic gonadotrophin (fβhCG) to detect aneuploidy has become an integral part of prenatal care. There have been numerous studies and reviews reporting the effectiveness of the serum PAPP-A to identify pregnancies at high risk for additional adverse perinatal outcomes (5-9). A review by Halscott et al concluded that the first trimester biomarkers do not have sufficiently high enough positive predictive values to be used for first trimester screening for the development of preeclampsia, fetal growth restriction, preterm birth or stillbirth (10). There are also studies suggesting there might be no association between serum PAPP-A and adverse pregnancy outcomes (11).

A comprehensive systematic review by Morris et al examined 44 studies including 169,637 pregnant women concluded that Down's serum screening analytes including low serum PAPP-A have been associated with pre-eclampsia and small for gestational age but the predictive accuracy was deemed to be low (12) . There are international guidelines highlighting association of PAPP-A and small for gestational age (4).

Thus, as we have become aware that complications of late pregnancy may have some relation with the first half of pregnancy, it seems reasonable to focus on this period to identify women at increased risk of poor pregnancy outcomes (2, 3).

1.1 Placenta:

The placenta is a unique and highly specialized organ of exchange in pregnancy that has an important role in the normal growth and development of the fetus. It acts to provide oxygen and nutrients to the fetus, whilst removing carbon dioxide and other waste products. It metabolizes several substances and can release metabolic products into maternal and/or fetal circulations (13-16). The placenta plays a pivotal role in protecting the fetus against certain xenobiotic molecules, infections and maternal diseases. The placenta releases hormones into both the maternal and fetal circulations to affect pregnancy, metabolism, fetal growth, parturition and other functions (13, 17).

1.2 Structure of Placenta:

The placenta is formed from two distinct genetic origins: the decidua (maternal part of placenta) that develops from the uterine tissue, and the chorion (fetal part of placenta),

which develops from the blastocyst (18). Between these two layers is a sinus called the intervillous space within the placenta that fills with maternal blood supplied by the uterine arteries. The maternal blood bathes tree-like structures called chorionic villi which originate from the chorion and serve as the interface where gas and nutrient exchange takes place between the fetal and maternal circulatory systems. Some villi span the distance of the intervillous space, serving as a place of attachment to the uterine wall and so are termed “anchoring” villi, whereas others float freely in the maternal blood and are termed “floating” villi (18, 19).

Chorionic villi are made up of several different cell types. These include the fetal endothelial cells lining the fetal blood vessels, the villous mesenchyme, the villous cytotrophoblast (CT) and the multinucleated syncytiotrophoblast (ST), which makes up the outermost cellular layer of the chorionic villi and is in direct contact with the maternal blood (10-12). The ST is maintained by differentiation and fusion of the highly proliferative CT, which makes up the layer immediately beneath the ST (6, 19, 20).

Extravillous trophoblast (EVT) is another trophoblast cell type that arises from the proliferation and differentiation of stem cells within the CT layer (10). The cells in this layer are key players in the crucial processes of normal placental development, namely EVT migration and invasion, and spiral artery remodeling. EVT cells penetrate the maternal decidua and first third of the myometrium where they invade and restructure the maternal uterine arteries (19, 21). This invasion also results in replacing the uterine vessel endothelial cells, transforming the blood vessels into spiral arteries of wider diameter which can accommodate the blood flow required to sustain pregnancy (19, 21,

22). This process occurs between 6 and 18 weeks of gestation, and is more or less complete before 20 weeks of gestation(23).

Thus, the placenta has an essential role in determining the outcome of pregnancy. Early prediction of potential placental insufficiency before the establishment of its dysfunction would prompt early preventive measures or treatment which could improve pregnancy outcomes.

CHAPTER 2: BACKGROUND

The Human placenta produces a wide variety of specific proteins, which do not occur or occur only in trace amounts in normal sera in non-pregnant state. During pregnancy, they appear in the maternal blood stream or their concentrations are strongly elevated (24). One of many such proteins is 'Pregnancy-associated plasma protein-A' (PAPP-A). It is also known as Pappalysin-1 A.

2.1 Pregnancy associated Plasma Protein A (PAPP-A)

PAPP-A was originally isolated in 1974, as one of four proteins of placental origin found in high concentrations in the blood of pregnant women (25). Soon after its discovery, PAPP-A was studied with relation to the adverse pregnancy outcomes in later pregnancies but failed to create any significant impact. A new period of PAPP-A research appeared after Brambati et al in 1991, described its decreased levels in first trimester of pregnancy with fetus affected by Down syndrome (26). Currently PAPP-A is a well-established marker used in the screening for Down's syndrome in the first trimester and there has been emerging evidence that serum PAPP-A levels early in pregnancy could predict adverse pregnancy outcomes (4, 27).

2.2 Structure of PAPP-A

PAPP-A is a glycoprotein that is encoded by the PAPP-A gene which is located on human chromosome 9q33.1(28). It is secreted as a disulphide-bound homodimer which has a molecular weight of 400,000 g/mol (29, 30).

The 1547-residue PAPP-A polypeptide (31) is secreted as a disulfide-bound dimer of 400 kDa (29). PAPP-A contains the zinc-binding motif and belongs to the metzincin superfamily of metalloproteinases which includes the astacins, the reprolysins, the serralysins, and the matrix metalloproteinases (21, 32). PAPP-A is the first member of a fifth metzincin family, the pappalysins (33). Along with a proteolytic domain, the PAPP-A subunit has three lin-notch repeats (LNR-1–3, each of 26–27 residues) and five complement control protein modules (CCP-1–5, each of 57–77 residues) who fecilitate binding of PAPP-A to heparin sulfates present on cell surfaces (31, 34).

In pregnancy serum, most of the PAPP-A (99%) (29) is covalently bound in a 2:2 complex to the 206-residue proform of eosinophil major basic protein, pro-MBP (35, 36).

During pregnancy, PAPP-A and proMBP are expressed in significant amounts in the human placenta (37). While PAPP-A is mainly expressed in the syncytiotrophoblast, ProMBP is expressed in extravillous cytotrophoblasts. ProMBP is secreted from these extravillous cytotrophoblasts without propeptide cleavage (38). Hence the PAPP- A/ proMBP complex formation occurs in the extracellular environment, possibly on the surface of the syncytiotrophoblast.

2.3 Mode of action of PAPP-A

PAPP-A enhances the bioavailability of insulin-like growth factor (IGF) locally by cleaving the inhibitors IGFBP-4 and -5 (insulin-like growth factor-binding protein-4 and -5) (39-42). IGF is mitogenic and anti apoptotic and is vital for the growth of human cells in most tissues (43, 44). It has a pivotal role in the development of the placenta and spiral artery remodelling as it stimulates CT proliferation and EVT migration in the first trimester (45).

2.3 Clinical application of PAPP-A

Detectable levels of maternal PAPP-A are demonstrated soon after the implantation and the levels of PAPP-A increase throughout pregnancy. It doubles in about 3–4 days during the first trimester and maximum levels are reached at term (46, 47).

Due to the exponential increase in PAPP-A levels during the first trimester, the interpretation of a given value would be very much dependent on the gestational age. The Common practice is to use the unit called MoM (Multiples of Median). A multiple of the median (MoM) is a measure of how far maternal PAPP-A concentration deviates from the expected normal median level for a pregnancy of the same gestational age. Hence MoM would be a gestational age independent expression of PAPP-A concentration. Along with gestational age, the following maternal and pregnancy-

associated characteristics are also known to affect the maternal serum concentration of PAPP-A: ethnicity, chronicity, assisted versus spontaneous conception, maternal pre-pregnancy weight, maternal smoking during pregnancy, parity, and history of previous pregnancy with trisomy 21, 18, or 13. MoM values would be further adjusted to the above factors as well.

Maternal serum PAPP-A is a well-established serum marker and is used in first-trimester screening programs for chromosomal abnormalities which are characterized by low maternal serum PAPP-A levels. There are no pathophysiologic explanations available for these low PAPP-A values. The performance of PAPP-A and free beta-hCG depends on gestational age in these screening programs.

Studies have tested the hypothesis that low maternal serum levels of PAPP-A in the first trimester can predict adverse pregnancy outcomes associated with poor placental function (6, 48-51). International Guidelines on "*The Investigation and Management of the Small for Gestational Age Fetus*" have recommended that pregnant women with a serum PAPP-A <0.4MoM (5^{th} centile) in the first trimester receive increased ultrasound surveillance for fetal growth disorders (4) . However, contradictory results have been observed in other publications (51, 52). A comprehensive systematic review concluded PAPP A has low accuracy in predicting small for gestation age fetus but could be a useful adjuvant when combined with other tests (12). Few studies have investigated the

association of first trimester fetal biometry [nuchal translucency (NT) and crown rump length (CRL)] with adverse outcomes, and their relationship with PAPP-A (5, 52).

The clinical applications of biomarkers including maternal PAPP-A is rapidly expanding field and there are variations in the research designs and inconsistencies in the outcomes assessed, hence, there is a lack of clear collated up-to-date summaries of the existing literature. There is still uncertainty about the best prediction and management strategies. A systematic review combined with a contemporary comprehensive study of a large population at a tertiary care hospital will improve our understanding in the relation between PAPP-A levels and pregnancy outcome. As measuring PAPP-A levels is part of first trimester screening of Down's syndrome, there would no extra cost in using it as a predictor of the pregnancy outcome if a correlation is established. This would provide an effective means when combined with interventions/surveillance to reduce adverse pregnancy outcome.

Chapter 3: Research questions addressed by this thesis

This thesis will aim to perform:

1. **A systematic review to assess the predictive accuracy of first trimester serum PAPP-A for** SGA and other adverse pregnancy outcomes measured in terms of pregnancy loss, preterm birth, and intra-uterine death (IUD) and hypertensive disorders of pregnancy.

   The aim of the review is to determine the predictive accuracy of first trimester serum PAPP-A for adverse pregnancy outcomes mentioned above.
   Thus:

   - *Population:* Pregnant women any health care setting, any level of risk.
   - *Tests:* Serum pregnancy associated plasma protein A measured in the first trimester (<14 weeks of pregnancy).
   - *Reference standard/outcome:* Birth weight, birth weight centile (population or customized), maternal (pre-eclampsia, pregnancy induced hypertension, gestational diabetes, abruption) and pregnancy outcomes (miscarriage, stillbirth, preterm delivery).
   - *Study design:* Observational test accuracy studies (cohorts, case-control prospective) allowing generation of 2x2 tables of accuracy. Case series <10

cases and case-control studies defined by reference standard outcome were excluded, these study designs have been shown to be associated with bias (49).

2. **Retrospective cohort study** to examine the association and to assess the predictive accuracy of maternal serum PAPP-A along with CRL and NT in first trimester for adverse pregnancy outcome in the population at BWNFT.

   Population: Pregnant women delivering at the Birmingham Women's Hospital NHS Foundation Trust (BWNFT) between 1st September 2011 and 31st March 2015.
   Setting: An NHS Foundation Trust in the West Midlands, which is a secondary and tertiary care hospital serving the region of West Midlands in the UK.
   Study design: A retrospective analysis of independent databases for PAPP-A in the first trimester (Down's syndrome screening), first trimester dating ultrasound and second trimester (CRIS) pregnancy outcome database (K2) and Genetics database.
   Outcomes: small for gestational age (birth weight <10th customized centile), and other adverse pregnancy outcomes e.g. stillbirth, infant death, preterm birth, and pre-eclampsia.

   Analysis: Unadjusted logistic regression analysis would be used to estimate the odds ratio (OR) for each binary outcome for two patients who differ by one unit of the potential prognostic factor (PAPP-A/NT/CRL). The three potential prognostic factors would be assessed in separate logistic regression models. Multivariable logistic regression analyses will be additionally adjusted for known prognostic factors and potential confounders. In the fully adjusted analyses, the linearity

assumption of the prognostic effects will be assessed and logistic regression analysis to develop prediction model.

CHAPTER 4: ASSOCIATION OF SERUM PAPP-A LEVELS IN FIRST TRIMESTER WITH SMALL FOR GESTATIONAL AGE NEONATE AND OTHER ADVERSE PREGNANCY OUTCOMES: SYSTEMATIC REVIEW AND META-ANALYSIS.

4.1 Introduction

Adverse pregnancy outcomes [stillbirth, preterm birth (PTB), small for gestational age (SGA), and hypertensive disorders of pregnancy] have a major psychological impact for the family as well as an increased cost for the healthcare system. Accurate methods of predicting these outcomes would allow health professionals to provide increased surveillance and offer optimum management, which could possibly improve the outcome of the pregnancy.

Pregnancy associated plasma protein A (PAPP-A) is a placental glycoprotein produced by syncytial trophoblast, which cleaves insulin-like growth factor binding protein 4 (IGFBP4) and is a positive regulator of insulin-like growth factors (IGFs) (53). Biochemical measurement of placental derived factors has been suggested as a means to improve fetal and maternal outcome of pregnancy. Previous studies have tested the hypothesis that low maternal serum levels of PAPP-A in the first trimester can predict adverse pregnancy outcomes associated with poor placental function (5, 6,

49-51). The recently published Royal College of Obstetricians and Gynaecologists (RCOG) Green top Guidelines assessed all the available evidence prior to their publication in 2013 and recommended that in women with a serum PAPP-A <0.415 multiples of the median (MoM) (5th centile) in the first trimester receive increased ultrasound surveillance for growth disorders (4). In 2010 first trimester combined screening was routinely introduced in the United Kingdom as the recommended screening for Down's syndrome (27). This test involves assay of PAPP-A between 10 and 13+6 weeks. Since this time, there has been a substantial increase in the number of published articles related to this placental analyte and thus a need to systematically review this evidence.

When assessing a biomarker, it is important to assess whether there is any prognostic association between the "analyte" and outcomes of interest before considering the predictive ability of the biomarker to predict the outcome of interest in an individual (54). The aim of this systematic review and meta-analysis is to improve our understanding of the association between first trimester maternal serum PAPP-A levels and pregnancy outcomes and where appropriate to evaluate the predictive ability for adverse pregnancy outcome.

4.2 Methods

A protocol driven systematic review was performed in accordance with published guidelines (55-59). The reporting of the review meets the criteria specified in the PRISMA guidance (59). The prisma checklist is provided in appendix 3.

4.2.1 Framing the question

A clearly defined research question is an essential element for the systematic review. This ensures that the review is correctly designed and that the question is fully answered. It has four key components: the population under study, the test or intervention, the reference standard or comparator and the type of study designs to be included. The questions posed in this systematic review are as follows:

- *Population:* Pregnant women any health care setting, any level of risk.
- *Tests:* Serum pregnancy associated plasma protein A measured in the first trimester (<14 weeks of pregnancy)
- *Reference standard/outcome:* Birth weight, birth weight centile (population or customized), maternal (pre-eclampsia, pregnancy induced hypertension, gestational diabetes, abruption) and pregnancy outcomes (miscarriage, stillbirth, preterm delivery).
- *Study design:* Observational test accuracy studies (cohorts, case-control prospective) allowing generation of 2x2 tables of accuracy. Case series <10 cases and case-control studies defined by reference standard outcome were excluded, these study designs have been shown to be associated with bias (60)

4.3 Identification of the literature

The search strategies used relevant medical subheadings (MeSH), text words and word variants for PAPP-A and each adverse pregnancy outcomes - small for gestational age, preterm birth, pre-eclampsia, stillbirth and gestational diabetes. The databases were searched from inception to March 2015.

4.3.1 Sources

A literature search was performed in electronic databases from inception till March 2015. No language restrictions were applied. We searched Embase, MEDLINE, CINAHL (current nursing and allied health literature) and Web of Science (grey literature) using combinations of relevant medical subject heading (MeSH) terms, keywords and word variants.

4.3.2 Definitions of adverse pregnancy outcomes:

1. Preeclampsia, defined as per ISSHHP guidelines as de-novo hypertension at or after 20 weeks' gestation (at least 2 readings of Blood Pressure >140 mmHg systolic or >90 mmHg diastolic) with proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L ['2 +'] on dipstick testing) (57). persistently elevated systolic (> 140 mmHg) or diastolic (> 90 mmHg) blood pressure and proteinuria (> 0.3 g/24h or spot urine protein to creatinine ratio ≥ 30 mg/ mmol) arising after the 20th week of pregnancy (61).

2. Small for gestational age: Defined as birth weight below the 10th, 5th, or 3rd percentile for gestational age (62).

3. Stillbirth: Fetal demise at or after 24 completed weeks

4. Preterm birth: Birth before 37 completed weeks of gestation.

5. Gestational Diabetes: Diabetes in pregnancy diagnosis was made on the basis of world health organisation recommendations i.e. one or more of the following criteria were met: Fasting plasma glucose ≥ 7.0 mmol/l (126 mg/ dl) or 2-hour plasma glucose ≥ 11.1 mmol/l (200 mg/dl) following a 75g oral glucose load or random plasma glucose ≥ 11.1 mmol/l (200 mg/ dl) (63).

The database search performed used terms for PAPP-A/pregnancy associated plasma protein A and combined with the following adverse pregnancy outcomes: miscarriage, SGA, PET, pre term birth, gestational diabetes. PAPP-A/ pregnancy associated plasma protein A was combined with each of the adverse pregnancy outcomes separately (along with their synonyms) using the AND operator. (The individual search strategies for adverse outcome are outlined in detail in appendix 1).

The reference lists of all included primary and review articles were examined to identify articles not captured by electronic searches. We also hand-searched selected specialist journals.

A comprehensive database collating all citations was constructed using Endnote 7 (Thomson Reuters) (64).

4.3.3 Study selection and data extraction

The extraction of a study's findings was conducted using a pre-designed extraction form (Appendix 2). Once the paper was selected after the first screening, further data from the selected papers were recorded on an Excel spreadsheet. Each selected paper was evaluated for its quality using two quality assessment tools for diagnostic accuracy studies namely QUADAS -2 (Quality assessment of diagnostic accuracy studies -2) STARD (Standards for reporting of diagnostic accuracy) and statistical data.

The titles and abstracts of the citations were scrutinized by three independent reviewers (Dr Rachel Katherine Morris (RKM), Dr Ashwini Bilagi (AB) and Pooja Devani (PD) partly in duplicate). No language restrictions were applied to the study. The reference lists of selected studies and review articles were checked and additional relevant articles were obtained. All foreign language papers were translated (see acknowledgements). Copies of full manuscripts of the citations that were likely to meet the selection criteria were obtained. The studies which met predefined and explicit criteria regarding populations, tests, reference standards and study design were selected. Data were extracted on study characteristics, quality assessment criteria and results for 2x2 tables (true positive, false positive, false negative, true negative) comparing the same threshold of PAPP-A with an individual outcome and entered in to an Excel spread sheet in duplicate by three reviewers (AB, RKM and PD). When disagreements occurred, the reviewers met and if a consensus could not be reached the opinion of a fourth reviewer (Professor Mark Kilby) was sought. In the case of duplicate publications, the most recent or up to date manuscript was selected.

4.3.4 Study Quality assessment

All studies meeting the pre-defined selection criteria were assessed for methodological and reporting quality, defined as confidence that the study design, conduct, analysis and reporting minimized any bias in the estimation of the association. Quality assessment was based on published guidelines for reporting of diagnostic accuracy studies (STARD) and methodological quality (QUADAS-2) (65-68). The details of guidelines are reported in appendix 3 and Appendix 4. The methodological quality items were adopted for the review question and two authors independently judged each quality item. In case of discrepancies, consensus was reached by discussion.

In the assessment of study quality, assessments were made in the domains of patient selection, index test, reference standard and flow and timing, assessing risk of bias and applicability as per QUADAS-2 (67). For the population, consecutive or random recruitment of pregnant women was ideal. Prospective recruitment was considered to introduce less bias than retrospective recruitment. The description of the population was considered ideal if there was sufficient information about the pregnant women given to assign a level of obstetric risk, and ideally this risk level was stated by the authors in the study's methods.

The quality of performance and reporting of the index standard (PAPP-A) was assessed considering the processes reported for storage of the maternal serum sample if needed and the immunoassay analyser used in the lab to quantify the levels of serum PAPP A. For the reference standard, any outcome relating to maternal, pregnancy or neonatal

outcome was considered and information collected on method of determination of reference standard, execution and blinding.

Ideal study designs were trials or cohort studies, case-control studies were only included when cases were not determined by references standard/outcome as it has been shown that this type of study design can affect accuracy (60). Verification bias was assessed using a flow chart for each study which documented the number of eligible women for the study, the number of women subjected to the index test, the number of women receiving the reference standard and the number of exclusions, withdrawals and uninterpretable results. Ideal verification was when all women could be accounted for and the number of eligible women progressing to the reference standard was >90%.

The assessment of quality is represented by a bar chart. No attempt was made to apply a quality score as this has been shown to have little validity and quality was not used as an aspect for inclusion/exclusion of studies from meta-analysis; instead an individual assessment was made and this was used to inform investigations into heterogeneity in results and sub-group analysis where appropriate (69).

4.3.5: Data synthesis and analysis

From the 2x2 tables the following were calculated with 95% confidence intervals (CI) for individual studies: odds ratio (OR), sensitivity, specificity and the likelihood ratios (LR). Results were pooled among groups of studies with similar characteristics, the

same threshold for the index test and same reference standard definition and threshold. Studies also reported a composite adverse pregnancy outcome, these studies were included in a meta-analysis if it could be ensured that individuals were only counted once and that the individual outcomes of the composite were all a similar magnitude and direction of effect across the studies (70). The OR was selected as the summary statistic, as it represents the effect of the exposure on the odds in an unbiased fashion and enables the results of both case-control and cohort studies to be included and provides a measure of the test's prognostic ability (71).

Data were first displayed as forest plots of the OR and 95% CI to allow a visual inspection for heterogeneity. Statistical heterogeneity was assessed using the I^2 statistic where I^2>50% is significant (72). Random effects meta-analysis was used throughout in anticipation of significant clinical and statistical heterogeneity. Where there were zero cells within a table a value of 0.5 was added to allow the calculation of log ORs and their variances for meta-analysis (73).

To explore for the presence of funnel plot asymmetry (small study effects), and thus potential publication bias, the Peters test was performed in each meta-analysis(74). Where there was a moderate statistically significant association between PAPP-A and an outcome measure (defined as OR>2 and 95% CI >1) then sensitivity, specificity and likelihood ratios were considered, using data from the 2x2 tables. Predictive summary measures were synthesized using the bivariate random effects prediction model where there were at least 4 studies in the meta-analysis and univariate meta-analysis where this was not possible (75). These measures assess the predictive ability of the test i.e. whether the test can accurately discriminate between those who do and those who do

not have the adverse outcome (sensitivity and specificity) and by how much a positive or negative test result modifies the odds of a poor outcome (likelihood ratios) (56). Throughout $p<0.05$ was statistical significance.

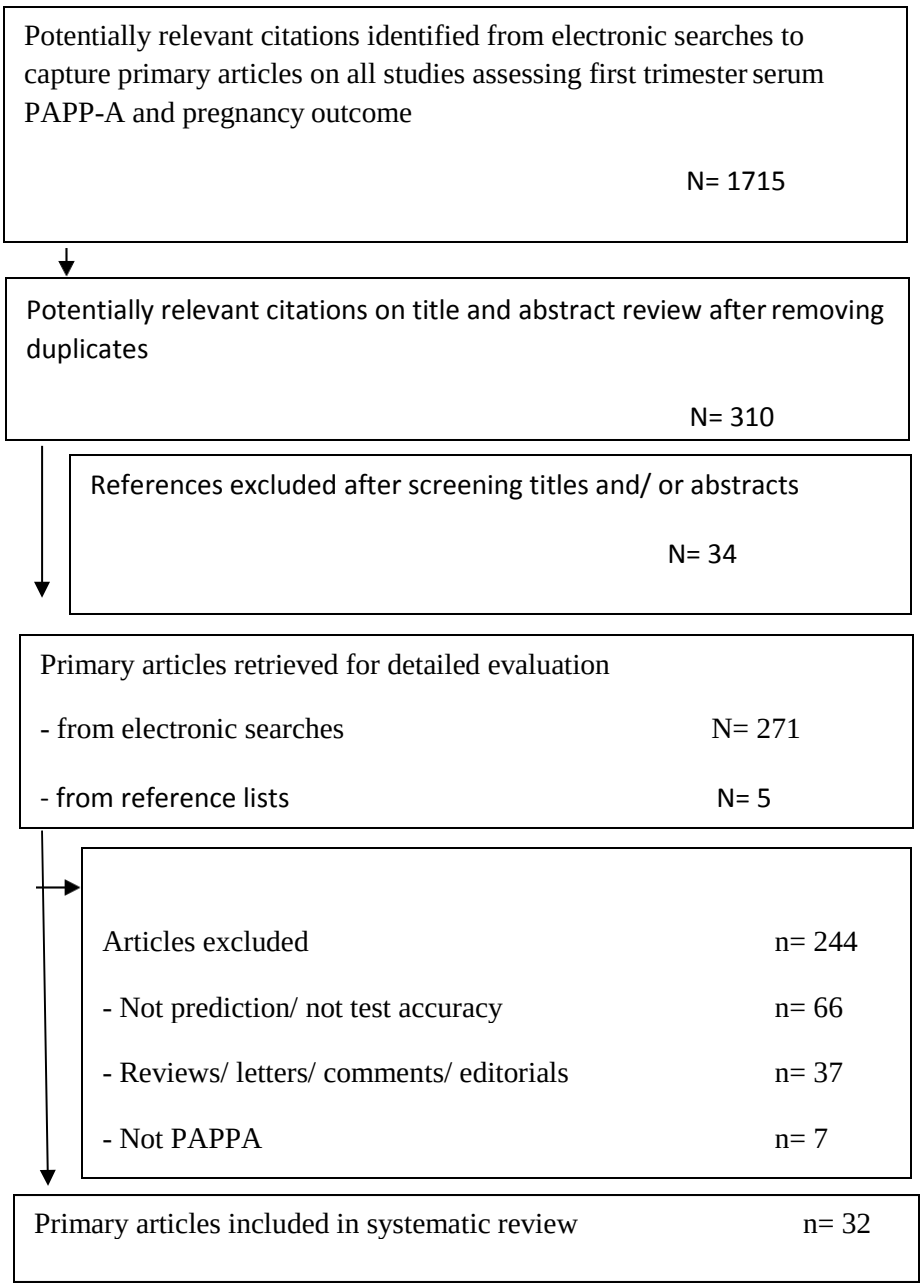
All analyses were performed in STATA 10.0 (StataCorp, College Station, TX, USA) using the metan, metandi and metabias commands (76-78). Univariate analyses were performed in Metadisc (79).

(80)

4.4: Results

Figure 1 demonstrates the study selection process with 32 studies being included reporting on 175,240 pregnancies (5, 6, 50, 52, 81-105). All studies were performed in secondary or tertiary care settings in a low risk or unselected population. All were singleton pregnancies except n=5 studies where it was not clear that multiple pregnancies were excluded. All but six studies excluded fetuses with chromosomal or structural anomalies.

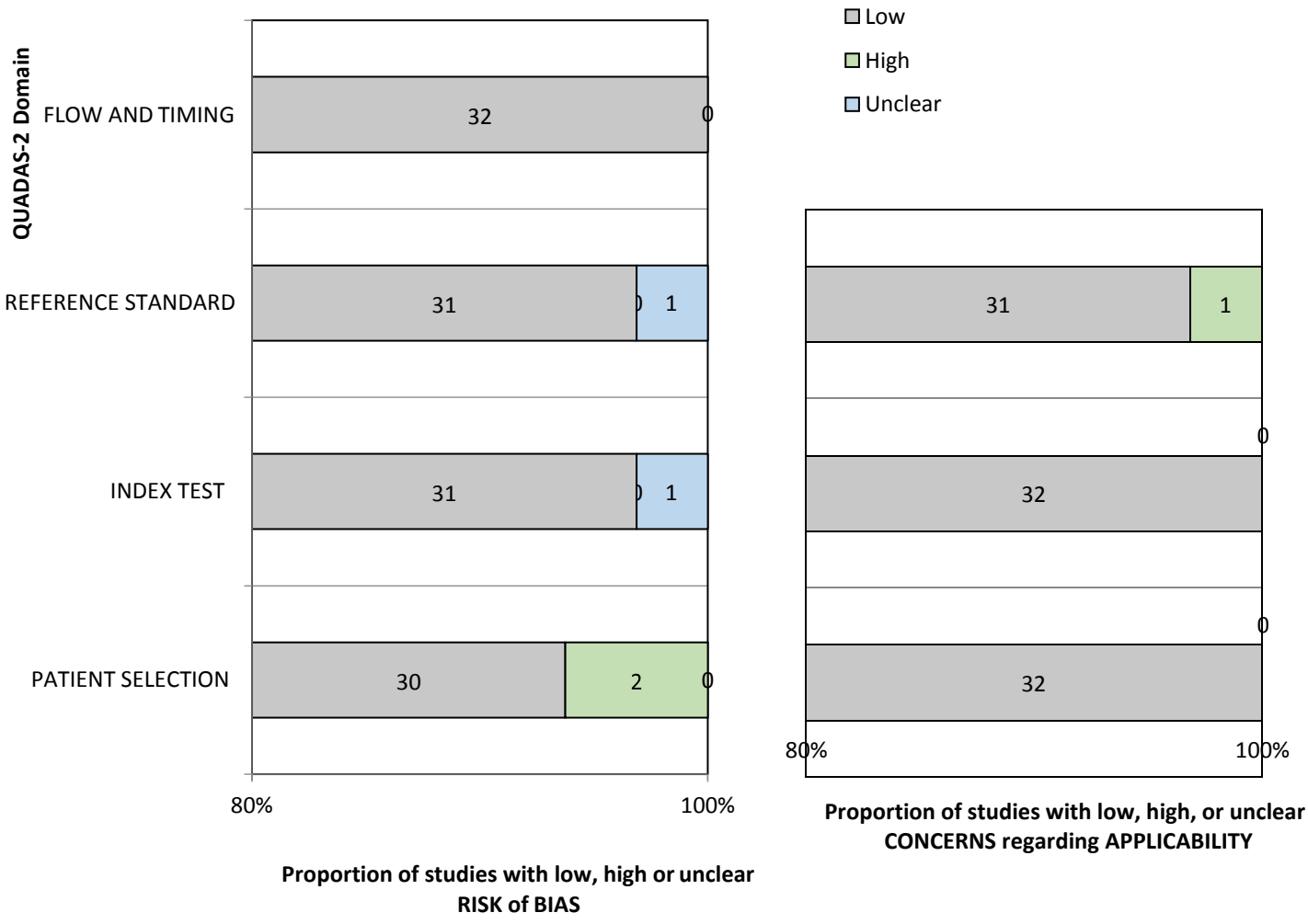
Figure 1: Study selection process for systematic review of association of prediction of first trimester serum pregnancy associated plasma protein A (PAPP-A) with adverse pregnancy outcome (inception to March 2015)



All studies were observational and non-interventional, designs were: n=23 cohort, n=5 case-control and n=4 unclear. Recruitment was prospective in n=13, retrospective in n=16 and unclear in n=3. PAPP-A was performed between 8-14 weeks and various thresholds were reported including centile cut-offs and multiples of the median (MoM). Outcomes included birth weight <10th (n=17 studies), <5th (n=15) or <3rd (n=3) centile and >90th (n=2) centile. Maternal outcomes included pre-eclampsia (n=11), pregnancy induced hypertension (n=6), preterm birth (<37 (n=22), <34 (n=2) and <32 (n=3) weeks), gestational diabetes (n=1), abruption (n=4) and pregnancy loss <24 weeks (n=4). Fetal outcomes included stillbirth >24 weeks (n=8). Six studies reported results for a composite adverse pregnancy outcome. The details of papers included in the study are listed in appendix 5. The characteristics of the included studies are described in appendix 6.

Figure 2 displays the bar charts for methodological quality. The left side bar diagram describes the risk of bias and the right side bar diagram explains the concerns regarding applicability. The left bar diagram has flow and timing, reference standard, index test and patient selection on y axis and on x axis are the number of papers. The right side bar diagram has reference standard, index test and patient selection on y axis and number of papers on the x axis. For patient selection two studies were assessed as high risk of bias (89, 102). In the other three domains (index test, reference standard and flow and timing) all studies were judged overall to have a low risk of bias. When assessing applicability, there was only one domain in which there was concern with one study having a high risk (80). The overall high quality of the included studies meant that sub-group analysis based on quality was not required.

Figure 2: Bar chart demonstrate methodological quality of included studies in systematic review of association of pregnancy associated plasma protein A with adverse pregnancy outcome assessed by QUADAS-2



4.4.1: Prognostic association

Table 1 summarizes the OR and 95% CI for all analyses. Assessing the previously recommended threshold of PAPP-A<5th centile in the first trimester (4, 106) gave increased odds of the following birth weight outcomes: Birth weight <10th centile OR 2.08 (95% CI 1.89 – 2.29), Birth weight <5th centile OR 2.83 (95% CI 2.52 – 3.18) and Birth weight <3rd centile OR 2.76 (95% CI 1.78 – 4.28). For other adverse pregnancy outcomes, the increased odds were: PET OR 1.94 (95% CI 1.63 – 2.30), PTB <37 weeks OR 2.09 (95% CI 1.87 – 2.33), pregnancy loss prior to 24 weeks OR 2.50 (95% CI 1.81– 3.47) and stillbirth after 24 weeks gestation OR 2.40 (95% CI 1.45 – 3.99).

For the composite, adverse outcome, the increased odds of an adverse outcome was OR 3.31 (95% CI 1.80 – 5.11). Where data was available to look at odds of an adverse outcome with PAPP-A<1st centile this demonstrated increasing odds with decreasing PAPP-A (Appendix 14). Three of the analyses demonstrated significant heterogeneity (Birthweight <10th, PET and PTB).

Table 1: Meta-analysis summary of studies for systematic review of association and prediction of first trimester maternal serum pregnancy associated plasma protein A (PAPPA) and adverse pregnancy outcomes.

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Birth weight												
<10th centile	17	65078										
<10th centile *	7	44316	1.88	1.72-2.05	0.16	0.14 -0.19	0.90	0.89 - 0.90	1.64	1.45 - 1.88	0.92	0.90 - 0.95
<5th centile *	12	59927	2.08	1.89-2.29	0.13	0.08 -0.2	0.94	0.90 - 0.96	1.96	1.58 -2.43	0.93	0.89 - 0.98
< 1st centile	2	39671	3.40	2.70 - 4.26	0.03	0.02 -0.04	0.99	0.99 - 0.99	3.49	2.51 - 4.89	0.98	0.98 - 0.99
<0.5MoM	3	4916	1.60	1.23 - 2.07	0.19	0.15 - 0.23	0.88	0.87 - 0.89	1.96	1.02 - 3.76	0.88	0.77 - 1.02
<0.3 MoM	2	3912	1.55	0.97 - 2.48	0.06	0.04 -0.09	0.96	0.96 - 0.97	1.93	0.72 - 5.20	0.97	0.90 - 1.04
Birth weight												
<5th centile	15	134825										
<10th centile	4	39714	2.29	2.01 - 2.60	0.20	0.18 - 0.22	0.90	0.90 - 0.90	2.17	1.64 - 2.87	0.90	0.85 - 0.94
<5th centile*	11	72245	2.83	2.52-3.18	0.22	0.10 - 0.41	0.92	0.84 - 0.96	2.65	2.35 - 2.99	0.85	0.74 - 0.98
<1st centile	2	45750	4.66	3.61 - 6.01	0.04	0.03 - 0.05	0.99	0.99 - 0.99	4.52	3.53 - 5.78	0.97	0.96 - 0.98
<0.5MoM	2	4550	2.12	1.53 - 2.95	0.25	0.19 - 0.32	0.86	0.85 - 0.87	1.99	1.23 - 3.22	0.84	0.68 -1.03
<0.3MoM	2	22464	3.13	2.30 - 4.26	0.12	0.09 -0.16	0.96	0.95 - 0.96	2.89	2.21 - 3.79	0.92	0.88 - 0.97

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Birth weight												
<3rd centile	3	8935										
<5th centile	2	8108	2.76	1.78 - 4.28	0.12	0.08 -0.18	0.95	0.95 - 0.96	2.58	1.75 - 3.79	0.93	0.88 -0.98
<0.5 MoM	2	3692	1.89	1.19 - 3.01	0.23	0.15 - 0.32	0.87	0.85 - 0.88	1.69	1.18 - 2.42	0.89	0.80 -0.99
<0.3 MoM	2	3692	2.68	1.37 - 5.27	0.10	0.05 - 0.17	0.96	0.96 - 0.97	2.53	1.37 - 4.67	0.94	0.88 -1.00
Birth weight												
>90th centile	2	35545										
<10th centile	2	35545	0.50	0.35 - 0.71	0.05	0.04 -0.08	0.90	0.90 -0.90	0.53	0.38 - 0.74	1.05	1.03 - 1.08
< 5th centile	2	35545	0.42	0.24 - 0.72	0.02	0.01 - 0.04	0.95	0.95 - 0.95	0.44	0.25 -0.75	1.03	1.02 - 1.04
Pre-eclampsia	11	71195										
< 10th centile	3	38956	1.42	1.18 - 1.72	0.14	0.12 - 0.16	0.90	0.89 - 0.90	1.55	1.06 - 2.27	0.94	0.88 - 1.01
< 5th centile*	8	132076	1.94	1.63-2.30	0.16	0.09 - 0.28	0.92	0.85 - 0.96	1.95	1.48 - 2.56	0.91	0.86 - 0.97
< 1st centile	2	45750	2.27	1.43 - 3.62	0.02	0.01 - 0.04	0.99	0.99 - 0.99	4.91	0.60 - 40.19	0.95	0.83 - 1.08

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Pregnancy induced hypertension	6	8562										
< 10th centile	2	5561	2.83	1.71 - 4.68	0.24	0.15 - 0.34	0.90	0.19 - 0.91	2.47	1.68 - 3.63	0.91	0.73 - 1.13
< 0.5 MoM	2	2124	5.07	2.78 - 9.27	0.47	0.31 - 0.62	0.86	0.84 - 0.87	2.80	0.25 - 31.57	0.43	0.03 -7.48
< 0.4 MoM	2	877	2.68	1.40 - 5.10	0.18	0.1 - 0.28	0.92	0.90 - 0.94	2.31	1.37 - 3.90	0.91	0.83 - 1.00
Pre-term birth												
<37 weeks	22	107324										
< 10th centile	3	38956	1.52	1.35 - 1.71	0.15	0.13 - 0.16	0.90	0.89 - 0.90	1.45	1.31 - 1.60	0.95	0.93 - 0.97
< 5th centile*	7	66133	2.09	1.87-2.33	0.16	0.09 - 0.29	0.91	0.83 - 0.96	1.84	1.41 - 2.39	0.92	0.87 - 0.98
< 1st centile	2	45750	3.63	2.89 - 4.55	0.03	0.03 - 0.04	0.99	0.99 - 0.99	4.28	1.50-12.25	0.97	0.94 - 1.00
< 0.6 MoM	2	4938	1.69	1.36 - 2.11	0.32	0.27 - 0.37	0.78	0.77 - 0.80	1.48	1.21 - 1.80	0.87	0.81 - 0.94
< 0.5 MoM	3	2946	3.02	2.16 - 4.22	0.30	0.23 - 0.37	0.87	0.86 - 0.88	2.31	0.62 - 8.55	0.75	0.52 - 1.09
< 0.4 MoM	3	12231	1.94	1.50 - 2.49	0.10	0.08 - 0.12	0.95	0.95 - 0.95	1.85	1.48 - 2.32	0.95	0.90 - 1.00
< 0.3 MoM	3	13060	2.11	1.50 - 2.95	0.05	0.04 - 0.07	0.98	0.98 - 0.98	1.86	0.95 - 3.64	0.98	0.96 - 1.00

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Pre-term birth												
<34 weeks	2	13012										
< 5th centile	2	13012	2.51	1.48 - 4.25	0.17	0.13 - 0.21	0.90	0.90 - 0.90	1.69	1.31 -2.16	0.93	0.88 - 0.97
< 1st centile	1	7769	2.37	0.57 - 9.81	0.02	0.02 - 0.07	0.99	0.99 - 0.99	2.34	0.58 - 9.41	0.99	0.96 - 1.02
Pre-term birth												
<32 weeks	3	42690										
<10th centile	2	35623	1.82	1.35 - 2.45	0.17	0.13 - 0.21	0.90	0.90 - 0.90	1.69	1.31 - 2.16	0.93	0.88 - 0.97
< 5th centile	3	42690	2.25	1.60 - 3.17	0.12	0.09 - 0.16	0.95	0.94 - 0.95	1.99	1.49 - 2.65	0.94	0.91 - 0.98
< 1st centile	1	33395	3.26	1.60 - 6.65	0.03	0.01 - 0.06	0.99	0.99 - 0.99	3.19	1.6 - 6.36	0.98	0.96 - 1.0

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Stillbirth >24 weeks	8	47916										
< 10th centile	2	33593	1.84	1.08 - 3.12	0.17	0.10 - 0.26	0.90	0.90 - 0.90	4.74	0.43 - 52.33	0.85	0.43 - 1.70
< 5th centile*	5	44575	2.40	1.45-3.99	0.18	0.08 - 0.36	0.88	0.80 - 0.94	1.58	0.67 - 3.71	0.92	0.78 - 1.09
<1st centile	1	33395	3.04	0.96 - 9.63	0.03	0.01-0.09	0.99	0.99 - 0.99	2.97	0.97 - 9.09	0.98	0.94 - 1.01
< 0.5 MoM	2	2119	5.74	0.81 - 40.70	0.50	0.01 - 0.99	0.85	0.84 - 0.87	4.10	1.22 - 13.70	0.71	0.22 - 2.26
Pregnancy loss ≤24 weeks	4	49986										
< 10th centile	2	38692	2.12	1.62 - 2.77	0.19	0.15 - 0.24	0.90	0.90 -0.90	1.91	1.53 - 3.37	0.90	0.85 - 0.95
< 5th centile	2	38692	2.50	1.81 - 3.47	0.12	0.09 - 0.16	0.95	0.95 - 0.95	2.25	1.47 - 3.46	0.94	0.99 - 1.00
<1st centile	1	33395	5.48	3.28 - 9.17	0.05	0.03 - 0.09	0.99	0.99 - 0.99	5.24	3.21 - 8.53	0.96	0.93 - 0.98
Gestational diabetes	1	5243										
< 5th centile	1	5243	4.17	2.00 - 8.69	0.18	0.09- 0.32	0.95	0.94 - 0.96	3.59	1.97 - 6.55	0.86	0.75 - 0.98

Pregnancy outcome/ PAPPA threshold	Number of included studies	Number included in analyses	Odds ratio	95% Confidence interval	Sensitivity	95% Confidence interval	Specificity	95% Confidence Interval	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval
Abruption	4	6368										
< 5th centile	2	2565	2.73	0.81 - 9.23	0.31	0.09 - 0.61	0.82	0.8 - 0.83	2.74	0.62 - 12.17	0.80	0.56 - 1.15
Composite adverse outcome	6	15930										
< 10th centile	2	1076	4.50	2.55 - 7.95	0.29	0.18 - 0.41	0.92	0.9 - 0.93	3.48	2.28 - 5.32	0.78	0.67 - 0.91
< 5th centile	3	13431	3.31	2.76 - 3.97	0.12	0.1 - 0.14	0.96	0.96 - 0.96	3.05	2.59 - 3.59	0.92	0.9 - 0.93
< 0.4 MoM	2	877	3.03	1.80 - 5.11	0.17	0.12 - 0.24	0.93	0.91 - 0.95	2.60	1.69 - 4.0	0.89	0.77 - 1.02

PAPPA - pregnancy associated plasma protein A

MoM multiples of median

* bivariate meta-analysis

Forest plots for the main analyses are shown in Figures 3 to 8. Inspection of the forest plots and table of characteristics could demonstrate no obvious cause for the demonstrated significant heterogeneity (Birthweight <10th, PET and PTB). Peter's test revealed no significant evidence of small study effect across all analyses (range p=0.39 – p=0.67).

Figure 3: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <10th centile with birth weight <10th centile

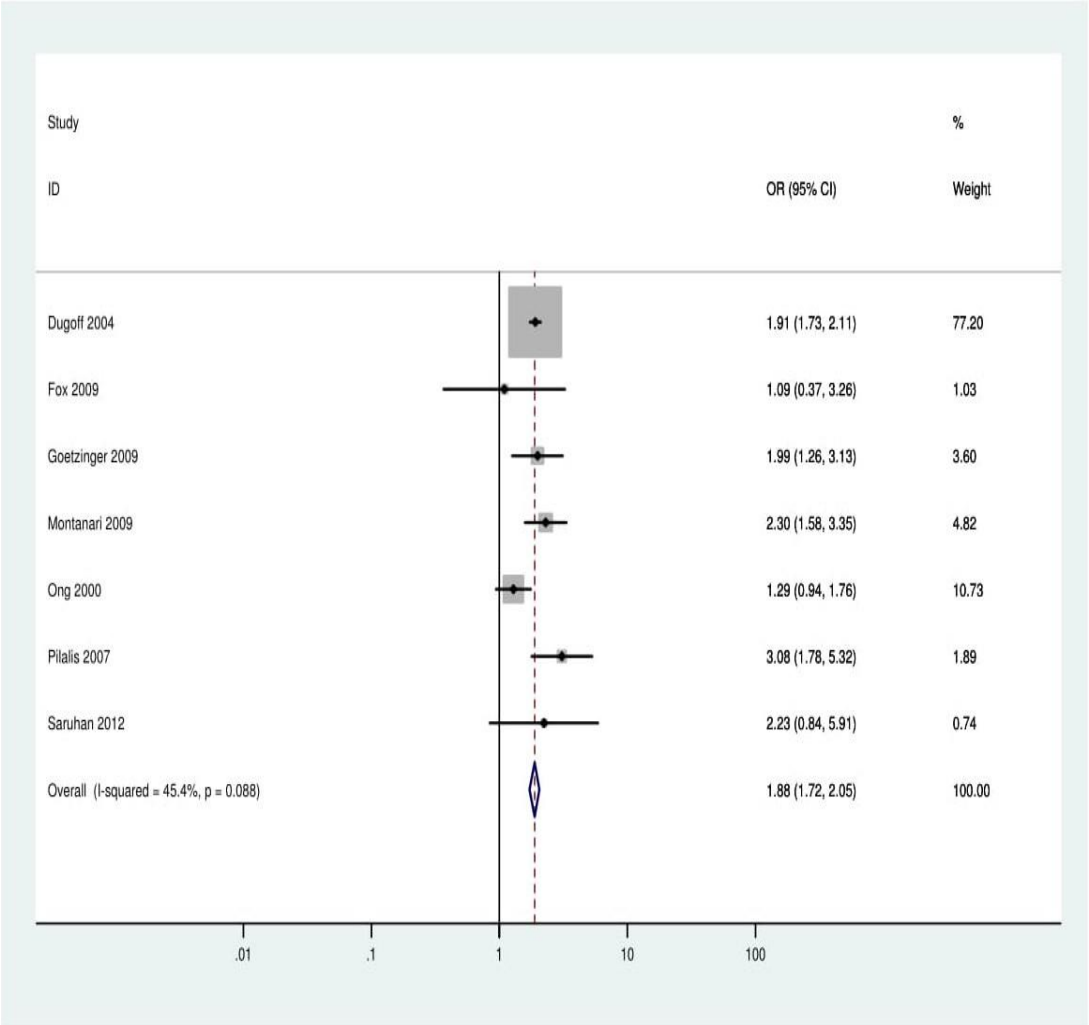


Figure 4: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <5th centile with birth weight <10th centile.

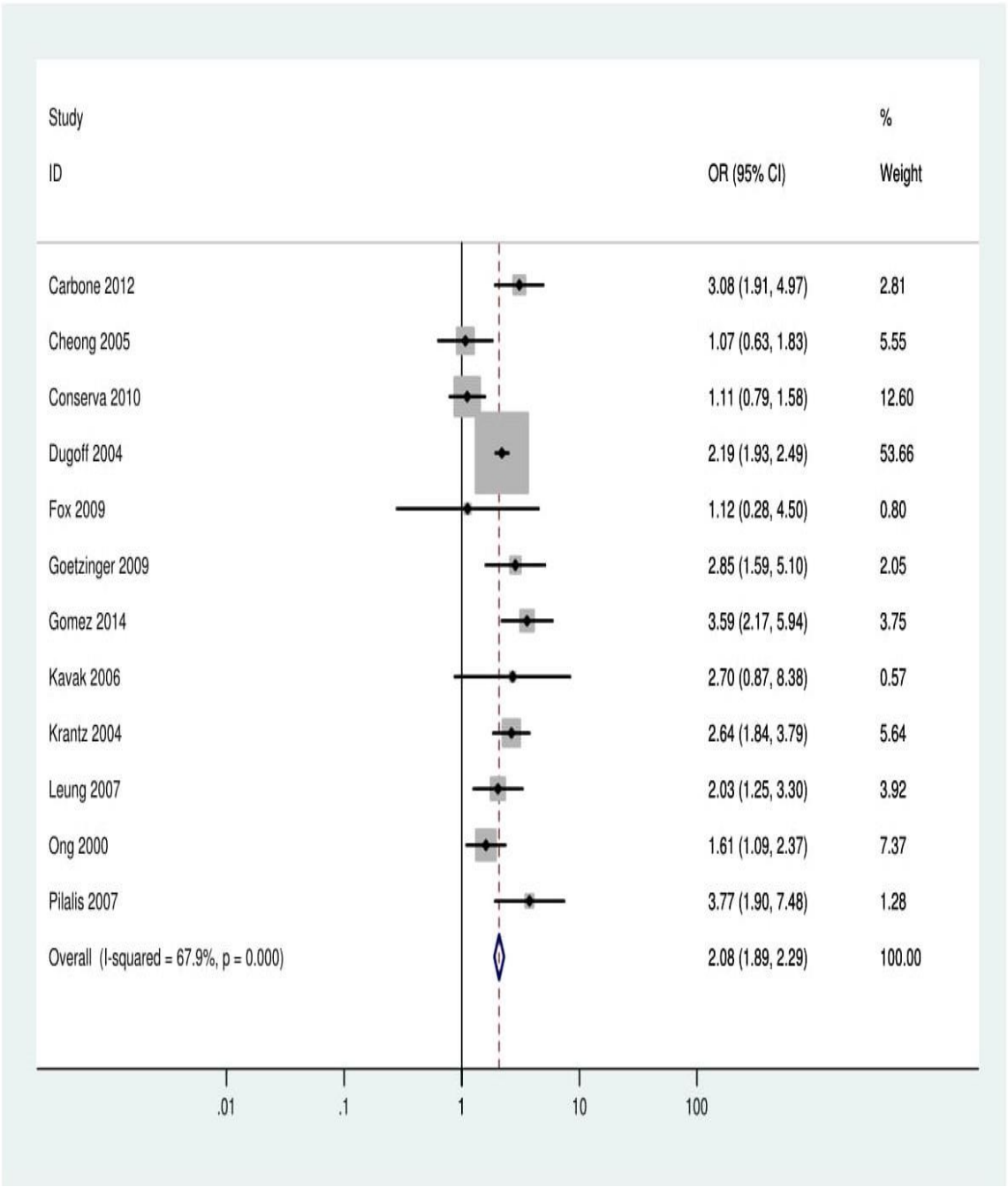


Figure 5: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <5th centile with birth weight <5th centile.

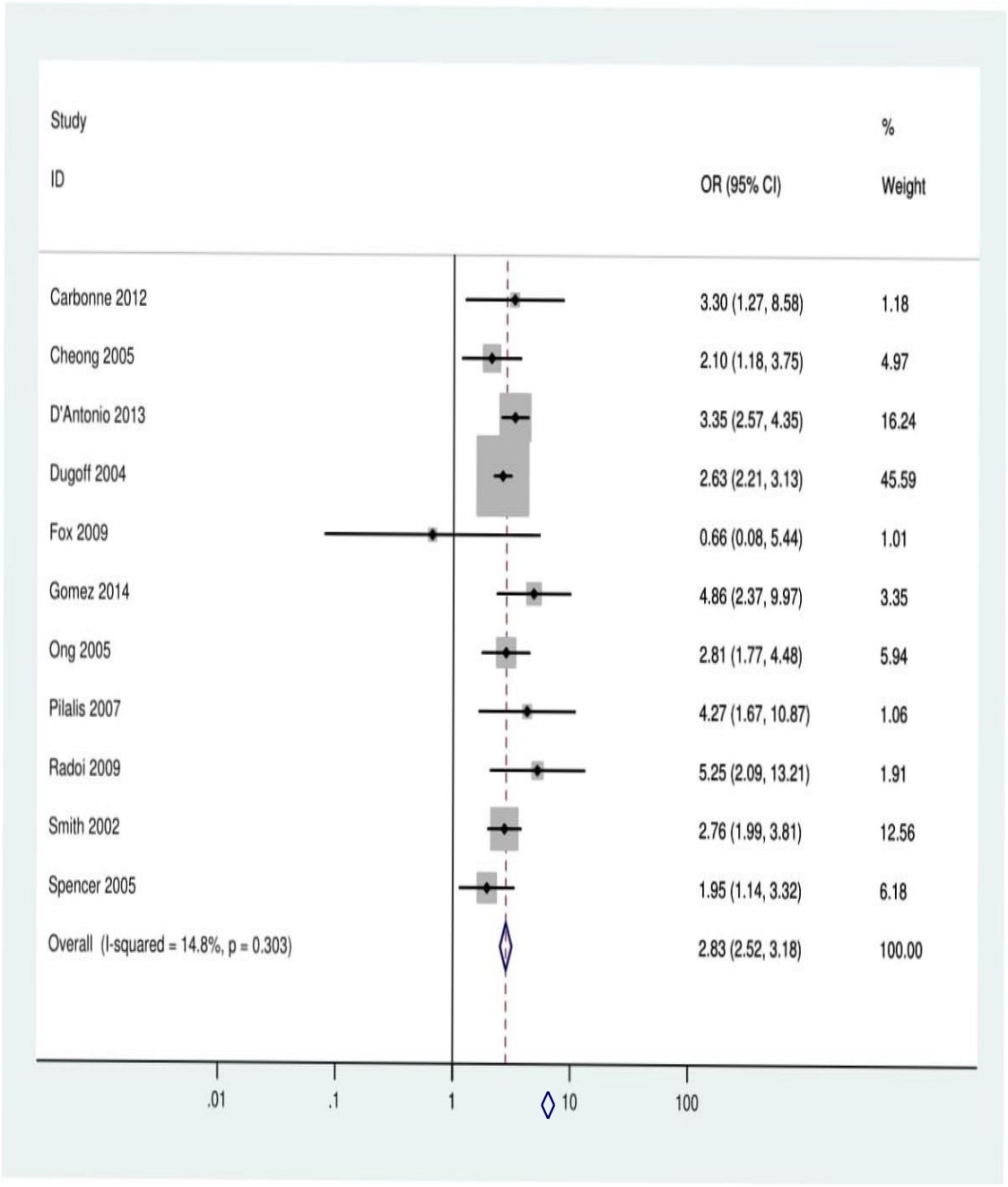


Figure 6: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <5th centile with pre-eclampsia

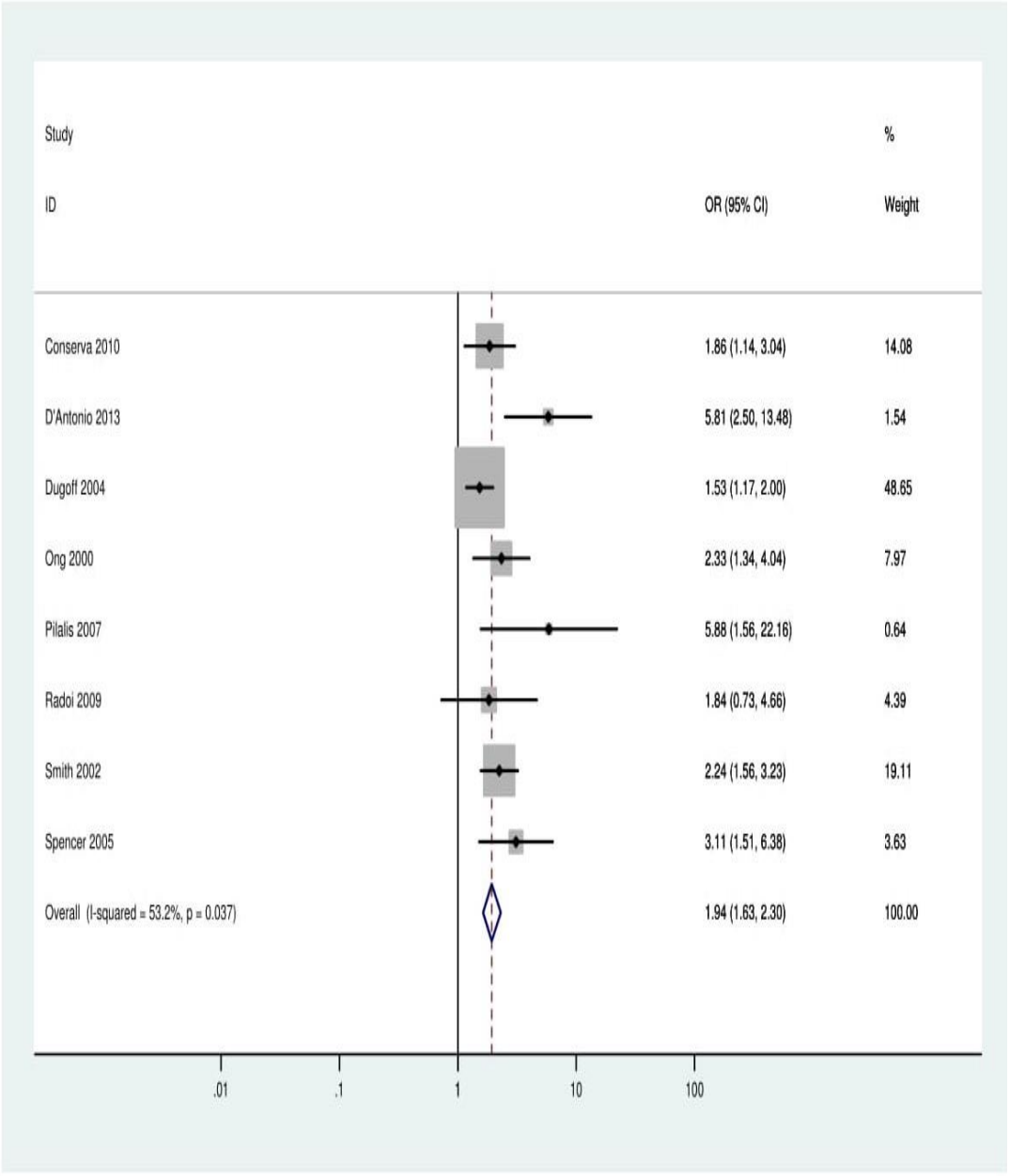


Figure 7: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <5th centile with pre-term birth <37 weeks

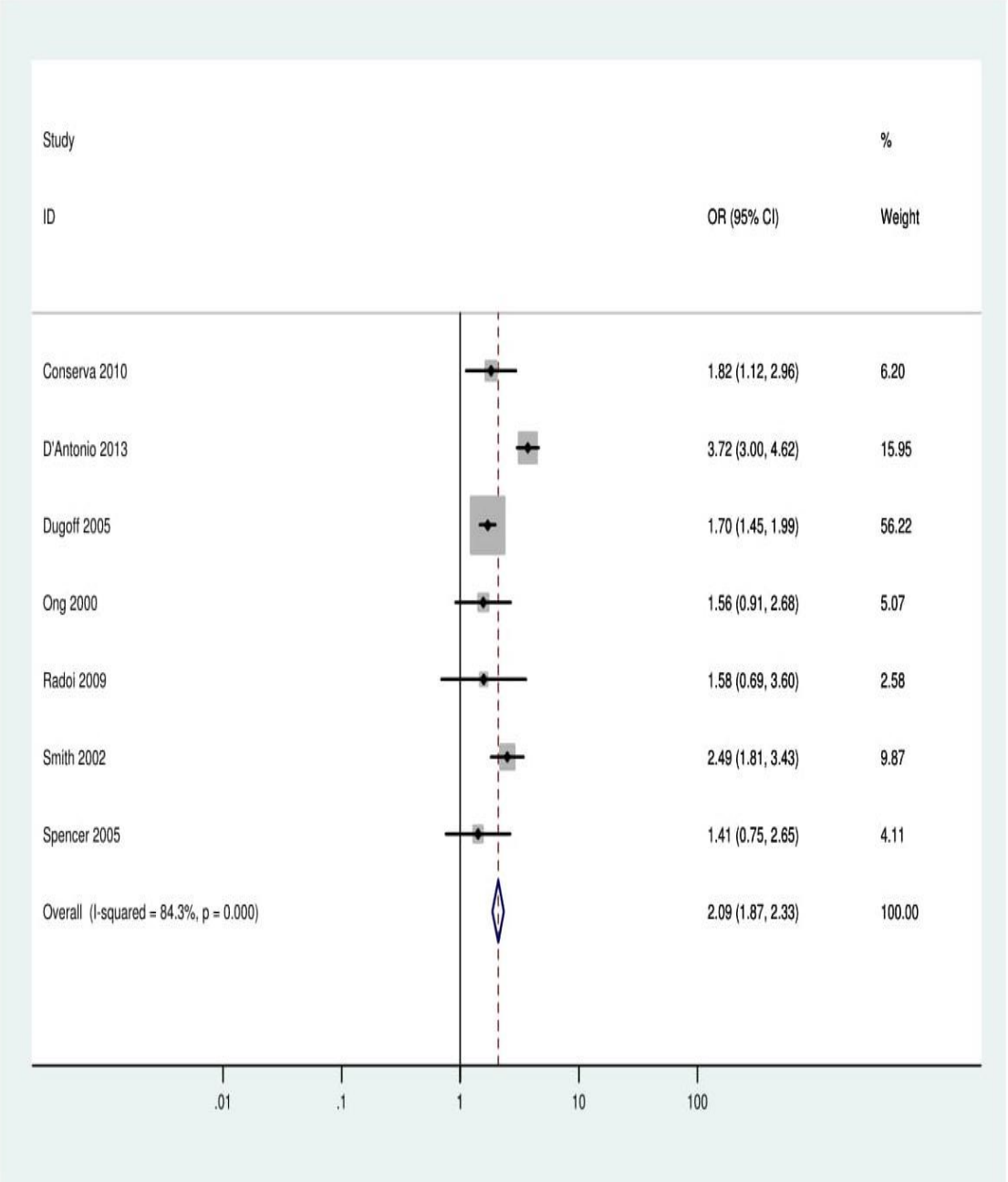
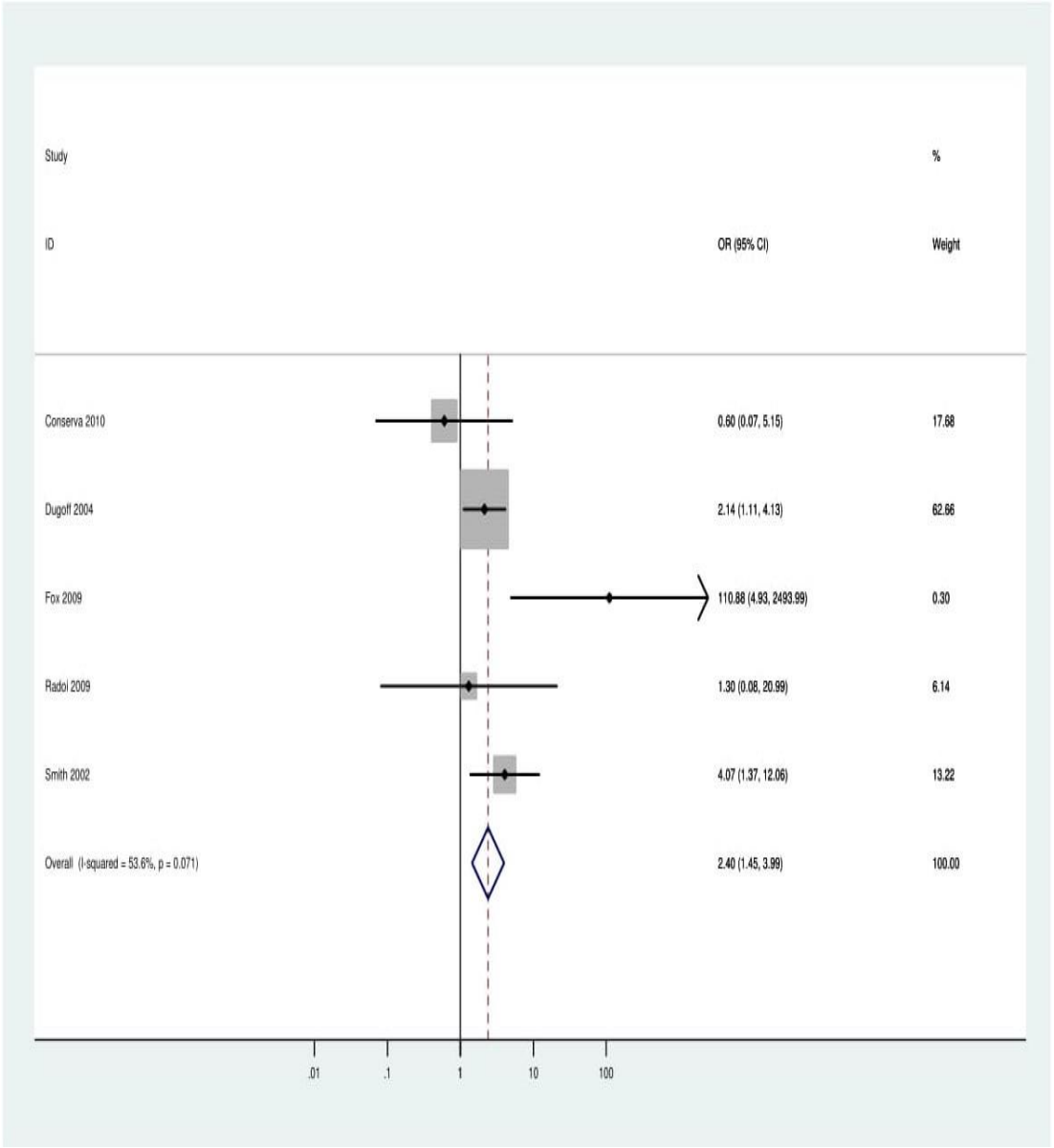


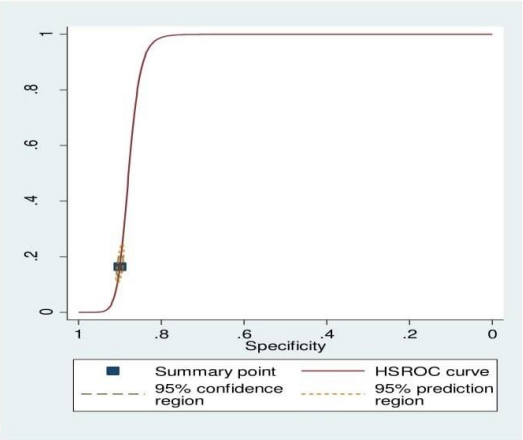
Figure 8: Forest plot for association (odds ratio) of pregnancy associated plasma protein A (PAPP-A) <5th centile with stillbirth >24 weeks



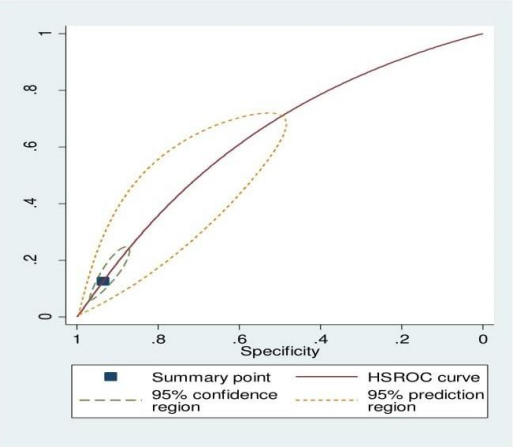
4.4.2: Predictive ability

Table 1 also summarises the sensitivity, specificity, likelihood ratios and 95% CI for all analyses. Bivariate meta-analysis was possible for 6 test-outcome combinations: PAPP-A <10th centile and birth weight <10th; PAPP-A <5th centile and birth weight 10th and <5th centile, pre-eclampsia, preterm birth <37 weeks and stillbirth >24 weeks and the hierarchical summary receiver operating characteristic curves (HSROC) are shown in Figure 7. Considering those analyses where a moderate association had been demonstrated (OR > 2.0 and lower CI > 1.0) the following predictive abilities were demonstrated all with a threshold of PAPP-A < 5th centile: Birthweight <10th centile LR+ve 1.96 (95% CI 1.58 -2.43), LR-ve 0.93 (95% CI 0.89 – 0.98); Birthweight <5th centile LR+ve 2.65 (95% CI 2.35 -2.99), LR-ve 0.85 (95% CI 0.74 – 0.98); PTB <37 weeks LR+ve 1.84 (95% CI 1.41 – 2.39), LR-ve 0.92 (95% CI 0.87 – 0.98) and stillbirth >24 weeks LR+ve 1.58 (95% CI 0.67 – 3.71) and LR-ve 0.92 (95% CI 0.78 – 1.09).

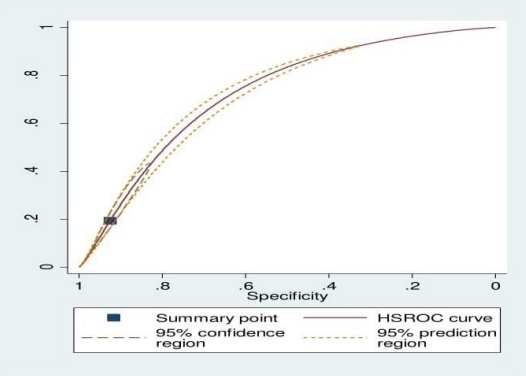
Figure 9: Hierarchical summary receiver operating curve (HSROC) for pregnancy associated plasma protein A (PAPP-A) and adverse pregnancy outcome



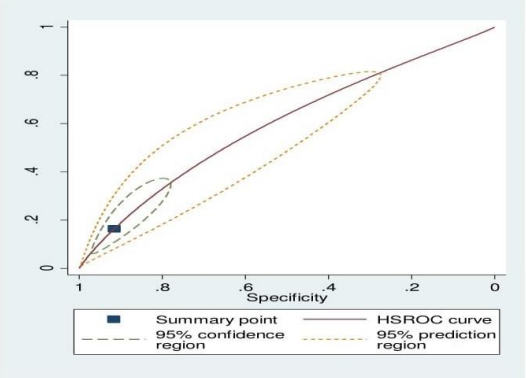
A: PAPPA <10th centile and birth weight <10th centile.



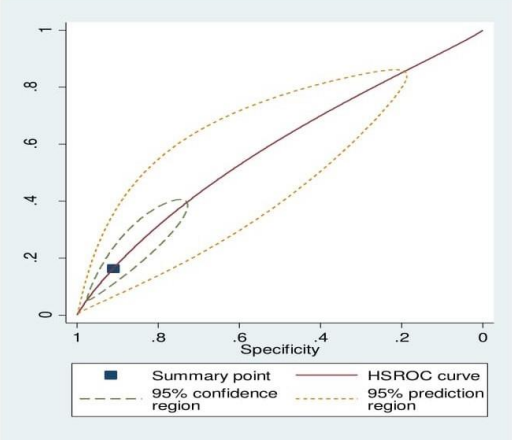
B: PAPPA < 5th centile and birth weight < 10th centile.



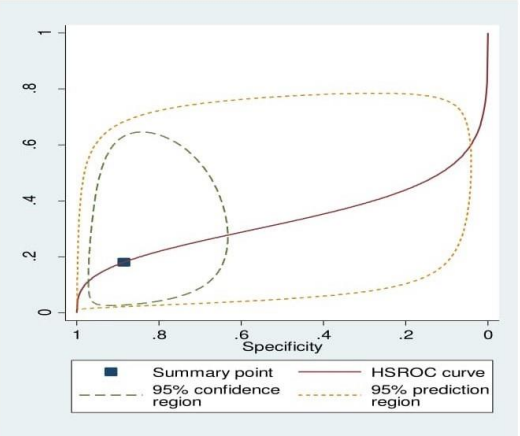
C: PAPPA <5th centile and birth weight < 5th centile.



D: PAPPA <5th centile and pre-eclampsia.



E: PAPPA < 5th centile and pre-term birth <37 weeks.



F: PAPPA <5th centile and stillbirth >24 weeks.

4.3.3: Clinical Interpretation

The predictive ability of PAPP-A is poor in an individual with only a small increase and minimal or no decrease in likelihood of disease with a positive or negative test. This can be converted to a probability of an adverse outcome for a low risk nulliparous woman (i.e. no known prior risk) in an unselected population with 8000 deliveries a year after a positive test (i.e. posterior test probability) using a nomogram (http://araw.mede.uic.edu/cgibin/testcalc.pl) (Table 2).

Thus, following a PAPP-A in the first trimester less than $<5^{th}$ centile a woman would have a 1 in 5.6 chance of an SGA baby (birth weight $<10^{th}$ centile) and a 1 in 3.7 of any adverse outcome. With lower levels of PAPP-A <1st centile the risks are considerably increased with a 1 in 3.6 chance of an SGA baby, 1 in 11 chance of pre-eclampsia, 1 in 3.7 chance of preterm birth (<37 weeks), 1 in 10 chance of late miscarriage and a 1 in 72 chance of stillbirth.

Table 2: Clinical use of first trimester pregnancy associated plasma protein A.

Pregnancy outcome/ PAPP-A threshold	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval	Prevalence (%)	Posterior probability after positive test % (number with positive test who have outcome)	Posterior probability after negative test % (number with negative test without outcome)
PAPP-A <5th centile							
Birth weight<10th centile *	1.96	1.58 -2.43	0.93	0.89 - 0.98	10	18% (1 in 5.6)	9% (1 in 1.1)
Birth weight <5th centile*	2.65	2.35 - 2.99	0.85	0.74 - 0.98	5	12% (1 in 8.2)	4% (1 in 1.0)
Pre-eclampsia	1.95	1.48 - 2.56	0.91	0.86 - 0.97	2	4% (1 in 26)	2% (1 in 1.0)
Preterm birth <37 weeks	1.84	1.41 - 2.39	0.92	0.87 - 0.98	8	12% (1 in 8.1)	7% (1 in 1.1)
Preterm birth <34 weeks	1.69	1.31 -2.16	0.93	0.88 - 0.97	2.4	4% (1 in 25)	2% (1 in 1.0)
Preterm birth <32 weeks	1.99	1.49 - 2.65	0.94	0.91 - 0.98	1.4	3% (1 in 36)	1% (1 in 1.0)

Pregnancy outcome/ PAPP-A threshold	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval	Prevalence (%)	Posterior probability after positive test % (number with positive test who have outcome)	Posterior probability after negative test % (number with negative test without outcome)
Pregnancy loss < 24 weeks	2.25	1.47 - 3.46	0.94	0.99 - 1.00	2	4% (1 in 23)	2% (1 in 1.0)
Stillbirth>24 weeks*	1.58	0.67 - 3.71	0.92	0.78 - 1.09	0.47	1% (1 in 135)	0% (1 in 1.0)
Composite adverse outcome	3.05	2.59 - 3.59	0.92	0.9 - 0.93	11	27% (1 in 3.7)	10% (1 in 1.1)
PAPP-A <1st centile							
Birth weight <10th centile	3.49	2.51 - 4.89	0.98	0.98 - 0.99	10	28% (1 in 3.6)	10% (1 in 1.1)
Birth weight <5th centile	4.52	3.53 - 5.78	0.97	0.96 - 0.98	5	19% (1 in 5.2)	5% (1 in 1.1)
Pre-eclampsia	4.91	0.60 - 40.19	0.95	0.83 - 1.08	2	9% (1 in 11)	2% (1 in 1.0)
Preterm birth <37 weeks	4.28	1.50-12.25	0.97	0.94 - 1.00	8	27% (1 in 3.7)	8% (1 in 1.1)
Preterm birth <34 weeks	2.34	0.58 - 9.41	0.99	0.96 - 1.02	2.4	5% (1 in 18)	2% (1 in 1.0)

Pregnancy outcome/ PAPP-A threshold	Positive Likelihood ratio	95% Confidence Interval	Negative Likelihood ratio	95% Confidence Interval	Prevalence (%)	Posterior probability after positive test % (number with positive test who have outcome)	Posterior probability after negative test % (number with negative test without outcome)
Preterm birth <32 weeks	3.19	1.6 - 6.36	0.98	0.96 - 1.0	1.4	4% (1 in 23)	1% (1 in 1.0)
Pregnancy loss < 24 weeks	5.24	3.21 - 8.53	0.96	0.93 - 0.98	2	10% (1 in 10)	2% (1 in 1.0)
Stillbirth > 24 weeks	2.97	0.97 - 9.09	0.98	0.94 - 1.01	0.47	1% (1 in 72)	0% (1 in 1.0)

PAPP-A - pregnancy associated plasma protein

MoM multiples of median

* bivariate meta-analysis

$ Prevalence data obtained from ONS 2014 (http://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2015-07-15)

Pre-eclampsia prevalence from NICE guidelines "Hypertension in Pregnancy: the management of hypertensive disorders during pregnancy". National Collaborating Centre for Women's and Children's Health. 2010

Late miscarriage prevalence from Wyatt PR,OwolabiT, Meier C, Huang T.Age-specific risk of fetal loss observed in a second trimester serum screening population.Am J Obstet Gynecol 2005;192:240–6

Composite adverse outcome prevalence calculated from included studies

4.5: Discussion

4.5.1: Main Findings

Low maternal serum PAPP-A in the first trimester has an association with adverse pregnancy outcomes with a moderate association once levels are <5th centile for gestation and a stronger association <1st centile. The predictive values are poor, thus although women with a low PAPP-A are at increased risk of an adverse outcome, the vast majority of these women will have a normal pregnancy outcome and the majority of women with an adverse outcome will have a normal PAPP-A.

4.5.2: Strengths and Limitations

The strength of this review, and consequently the validity of the results and inferences made, lie in its methodology. This included complying with recommended techniques for quality assessment (57, 67), performing and interpreting meta-analyses and reporting of our findings (59, 75). Our search strategies were comprehensive and robust, evidenced by Peters test demonstrating no evidence of small study bias. We have considered all aspects of test performance and displayed both prognostic and predictive ability of the test as well as demonstrating how the test would perform in a sample population.

Limitations within the review relate in the first instance to limitations within the included studies. There was significant statistical heterogeneity in some analyses which could not be accounted for when examining clinical characteristics nor study design and

was thus unexplained. Within some analyses there was a lack of data and thus for some bivariate meta-analysis could not be performed and for others test performance had to be assessed from a single study. We recognise that there are other variables that should be considered when assessing risk and that for the clinical interpretation we have assumed a background prevalence of the adverse outcome. It is not known how risk factors in obstetrics interact and how they modify risk in an individual. It is reasonable to assume however that in a woman with multiple risk factors e.g. previous SGA baby the risk will be higher than those discussed. One limitation in the methodology employed is the need to consider PAPP-A as a dichotomous variable i.e. categorisation using a threshold.

This is a common technique in clinical research with dichotomization to simplify the analysis. This has limitations statistically as it can lead to a loss of power as much of the information is lost, classifying very similar factor values as different in opposite sides of the cut-off point and the concealment of a potential non-linear relationship between the outcome and the factor of interest (107-109). One technique to overcome this is individual patient data meta-analysis (IPD) which uses original source data at the participant level thus having many advantages such as being able to derive prognostic factor results directly, independent of study reporting and significance, and analyse continuous factors more appropriately (110, 111).

4.5.3: Interpretation

Prognostic factor research is important as it allows us to potentially improve outcome for patients by identifying modifiable factors by either intervention e.g.

delivery or by different management pathways e.g. surveillance. If treatments are available that may modify disease then prognostic factors may have a role in predicting differential treatment response (112). Even if a prognostic factor is insufficient as a stand-alone test, it may still add some independent prognostic value over other prognostic factors, and used in a multivariable prognostic model to help provide absolute risk predictions for women based on their individual characteristics (112). It is thus imperative to robustly and systematically assess prognostic factors as has been done in this review for PAPP- A.

Our results demonstrate evidence of associations between PAPP-A and adverse pregnancy outcome. Future work should thus include IPD meta-analysis as previously discussed to allow assessment of PAPP-A as a continuous variable and its relationship with other prognostic markers available during the pregnancy; first trimester (e.g. crown rump length, nuchal translucency), second trimester (e.g. fetal biometry, uterine artery Doppler) and third trimester (e.g. placental biomarkers, placental morphology) (113-117). Any prognostic model developed would then require validation in external data sets (118).

4.6: Conclusion

Low maternal serum PAPP-A in the first trimester has an association with adverse pregnancy outcome particularly if levels are very low (<1st centile). National guidelines have identified this evidence and suggested increased surveillance for impaired fetal growth (4) but there are no recommendations for surveillance for other adverse

outcomes e.g. miscarriage, preterm birth, pre-eclampsia nor for interventions due to the increased risk of stillbirth (e.g. induction at term). It must be recognised that for the individual predictive value are poor and thus the majority of adverse outcomes will occur in the group without an abnormally low PAPP-A and thus outside any guidelines for increased surveillance or intervention. Thus, future research is required to develop robust and accurate prediction models that can allow modern day obstetrics to practice truly stratified medicine (119).

CHAPTER 5: Association of maternal serum PAPP-A levels, nuchal translucency and crown rump length in first trimester with adverse pregnancy outcomes: Retrospective cohort study

5.1 Introduction

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

Pregnancy associated plasma protein A (PAPP-A) is a placental glycoprotein produced by syncytial trophoblast of the placenta, which cleaves insulin-like growth factor binding protein 4 (IGFBP4) and is a positive regulator of insulin-like growth factors (IGFs), potentially influencing fetal growth and wellbeing (53).

Studies have tested the hypothesis that low maternal serum levels of PAPP-A in the first trimester can predict adverse pregnancy outcomes associated with poor placental function (5, 6, 49-51). International Guidelines on "*The Investigation and Management of the Small for Gestational Fetus*" have recommended that pregnant women with a serum PAPP-A <0.4MoM (1st centile) in the first trimester receive increased ultrasound surveillance for fetal growth disorders (4). However, contradictory results have been

observed in publications (51, 120) and few studies have investigated the association of first trimester fetal biometry [nuchal translucency (NT) and crown rump length (CRL)] with adverse outcomes and their relationship with PAPP-A (5, 52). The objective of this study is to determine the relationship between serum pregnancy-associated plasma protein-A (PAPP-A), nuchal translucency (NT) and crown rump length (CRL) in first trimester and adverse pregnancy outcomes in a large cohort study.

5.2: Methods

5.2.1 Data collection

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

An ethical committee approval was applied using the online IRAS (integrated research application system) application form and emailing the confidentiality advisory group. Both approvals were granted and the approval letters are displayed in appendices 9 and 10.

All pregnant women who accepted first trimester aneuploidy screening and delivered in BWNFT were included in the study. First trimester aneuploidy screening is offered to all pregnant women between 11+2 to 14+1 weeks of gestation (crown–rump length measures from 45 mm to 84 mm) as part of the National Downs Syndrome Screening

Programme (27, 34). This involves measuring maternal serum levels of PAPP-A and free beta human chorionic gonadotrophin (free B HCG), along with the NT, and the pregnancy is dated based on CRL. All first trimester scans and measurements performed at BWNFT are performed by accredited sonographers as per National NEQAS guidelines. Analysis for PAPP-A was performed on the Auto-Delfia immunoassay platform (Perkin Elmer Ltd, Seer Green, UK). For the purpose of Down's syndrome screening, PAPP-A values are converted to multiples of the median for gestation. To prevent any loss of data and to remove the need for considering an absolute threshold, in this study PAPP-A values were considered as a continuous variable using the absolute value (U/L).

The data was collected from the following hospital-based, secure and confidential computerized databases. These databases along with the subject demographics, captured the following information:

1. K2 database: Recorded the peri- partum events.
2. Biochemistry database: Recorded of the serum PAPP- A levels, NT and CRL along with the gestational age and number of fetuses in the pregnancy. It also noted if the pregnancy was result of a donor egg.
3. Genetics database: Recorded abnormal karyotype from invasive prenatal testing.

The data from databases was exported in to respective Microsoft Excel formats. Subject's hospital number was used as a common denominator linking all the databases. Where available, date of birth and/or NHS number was used for cross referencing.

All the excel spread sheets were merged by using 'merge the tables' software from Able bits excel software support website (121).

The Biochemistry and K2 databases were merged first. Maternal Hospital numbers were used as common denominators to merge the two excel spread sheets. It was cross referenced by baby's date of birth and Maternal NHS number. K2 data was captured from 1st August 2011 to 31st March 2015 and biochemistry data was collected from January 2011 to October 2014.

Patients having multiple pregnancy with in the study period were highlighted. Mismatching duplicates were manually checked and removed using baby date of birth and date of PAPP-A test. Pregnancies by donor egg were removed as details such as mother's age were details of the donor, not the carrier. To this data the genetics data was linked up and all the patients who had had abnormal karyotypes were highlighted and removed from the study population.

Thus, the master sheet containing the data from all different databases was created and finally the data was anonymised by assigning research number to each patient in place of hospital number and NHS number.

5.2.2 Definitions of maternal and obstetric characteristics

Preterm birth (PTB) was defined as live delivery prior to 37 weeks, both spontaneous and iatrogenic. Pre-eclampsia (PE) was defined according to the International Society for the study of Hypertension in Pregnancy (ISSHP) definition as *de-novo* hypertension at or after 20 weeks gestation (at least 2 readings of Blood Pressure >140 mmHg systolic or >90 mmHg diastolic) with proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L [‘2 +’] on dipstick testing)(57). Small for gestational age (SGA) was defined as birthweight below the 10th percentile of the customised growth chart (121). Miscarriage was defined as fetal demise before 24 weeks of gestation. Stillbirth was defined as intrauterine death after 24 completed weeks of pregnancy. Perinatal death was defined as fetal or neonatal death between 24 weeks of gestation and 7 days after birth. Neonatal death was defined as death between birth and 28 days.

5.2.3 Data Analysis

5.2.3a Mother and fetus demographics and clinical features

Distributions of demographic characteristics and known prognostic factors were summarised for the following variables: maternal age at test, gestational age at test, parity, body mass index (BMI), deprivation category (Index of Multiple Deprivation 2010– IMD (122), calculated using National Perinatal Epidemiology Unit calculator), ethnicity, assisted conception (IVF), smoking status, pre-pregnancy insulin-dependent diabetes mellitus, and gender of the baby. Mean and standard deviation (SD), or median

and interquartile range (IQR), is reported for continuous variables, according to whether the variables were normally distributed. The number and percentage are reported for categorical variables.

5.2.3b Analysis of prognostic association with outcomes

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

Again, for each of the three factors separately, multivariable logistic regression analyses were fitted to examine the odds ratio adjusted for the known (or likely) existing prognostic factors of maternal age (years), parity, BMI, smoking status, IVF, ethnicity, deprivation category and gestational diabetes. This provided the adjusted odds ratio for a 1-unit increase in each factor, to reveal their independent prognostic value over and above other factors (Appendix 13).

Then, for each outcome, the three factors were analysed in combination in one multivariable logistic regression model, whilst adjusting for the other factors detailed above, to explore whether the prognostic value of each factor is the same after adjusting for the other two potential prognostic factors. The linearity assumption of all continuous variables was assumed for these multivariable models.

Therefore, finally, the fully adjusted models with all three potential factors in combination were fitted again; however, additionally, the linearity assumption of the prognostic effects for the three factors of interest was assessed, and alternative functional forms were considered if the assumption was violated. A linear relationship was specified for all three prognostic factors of interest. The functional form was chosen using fractional polynomials, where all possible fractional polynomials up to the second degree were considered based on their statistical significance (123). A linear relationship was specified for the other continuous covariates (maternal age and BMI).

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

5.2.3c Handling of missing data

The percentages of missing values for each covariate and outcome were calculated. Missing data was imputed with multiple imputation with chained equations with 35 imputed datasets equal to the percentage of missing data(124). For non-normally distributed variables, predictive mean matching was used to impute the missing data (125, 126). The imputation model contained all complete outcomes and covariates that were included in the multivariable analyses. Rubin's rules were used to combine the

parameter estimates and standard errors into a single inference (126). A complete-case analysis was also conducted as a sensitivity analysis.

Estimates of prognostic effects are reported as odds ratios (OR) with 95% confidence intervals (CI) and p-values. Analyses were performed using Stata version 14 (127).

5.3 Results

Total number of women delivered in this time frame was 30,099 (as recorded in K2 software). Biochemistry data from first trimester serum PAPP-A levels was captured from 1st January 2011 to 10th October 2014. This had PAPP-A levels for 14352 women. After combining K2 data and the biochemistry data we had 12837 women who had PAPP-A levels done and their pregnancy outcome was recorded. This difference of 1515 women could be attributed to the patient flow from and to other hospitals for delivery. Ten pregnancies conceived with donor eggs were excluded. Three pregnancies with an unclear pregnancy outcome showing death of the babies post 28 days were excluded since this outcome would not be routinely recorded in the databases. After excluding 232 multiple pregnancies, the final study cohort was of 12,592 singleton pregnancies (Table 3).

Table 3: Mother and fetus demographics and clinical features at the test

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

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

As depicted in Table 3, the mean maternal age and median BMI were 30.6 (SD 5.6) and 25.1 (IQR 22.4 to 28.8), respectively. Majority of the women were White (62.6%) followed by South Asians (19.9%). Mean gestational age at first trimester ultrasound was 88.2 days (SD 4.3). About 2% (n=250) of pregnancies were the result of assisted conception. About 13% (n=1569) women were smokers and about 84% (n=10611) women did not smoke while about 3% (n= 412) women stopped smoking during pregnancy. Nearly half (43%, n=5495) patients lived in the most deprived areas (deprivation score ≥ 34.18) and 3.6% (n=455) patients lived in the least deprived areas (deprivation score ≤ 8.49). Gender distribution was almost equal in the fetuses (48.6% male and 51.1% female).

Table 4: Number of events for each outcome in singleton pregnancies N=12,592

	Number of events (%)
Pre-term labour (<37 weeks)	852 (6.77)
Pre-eclampsia	352 (2.80)
SGA (<10th customised centile)	1824 (14.49)
Miscarriage (death prior to birth <24 weeks gestation)	73 (0.58)
Stillbirth (death prior to birth >24 weeks gestation)	37 (0.29)
Perinatal death* (Death between 24 weeks gestation and 7 days after birth)	73 (0.58)
Neonatal death$^{\$}$ (Death between birth and 28 days)	38 (0.31)

* Perinatal death includes stillbirths; $^{\$}$ neonatal death includes babies that die between birth and 7 days after birth that are captured within the perinatal death category.

Table 4 displays the number of events for each outcome for the cohort. Of 12592 women, 852 had preterm birth (6.8%). This compares lower than the national rate of about 11%) (128). 352 patients had pre-eclampsia (both mild and severe) (2.8%). This is comparable with the national rates of about 2 % for severe pre eclampsia and about 6 % for mild pre eclampsia (129). 1824 babies were SGA (14.5%) which is higher than expected (> 10 %) of the cohort. There were 73 pregnancies that ended in miscarriage (0.6%)and 37 stillbirths (0.3%)which is lower than national rate of 0.5% (130). There were 38 neonatal deaths (0.31%) of which 36 were early and thus giving a total of 73 perinatal deaths (0.6%).

Table 5: Number and percentage of missing covariate data for singleton pregnancies

	n (%) missing data
PAPP-A (U/L)	0 (0)
Nuchal translucency (mm)	2 (0.02)
Crown rump length (mm)	2 (0.02)
Mother's age (years)	0 (0)
Gestational age at birth (days)	0 (0)
Parity (number)	567 (4.5)
BMI	4466 (35.5)
Deprivation score*	175 (1.4)
Ethnicity	0 (0)
Assisted conception	0 (0)
Smoking status	0 (0)
Gestational diabetes mellitus	0 (0)

BMI was missing in 4466 (35.5%) records, parity was missing in 567 (4.5%) records and deprivation score was missing in 175 (1.4%) records. There were missing data for 152 (1.2%) fetal weight and four (0.03 %) neonatal outcomes (table 5). Multiple imputation was performed for missing BMI (height, weight), parity and deprivation score values. Although some variables were normally distributed, predictive mean matching was used for all variables because imputation with chained regression analysis imputed unrealistic values for weight and height.

Results analysis:

5.3.1 PAPP-A results

Table 6: PAPP-A: results from unadjusted logistic regression

Outcome	Odds Ratio	95% Confidence Interval OR	p-value
SGA	0.874	0.849 to 0.900	<0.0001
Pre-term labour	0.934	0.899 to 0.970	<0.0001
Pre-eclampsia	0.915	0.862 to 0.971	0.004
Miscarriage	0.968	0.858 to 1.092	0.598
Stillbirth	0.810	0.655 to 1.002	0.052
Perinatal death	0.927	0.815 to 1.054	0.245
Neonatal death	1.032	0.886 to 1.201	0.686

An unadjusted OR for PAPP-A for the outcome of SGA means that for a one unit increase in PAPP-A (U/L), the odds are lower by 13% (table 6). This is a highly statistically significant result with a 95% CI that suggests there is between 10.0% and a 15.1% lower odds of SGA for a one unit increase in PAPP-A ($p<0.0001$). Similar conclusions can be drawn for the association between PAPP-A and pre-term birth [OR 0.93 (95%CI 0.90, 0.97), $p<0.0001$]. In addition, PAPP-A and pre-eclampsia demonstrated a similar relationship [OR 0.92 (95% CI 0.86, 0.97), $p=0.004$]. The results for stillbirth were in the same direction and quantitatively similar, although the CI was slightly wider and the p-value was just above the 5% [OR 0.81 (95% CI 0.66, 1.0), $p=0.052$]. There was no evidence of a strong association between PAPP-A and miscarriage, perinatal death or neonatal death.

Multivariable analysis was performed after adjusting for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

The results for each adverse event are as follows:

Table 7: PAPP-A: Results from adjusted logistic regression for SGA (N-12,300)

SGA (<10th customised centile)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.878	0.851 to 0.906	<0.0001
Mother's age (years)		1.016	1.005 to 1.026	0.003
BMI		1.000	0.988 to 1.012	0.996
Parity	0	1.178	1.054 to 1.316	0.004
	>4	1.015	0.627 to 1.644	0.952
Ethnicity	South-Asian	0.764	0.616 to 0.947	0.014
	Oriental	0.695	0.529 to 0.896	0.006
	Other Mixed	0.689	0.529 to 0.896	0.006
	White	0.650	0.535 to 0.792	<0.0001
Deprivation score categories	2	1.120	0.790 to 1.590	0.524
	3	1.033	0.758 to 1.406	0.838
	4	1.037	0.763 to 1.410	0.817
	5	1.120	0.884 to 1.616	0.245
Smoking status	Yes	2.547	2.218 to 2.926	<0.0001
	Stopped	1.153	0.870 to 1.534	0.327
IVF		0.661	0.432 to 1.011	0.056
Gestational diabetes		0.987	0.780 to 1.219	0.903

N=12,300

In this adjusted analysis in Table 7, there is evidence of an association between PAPP-A and SGA with an odds ratio estimate of 0.87. The estimate is statistically significant with a 95% confidence interval of 0.85 to 0.90, suggesting between 10% and 15% lower odds of SGA ($p<0.0001$) for a one unit increase in PAPP-A, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 8: PAPP-A: Results from adjusted logistic regression for preterm birth(<37 weeks, N=12,454)

Preterm birth (<37 weeks)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.924	0.884 to 0.965	<0.0001
Mother's age (years)		1.001	0.995 to 1.024	0.215
BMI		0.995	0.978 to 1.012	0.568
Parity	0	1.027	0.872 to 1.211	0.747
	>4	1.015	0.627 to 1.644	0.952
Ethnicity	South-Asian	0.683	0.508 to 0.918	0.011
	Oriental	0.409	0.222 to 0.752	0.004
	Other Mixed	0.665	0.461 to 0.960	0.029
	White	0.624	0.478 to 0.816	0.001
Deprivation score categories	2	0.866	0.526 to 1.424	0.571
	3	0.874	0.570 to 1.339	0.536
	4	0.921	0.603 to 1.405	0.702
	5	1.065	0.704 to 1.611	0.765
Smoking status	Yes	1.641	1.333 to 2.021	<0.0001
	Stopped	0.907	0.578 to 1.422	0.670
IVF		1.083	0.642 to 1.828	0.764
Gestational diabetes		1.323	1.000 to 1.748	0.050

N=12,454

Table 8 showing adjusted analysis, there is evidence of lower odds of a preterm birth as PAPP-A increases with an odds ratio estimate of 0.92. The estimate is statistically significant with a 95% confidence interval of 0.90 to 0.96, suggesting between 4% and

10% lower odds of preterm birth (p=0.001) for a one unit increase in PAPP-A, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 9: PAPP-A: Results from adjusted logistic regression for PET (N=12,322)

Pre-eclampsia toxaemia		**Odds Ratio**	**95% Confidence Interval OR**	**p-value**
PAPP-A (U/L)		0.906	0.848 to 0.968	0.003
Mother's age (years)		1.015	0.993 to 1.037	0.185
BMI		1.046	1.022 to 1.070	<0.0001
Parity	0	2.634	2.059 to 3.369	<0.0001
	>4	1.000	-	-
Ethnicity	South-Asian	0.569	0.378 to 0.857	0.007
	Oriental	0.282	0.109 to 0.729	0.009
	Other Mixed	0.441	0.252 to 0.773	0.004
	White	0.519	0.360 to 0.748	<0.0001
Deprivation score categories	2	0.700	0.371 to 1.320	0.270
	3	0.718	0.421 to 1.224	0.223
	4	0.625	0.365 to 1.068	0.086
	5	0.824	0.491 to 1.384	0.464
Smoking status	Yes	0.573	0.371 to 0.886	0.012
	Stopped	0.941	0.520 to 1.702	0.841
IVF		1.662	0.954 to 2.896	0.073
Gestational diabetes		0.901	0.584 to 1.391	0.639

N=12,322

Where the odds ratios are omitted in results tables, there were no events for the categories amongst those individuals included in the analysis. In this analysis in table 7, there was no estimate of an odds ratio for parity >4 compared to parity of 1-4 because there were no events of pre-eclampsia toxaemia for those that have a parity >4.

In this adjusted analysis, there is evidence of lower odds of a pre-eclampsia as PAPP-A increases with an odds ratio estimate of 0.908 (table 9). The estimate is statistically significant with a 95% confidence interval of 0.85 to 0.97, suggesting between 3% and 15% lower odds of pre-eclampsia (p=0.004) for a one unit increase in PAPP-A, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 10: PAPP-A: Results from adjusted logistic regression for miscarriage (<24 weeks gestation, N=10,404)

Miscarriage (death <24 weeks gestation)		Odds Ratio	95% Confidence Interval (	p-value
PAPP-A (U/L)		1.008	0.839 to 1.212	0.929
Mother's age (years)		0.966	0.903 to 1.033	0.315
BMI		1.045	0.985 to 1.111	0.146
Parity	0	0.538	0.253 to 1.144	0.108
	>4	1.000	-	-
Ethnicity	South-Asian	1.404	0.364 to 5.411	0.622
	Oriental	1.000	-	-
	Other Mixed	1.608	0.351 to 7.363	0.541
	White	0.896	0.250 to 3.210	0.866
Deprivation score categories	2	1.000	-	-
	3	0.845	0.098 to 7.302	0.878
	4	1.734	0.221 to 13.586	0.600
	5	1.207	0.154 to 9.451	0.858
Smoking status	Yes	0.762	0.255 to 2.277	0.626
	Stopped	0.821	0.110 to 6.123	0.848
IVF		2.230	0.288 to 17.294	0.443
Gestational diabetes		1.000	-	-

N=10,404

In this adjusted analysis in table 10, there is no evidence of an association between miscarriage and PAPP-A after adjusted for the other factors since the odds ratio estimate is 1.010 (95% CI: 0.84 to 1.21; p=0.912

Table 11: PAPP-A: Results from adjusted logistic regression for stillbirth (>24 weeks gestation) (singleton pregnancies, N =9,753)

Stillbirth (death >24 weeks gestation)		Odds Ratio	95% Confidence Interval OI	p-value
PAPP-A (U/L)		0.714	0.520 to 0.981	0.038
Mother's age (years)		0.983	0.904 to 1.069	0.693
BMI		1.003	0.925 to 1.087	0.945
Parity	0	1.983	0.825 to 4.769	0.126
	>4	1.000	-	-
Ethnicity	South-Asian	1.998	0.239 to 16.710	0.523
	Oriental	1.000	-	-
	Other Mixed	0.867	0.053 to 14.112	0.920
	White	1.227	0.154 to 9.765	0.847
Deprivation score categories	2	0.540	0.067 to 4.351	0.562
	3	1.099	0.382 to 3.162	0.861
	4	0.964	0.342 to 2.715	0.944
	5	1.000	-	-
Smoking status	Yes	1.000	-	-
	Stopped	0.992	0.131 to 7.512	0.994
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=9,75

In Table 11, there is evidence of lower odds of a stillbirth as PAPP-A increases with an odds ratio estimate of 0.723. The estimate is statistically significant with a 95% confidence interval of 0.53 to 0.99, suggesting between 1% and 47% lower odds of

stillbirth (p=0.044) for a one unit increase in PAPP-A, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 12: PAPP-A: Results from adjusted logistic regression for perinatal death (death between 24 weeks gestation and 7 days after birth) (singleton pregnancies, N=10,898)

Perinatal death (death between 24 weeks gestation and 7 days after birth)		**Odds Ratio**	**95% Confidence Interval OR**	**p-value**
PAPP-A (U/L)		0.880	0.733 to 1.058	0.173
Mother's age (years)		0.961	0.903 to 1.023	0.211
BMI		1.063	1.010 to 1.118	0.020
Parity	0	2.246	1.166 to 4.328	0.016
	>4	1.000	-	-
Ethnicity	South-Asian	0.567	0.218 to 1.454	0.236
	Oriental	0.369	0.044 to 3.077	0.357
	Other Mixed	0.345	0.089 to 1.338	0.124
	White	0.219	0.089 to 0.538	0.001
Deprivation score categories	2	2.279	0.812 to 6.394	0.118
	3	0.967	0.395 to 2.369	0.942
	4	1.184	0.556 to 2.518	0.662
	5	1.000	-	-
Smoking status	Yes	0.830	0.280 to 2.454	0.736
	Stopped	1.375	0.323 to 5.845	0.667
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,898

Table 12 shows there is no evidence of an association between perinatal death and PAPP-A after adjusting for the other factors since the odds ratio estimate is 0.88 (95% CI: 0.73 to 1.05; p=0.164).

Table 13: PAPP-A: Results from adjusted logistic regression for neonatal death (death between birth and 28 days after birth) (singleton pregnancies, N=10,876)

Neonatal death (death between birth and 28 days)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		1.043	0.847 to 1.287	0.687
Mother's age (years)		0.949	0.865 to 1.040	0.261
BMI		1.122	1.051 to 1.198	0.001
Parity	0	2.360	0.8908 to 6.132	0.078
	>4	1.000	-	-
Ethnicity	South-Asian	0.464	0.139 to 1.555	0.213
	Oriental	0.659	0.073 to 5.956	0.711
	Other Mixed	0.267	0.052 to 1.371	0.114
	White	0.069	0.019 to 0.246	<0.0001
Deprivation score categories	2	5.652	1.607 to 19.884	0.007
	3	0.384	0.048 to 3.078	0.368
	4	1.241	0.420 to 3.663	0.696
	5	1.000	-	-
Smoking status	Yes	3.041	0.895 to 10.329	0.075
	Stopped	2.271	0.284 to 18.145	0.439
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,876

Table 13 shows there is no evidence of an association between neonatal death and PAPP-A after adjusted for the other factors since the odds ratio estimate is 1.03 (95% CI: 0.83 to 1.27; p=0.786).

Summary of results:

a) PAPP-A

In both the unadjusted analyses and the adjusted analyses, there is significant evidence of lower odds of SGA, premature labour, pre-eclampsia and stillbirth as PAPP-A increases. There is no evidence of a significant association between PAPP-A and miscarriage, perinatal death or neonatal death.

5.3.2 NT result

Similar analysis was carried out looking for relation of NT and CRL with adverse pregnancy outcomes. Detailed analysis and the tables can be found in appendices 11 and 12. Summary of the analysis is as below:

For NT, in the unadjusted analyses (Appendix 11: Table 14a), for higher values of NT there was a strong association with an increased odds of miscarriage [OR 1.94 (95% CI 1.54, 2.45), p<0.0001], and a significant decreased odds of SGA [OR 0.81 (95% CI 0.72, 0.91), p<0.0001]. There was also some evidence that higher values are associated with an increased risk of PTB [OR 1.15 (95% CI 1.0, 1.32), p=0.053] though the CI overlapped one.

After multivariable analysis, there was independent prognostic value of NT for SGA [OR 0.8 (95% CI 0.71, 0.90), p<0.001] (Appendix 11 Table 14b), and for miscarriage [OR 1.75 (95% CI 1.12, 2.72), p=0.013] (Appendix 11: Table 14e). There was no significant relationship between NT and PTB, PE, stillbirth, perinatal or neonatal death in the unadjusted or adjusted analyses (Appendix 11 Tables 14a-h).

5.3.3 CRL Results

For CRL in the unadjusted analysis there was no significant association with any of the outcomes (Appendix 12: Table 15a). There was a borderline statistical significance for SGA which remained after adjustment [OR 0.99 (95% CI 0.99, 1.00, p=0.051)] (Appendix 12: Table 15b), but the magnitude of the OR was close to one. After adjustment for other known predictors and potential confounders, there was evidence of a strong association between CRL and stillbirth [OR 0.94 (95% CI 0.89, 0.99), p=0.027], thus between 1% and 11% lower odds of stillbirth for a one unit increase in CRL (Appendix 12: Table 15f). The adjusted analyses for PTB, PE, miscarriage, perinatal death and neonatal death demonstrated no significant association with CRL (Appendix 12: Tables 15-c,d,e,g,h).

5.3.4 Adjustment for all three potential prognostic factors

Finally, the fully adjusted models with all three potential factors in combination were fitted again. The results are as follows.

In the adjusted analysis shown in Table 16a, the odds of SGA are statistically significantly lower as PAPP-A and NT increase, with odds ratio estimates of 0.86 (95% CI: 0.83 to 0.89; p<0.0001), and 0.80 (95% CI: 0.70 to 0.92; p=0.001), respectively. The odds of SGA are also statistically significantly higher as CRL increases with an odds ratio estimate of 1.01 (95% CI: 1.00 to 1.02; p=0.003).

In adjusted analysis in Table 16b, the odds of preterm birth are statistically significantly lower as PAPP-A increases, with an odds ratio estimates of 0.91 (95% CI: 0.87 to 0.95; p<0.0001). The association between NT and preterm birth and between CRL and preterm birth are not statistically significant (similar to the analyses without the other two potential prognostic markers).

Table 16 a: PAPP-A, NT and CRL: Results from adjusted logistic regression for SGA (singleton pregnancies, N=12,299)

SGA (<10th customised centile)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.869	0.839 to 0.899	<0.0001
NT (mm)		0.795	0.696 to 0.909	0.001
CRL (mm)		1.011	1.003 to 1.018	0.004
Mother's age (years)		1.015	1.005 to 1.026	0.003
BMI		0.999	0.987 to 1.012	0.922
Parity	0	1.178	1.054 to 1.316	0.004
	>4	1.026	0.633 to 1.661	0.918
Ethnicity	South-Asian	0.762	0.614 to 0.945	0.013
	Oriental	0.709	0.491 to 1.024	0.067
	Other Mixed	0.686	0.527 to 0.893	0.005
	White	0.651	0.535 to 0.792	<0.0001
Deprivation score categories	2	1.124	0.792 to 1.594	0.512
	3	1.024	0.752 to 1.394	0.882
	4	1.031	0.758 to 1.402	0.845
	5	1.192	0.882 to 1.611	0.253
Smoking status	Yes	2.542	2.212 to 2.921	<0.0001
	Stopped	1.158	0.870 to 1.541	0.314
IVF		0.656	0.429 to 1.003	0.052
Gestational diabetes		0.978	0.791 to 1.208	0.834

N=12,299

Table 16b: PAPP-A, NT and CRL: Results from adjusted logistic regression for preterm birth (singleton pregnancies, N=12,453)

Preterm birth (<37 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.915	0.872 to 0.960	<0.0001
NT (mm)		1.124	0.946 to 1.335	0.183
CRL (mm)		1.003	0.992 to 1.013	0.632
Mother's age (years)		1.009	0.994 to 1.024	0.232
BMI		0.993	0.976 to 1.011	0.444
Parity	0	1.033	0.876 to 1.219	0.694
	>4	0.982	0.489 to 1.974	0.960
Ethnicity	South-Asian	0.675	0.502 to 0.908	0.009
	Oriental	0.398	0.216 to 0.732	0.003
	Other Mixed	0.658	0.456 to 0.950	0.025
	White	0.615	0.470 to 0.804	<0.0001
Deprivation score categories	2	0.868	0.528 to 1.428	0.578
	3	0.872	0.569 to 1.336	0.528
	4	0.924	0.605 to 1.410	0.712
	5	1.067	0.706 to 1.614	0.758
Smoking status	Yes	1.618	1.313 to 1.993	<0.0001
	Stopped	0.899	0.523 to 1.410	0.642
IVF		1.070	0.634 to 1.807	0.800
Gestational diabetes		1.296	0.978 to 1.716	0.071

N=12,453

In adjusted analysis as seen in Table 16c, the odds of pre-eclampsia are statistically significantly lower as PAPP-A increases and statistically significantly lower as CRL increases, with odds ratio estimates of 0.88 (95% CI: 0.82 to 0.95; p=0.001), and 1.02 (95% CI: 1.01 to 1.04; p=0.006), respectively. There is no association between pre-eclampsia and NT.

In this adjusted analysis (Table 16d), the odds of miscarriage are statistically significantly higher as NT increases, with an odds ratio estimates of 1.67 (95% CI: 1.01 to 2.76; p=0.047). The association between PAPP-A and miscarriage and also between CRL and miscarriage are not statistically significant (similar to the analyses without the other two potential prognostic markers).

Table 16c: PAPP-A, NT and CRL: Results from adjusted logistic regression for pre-eclampsia (singleton pregnancies, N=12,321)

Pre-eclampsia		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.876	0.815 to 0.942	<0.0001
NT (mm)		0.782	0.584 to 1.046	0.097
CRL (mm)		1.023	1.007 to 1.039	0.004
Mother's age (years)		1.013	0.992 to 1.035	0.239
BMI		1.043	1.019 to 1.067	<0.0001
Parity	0	2.641	2.065 to 3.378	<0.0001
	>4	1.000	-	-
Ethnicity	South-Asian	0.561	0.375 to 0.845	0.006
	Oriental	0.286	0.111 to 0.740	0.010
	Other Mixed	0.435	0.248 to 0.763	0.004
	White	0.512	0.355 to 0.739	<0.0001
Deprivation score categories	2	0.709	0.375 to 1.337	0.288
	3	0.715	0.419 to 1.220	0.218
	4	0.623	0.364 to 1.065	0.084
	5	0.818	0.487 to 1.374	0.448
Smoking status	Yes	0.568	0.367 to 0.878	0.011
	Stopped	0.938	0.518 to 1.697	0.832
IVF		1.605	0.921 to 2.798	0.095
Gestational diabetes		0.898	0.582 to 1.387	0.629

N=12,321

Table 16d: PAPP-A, NT and CRL: Results from adjusted logistic regression for miscarriage (singleton pregnancies, N=10,404)

Miscarriage (death <24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.945	0.771 to 1.159	0.588
NT (mm)		1.669	1.007 to 2.764	0.047
CRL (mm)		1.024	0.980 to 1.070	0.285
Mother's age (years)		0.963	0.900 to 1.030	0.272
BMI		1.036	0.974 to 1.102	0.261
Parity	0	0.555	0.261 to 1.183	0.127
	>4	1.000	-	-
Ethnicity	South-Asian	1.287	0.337 to 4.963	0.714
	Oriental	1.000	-	-
	Other Mixed	1.459	0.317 to 6.713	0.627
	White	0.801	0.223 to 2.879	0.734
Deprivation score categories	2	1.000	-	-
	3	0.866	0.100 to 7.500	0.896
	4	1.758	0.224 to 13.783	0.591
	5	1.187	0.151 to 9.308	0.870
Smoking status	Yes	0.725	0.242 to 2.175	0.566
	Stopped	0.756	0.101 to 5.662	0.785
IVF		2.046	0.262 to 15.958	0.494
Gestational diabetes		1.000	-	-

N=10,404

Table 16e: PAPP-A, NT and CRL: Results from adjusted logistic regression for stillbirth (singleton pregnancies, n=9,753)

Stillbirth (death >24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		0.785	0.563 to 1.093	0.152
NT (mm)		1.191	0.447 to 3.176	0.726
CRL (mm)		0.953	0.894 to 1.016	0.142
Mother's age (years)		0.988	0.910 to 1.075	0.787
BMI		1.011	0.933 to 1.096	0.787
Parity	0	1.968	0.816 to 4.743	0.132
	>4	1.000	-	-
Ethnicity	South-Asian	2.039	0.243 to 17.087	0.511
	Oriental	1.000	-	-
	Other Mixed	0.903	0.055 to 14.698	0.943
	White	1.268	0.159 to 10.117	0.823
Deprivation score categories	2	0.517	0.064 to 4.172	0.536
	3	1.092	0.380 to 3.140	0.870
	4	0.961	0.341 to 2.704	0.940
	5	1.000	-	-
Smoking status	Yes	1.000	-	-
	Stopped	1.030	0.136 to 7.808	0.977
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=9,753

There is no statistically significant evidence of associations between PAPP-A, NT or CRL and stillbirth after adjusting for each other and the other known predictors.

Table 16f: PAPP-A, NT and CRL: Results from adjusted logistic regression for perinatal death (singleton pregnancies, N=10,898)

Perinatal death (death between 24 weeks gestation and 7 days after birth)		**Odds Ratio**	**95% Confidence Interval OR**	**p-value**
PAPP-A (U/L)		0.921	0.757 to 1.119	0.408
NT (mm)		1.176	0.565 to 2.448	0.665
CRL (mm)		0.973	0.931 to 1.017	0.226
Mother's age (years)		0.964	0.906 to 1.026	0.247
BMI		1.070	1.013 to 1.123	0.014
Parity	0	2.237	1.159 to 4.315	0.016
	>4	1.000	-	-
Ethnicity	South-Asian	0.574	0.222 to 1.486	0.253
	Oriental	0.368	0.044 to 3.081	0.357
	Other Mixed	0.355	0.092 to 1.377	0.134
	White	0.224	0.091 to 0.551	0.001
Deprivation score categories	2	2.214	0.789 to 6.217	0.131
	3	0.961	0.393 to 2.354	0.931
	4	1.170	0.550 to 2.488	0.684
	5	1.000	-	-
Smoking status	Yes	0.838	0.283 to 2.479	0.749
	Stopped	1.407	0.330 to 5.990	0.644
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,898

There is no statistically significant evidence of associations between PAPP-A, NT or CRL and perinatal death after adjusting for each other and the other known predictors.

Table 16g: PAPP-A, NT and CRL: Results from adjusted logistic regression for neonatal death (singleton pregnancies, N=10,876)

Neonatal death (death between birth and 28 days)		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (U/L)		1.059	0.842 to 1.331	0.624
NT (mm)		1.117	0.377 to 3.312	0.842
CRL (mm)		0.989	0.931 to 1.052	0.733
Mother's age (years)		0.950	0.866 to 1.041	0.270
BMI		1.124	1.052 to 1.200	0.001
Parity	0	2.355	0.906 to 6.123	0.079
	>4	1.000	-	-
Ethnicity	South-Asian	0.470	0.140 to 1.579	0.222
	Oriental	0.658	0.072 to 5.993	0.711
	Other Mixed	0.271	0.053 to 1.397	0.119
	White	0.069	0.020 to 0.250	<0.0001
Deprivation score categories	2	5.453	1.550 to 19.187	0.008
	3	0.345	0.043 to 2.757	0.316
	4	1.162	0.394 to 3.427	0.786
	5	1.000	-	-
Smoking status	Yes	3.044	0.897 to 10.337	0.074
	Stopped	2.294	0.286 to 18.381	0.434
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,87

There is no statistically significant evidence of associations between PAPP-A, NT or CRL and neonatal death after adjusting for each other and the other known predictors.

FUNCTIONAL FORM OF POTENTIAL PROGNOSTIC FACTORS

Table 16h: PAPP-A, NT and CRL: Results from adjusted logistic regression for SGA assessing functional form of PAPP-A, NT and CRL (singleton pregnancies, N=12,299)

SGA (<10th customised centile)		Odds Ratio	95% Confidence Interval OR	p-value
Log_e(PAPP-A) (U/L)		0.636	0.580 to 0.697	<0.0001
NT (mm)		0.792	0.693 to 0.905	0.001
CRL (mm)		1.014	1.005 to 1.021	<0.0001
Mother's age (years)		1.016	1.005 to 1.026	0.003
BMI		0.996	0.983 to 1.008	0.487
Parity	0	1.187	1.063 to 1.326	0.002
	>4	1.040	0.642 to 1.686	0.873
Ethnicity	South-Asian	0.761	0.614 to 0.944	0.013
	Oriental	0.712	0.493 to 1.027	0.069
	Other Mixed	0.687	0.528 to 0.894	0.005
	White	0.646	0.531 to 0.786	<0.0001
Deprivation score categories	2	1.120	0.789 to 1.589	0.528
	3	1.024	0.751 to 1.395	0.882
	4	1.029	0.756 to 1.399	0.856
	5	1.192	0.881 to 1.612	0.255
Smoking status	Yes	2.496	2.171 to 2.869	<0.0001
	Stopped	1.152	0.866 to 1.534	0.331
IVF		0.648	0.423 to 0.992	0.046
Gestational diabetes		0.966	0.781 to 1.194	0.747

N=12,299

The log transformation was the statistically best fitting functional form for PAPP-A in this analysis in Table 16h. The linear function was selected for nuchal translucency and crown rump length. Again, all three factors of interest are statistically associated with SGA.

For crown rump length and nuchal translucency the linear function was best. The best functional form for PAPP-A was 1/sqrt (PAPP-A). Again, only PAPP-A was statistically significantly associated with preterm birth(Table 16i).

Table 16i: PAPP-A, NT and CRL: Results from adjusted logistic regression for preterm birth assessing functional form of PAPP-A, NT and CRL (singleton pregnancies, N=12453)

Preterm birth (<37 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
$1/\sqrt{\text{PAPPA (U/L)}}$		2.329	1.669 to 3.250	<0.0001
NT (mm)		1.117	0.941 to 1.326	0.204
CRL (mm)		1.005	0.994 to 1.015	0.386
Mother's age (years)		1.009	0.995 to 1.024	0.218
BMI		0.990	0.973 to 1.008	0.276
Parity	0	1.039	0.881 to 1.225	0.648
	>4	0.999	0.497 to 2.009	0.999
Ethnicity	South-Asian	0.682	0.509 to 0.914	0.011
	Oriental	0.403	0.219 to 0.741	0.003
	Other Mixed	0.663	0.4650to 0.956	0.028
	White	0.616	0.473 to 0.804	<0.0001
Deprivation score categories	2	0.862	0.524 to 1.419	0.560
	3	0.871	0.570 to 1.335	0.526
	4	0.920	0.602 to 1.404	0.698
	5	1.063	0.703 to 1.609	0.771
Smoking status	Yes	1.589	1.290 to 1.956	<0.0001
	Stopped	0.896	0.571 to 1.406	0.634
IVF		1.057	0.626 to 1.786	0.835
Gestational diabetes		1.281	0.966 to 1.697	0.085

N=12,453

16j: PAPP-A, NT and CRL: Results from adjusted logistic regression for pre-eclampsia assessing functional form of PAPP-A, NT and CRL (singleton pregnancies, N=12,321)

Pre-eclampsia		Odds Ratio	95% Confidence Interval OR	p-value
PAPP-A (u/l)		0.879	0.817 to 0.945	0.001
NT (mm)		0.792	0.592 to 1.060	0.117
CRL (mm)		1.023	1.007 to 1.039	0.006
Mother's age (years)		1.013	0.992 to 1.035	0.223
BMI		1.042	1.017 to 1.067	0.001
Parity	0	2.674	2.099 to 3.407	<0.0001
	>4	1.000	-	-
Ethnicity	South-Asian	0.557	0.369 to 0.839	0.005
	Oriental	0.288	0.111 to 0.746	0.010
	Other Mixed	0.403	0.227 to 0.713	0.002
	White	0.494	0.342 to 0.712	0.000
Deprivation score categories	2	0.710	0.376 to 1.340	0.291
	3	0.713	0.417 to 1.217	0.215
	4	0.602	0.351 to 1.032	0.065
	5	0.783	0.466 to 1.316	0.356
Smoking status	Yes	0.623	0.355 to 1.092	0.099
	Unknown	1.034	0.828 to 1.292	0.765
IVF		1.630	0.935 to 2.843	0.085
Gestational diabetes		0.834	0.531 to 1.309	0.430

N=12,321

5.3.4: PAPP-A, NT and CRL in combination

Assuming linear functions for all continuous variables, the three potential prognostic factors were then considered in combination with adjustment for confounders and known prognostic factors as discussed (Tables 16a-j). For SGA, this analysis demonstrated statistically significant associations with PAPP-A [OR 0.86 (95% CI 0.83, 0.89), p<0.0001)]; NT [OR 0.80 (95% CI 0.70, 0.92); p=0.001] and CRL [OR 1.01 (95% CI 1.00, 1.02); p=0.003)] (Table 16h).

For preterm birth (Table 16i), only PAPP-A was statistically significantly associated with reduced odds [OR 0.91 (95% CI 0.87, 0.95), p<0.0001], as seen when the factors were considered individually. For pre-eclampsia, PAPP-A was still significantly associated [OR 0.88 (95% CI 0.82, 0.95); p=0.001] and now there was evidence of increased odds of pre-eclampsia as CRL increases [OR 1.02 (95% CI 1.01, 1.04); p=0.006] (Table 16j).

There remained a statistically significantly increased odds of miscarriage as NT increases [OR 1.67 (95% CI 1.01, 2.76), p=0.047], and no evidence of associations between miscarriage and PAPP-A or CRL (Table 16d). There were no statistically significant associations between stillbirth and any of PAPP-A, NT or CRL, unlike the individual models (Table 16e). There was no evidence of associations between any of the three factors of interest and perinatal or neonatal death (Table 16f-g).

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

5.4: Discussion

5.4.1: Main Findings

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

5.4.2 Strengths and Limitations:

Our study has several strengths. This is a large cohort study looking at multiple pregnancy outcomes providing reproducible statistical results. The UK is a country where high quality and homogenous universal health care is provided to its residents free of charge irrespective of socioeconomic and other statuses. This made our cohort a reliable representative of the population avoiding bias due to skewed demographics.

Certain factors that are known to affect pregnancy outcome, such as ethnicity, parity, maternal age and BMI, socio economic deprivation, smoking status and pre-pregnancy insulin-dependent diabetes mellitus, have been adjusted for in our analysis. We have made an effort to look for lesser researched possible associations such as miscarriage and neonatal/perinatal death. Despite these strengths, our study is not without limitations. The data for potential confounding factors (existing prognostic factors) was limited to that which is routinely collected in our electronic maternity record as this was a retrospective study. Although the databases used were not designed for this particular study they are populated by qualified health professionals and data was obtained from multiple sources to allow cross-referencing and checking of outcomes. The biochemistry data is part of the National Screening Programme and thus subject to the relevant quality assurance (UK National external quality assurance scheme (UKNEQAS), Edinburgh Royal Infirmary, UK and Downs syndrome screening quality assurance and support service (DQASS), University of Plymouth UK). Our sample was determined by the number of patients available with an electronic record and outcome data and thus not determined by a sample size calculation. Most confidence intervals were quite narrow, but we recognise that non-significant findings do not necessarily mean that no prognostic association exists, and may simply reflect a low power to detect genuine associations. Nevertheless, many confidence intervals were relatively narrow and the prognostic associations identified were often strongly significant (131)

The clinical utilisation of CRL as an individual prognostic factor (i,e outside of a model using it as a continuous factor) is less clear as standard care in UK is for a single first trimester ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk.

Previous studies have assessed the prognostic value of difference in expected to observed CRL based on the last menstrual period or observed versus expected change in CRL in the first trimester and CRL as a continuous factor in multivariable analysis (52) (132, 133). The use of CRL to date a pregnancy assumes that there is no growth variation within the first trimester nor association with factors such as fetal sex, maternal age or ethnicity(133, 134). A study from the Netherlands demonstrated that CRL in the first trimester was associated with an increased risk of adverse birth outcomes ad postnatal growth acceleration (133). As standard care in the UK is only to offer one first trimester ultrasound, thus it is not possible to assess CRL change and the use of the CRL to date the pregnancy does not allow the assessment of observed to expected CRL. We thus wished to assess whether CRL, measured between the 11+2 to 14+1 week window, assessed as a continuous variable had a relationship with adverse pregnancy outcome i.e. in particular assessing extremes of the continuum.

5.5 Interpretation

The aim was to provide more evidence toward establishing if the prognostic factors of interest can be used to further inform the management of potential adverse outcomes, for example by increased surveillance for pregnant women at greater risk. The results showed evidence of associations between the potential prognostic factors and several outcomes, and the associations remained largely the same when the factors were considered in combination. Future work is now important to establish whether the findings from all prognostic factor studies are consistent by synthesizing the evidence. The evidence from this study supports the need for women with pregnancies with a low PAPP-A and increased NT being under Consultant led care and the recommendation within the RCOG guidelines (4) for increased surveillance

for SGA in pregnancies with a low PAPP-A and supports this being extended to pregnancies in the first trimester with an increased NT. At present until a model is developed that can incorporate these factors as continuous variables it would be appropriate to use accepted thresholds of<5th centile for PAPP-A and >99th centile for NT. Due to the association with low PAPP-A and PTB and PE these pregnancies should be assessed comprehensively for other risk factors for PTB and consideration given to the commencement of aspirin prior to 16 weeks. The clinical utilisation of CRL as an individual prognostic factor (i.e. outside of a model using it as a continuous factor) is less clear as standard care in the UK is for a single first trimester ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk. Thus, it is not possible to determine whether a CRL is larger or smaller than expected.

Independent prognostic factors have a broad array of potential uses in both clinical practice and health research (112). For instance, they help to define disease at diagnosis; they may be modifiable for interventions to improve outcomes; they aid the design and analysis of trials; they are confounders to consider in observational studies and unbalanced trials; and they are the building blocks of prognostic models (112). Prognostic factor research is therefore important to discover and evaluate such factors.

We emphasize that our multivariable models were fitted to examine if there is evidence of an independent association between the potential prognostic factors of interest and the maternal and fetal outcomes after adjustment for known prognostic factors. Our objective was to assess the prognostic factors themselves and not on an overall prognostic model for individual risk prediction. This is especially important since there was no external data to validate such a model (118). Future work could use new datasets to develop individual risk prediction models to tailor treatment choices to the

individual. Such models should build on the findings of this study, in terms of the prognostic factors that were identified as important.

5.6: Conclusion

The evidence from this study supports the need for women with pregnancies with a low PAPP-A and increased NT being under Consultant led care and the recommendation within the international guidelines for increased surveillance for SGA in pregnancies with a low PAPP-A and supports this being extended to pregnancies in the first trimester with an increased NT. At present until a model is developed that can incorporate these factors as continuous variables it would be appropriate to use accepted thresholds of <5th centile for PAPP-A and >99th centile for NT. Due to the association with low PAPP-A and PTB and PE these pregnancies should be assessed comprehensively for other risk factors for PTB and consideration given to the commencement of aspirin prior to 16 weeks. The clinical utilization of CRL as an individual prognostic factor (i.e. outside of a model using it as a continuous factor) is less clear as standard care in the UK is for a single first trimester ultrasound to incorporate dating (using CRL) and NT for aneuploidy risk. Thus, it is not possible to determine whether a CRL is larger or smaller than expected.

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

CHAPTER 6: CLINICAL APPLICATION

6.1 Introduction

Studies have tested the hypothesis that low maternal serum levels of PAPP-A in the first trimester are prognostic factors for adverse pregnancy outcomes associated with poor placental function (5, 6, 49, 50) . International Guidelines on "The Investigation and Management of the Small for Gestational Fetus" have recommended that pregnant women with a serum PAPP-A < 0.4MoM (5th centile) in the first trimester receive increased ultrasound surveillance for fetal growth disorders (135).

6.2 Statistics

In our population at Birmingham Women's Hospital, the retrospective cohort study from 2011-2015 included over 12,000 women and found that there was a significant relationship between PAPP-A and adverse pregnancy outcome and after multivariable analysis (i.e. adjusting for other factors) there was a lower odds of SGA [adjusted odds ratio (OR) 0.87 (95% CI 0.85,0.90)], preterm birth <37 weeks (PTB) [OR 0.92 (95%CI 0.90,0.96)], pre-eclampsia (PE) [0.91 (95% CI 0.85,0.97)] and stillbirth [OR 0.72 (95% CI 0.53,0.99)] as PAPP-A increases .

This equated in our population at Birmingham Women's Hospital to the following:

- **Background risks:** 3 out of 100 women got pre-eclampsia, 6 out of 100 had miscarriage (12-24 weeks), 35 out of 10000 had a stillbirth; 67 out of 10000 had a perinatal death and 33 out of 10000 had a neonatal death, 8 out of 100 had a preterm delivery, 16 out of 100 a small baby (<10th centile).

- **Risk if PAPP-A ≤5th centile:** 4 out of 100 got pre-eclampsia, 1 out of 100 had a miscarriage, 1 out of 100 had a stillbirth, 15 out of 1000 had a perinatal death, 6 out of 1000 a neonatal death, 26 out of 100 a small baby.

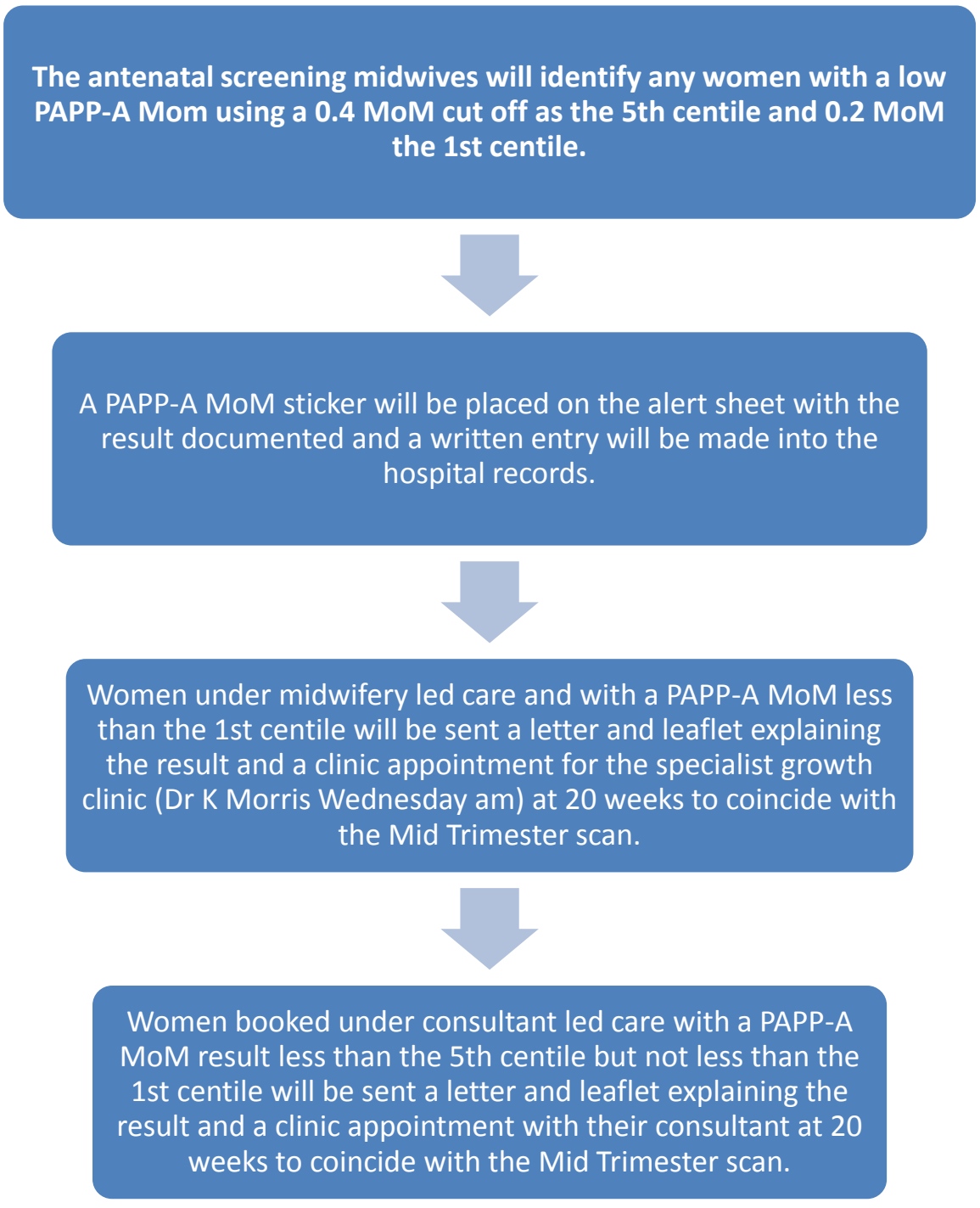
- **Risk if PAPP-A ≤ 1st centile:** 2 out of 100 had PET, 3 out of 100 had a miscarriage, 3 out of 100 had a stillbirth, 3 out of 100 had a perinatal loss, 34 out of 100 had a small baby and 27 out of 100 had a preterm delivery.

6.3 Low PAPP-A SGA pathway

The above findings and national recommendations for PAPP-A MoM to be included in the risk assessment for SGA in the RCOG SGA guideline, BWNFT will offer all women with a PAPP-A <5th centile serial growth scans. At present women will not be offered a PAPP-A estimation as part of routine care as the test has not been approved nor funded for clinical use in this manner. Women will therefore only be identified as part of the combined test for fetal chromosome anomaly screening (trisomies 21,18 and 13).

This pathway only applies to women with an isolated low PAPPA i.e. whose combined test gives a low risk for trisomy 21, 18 and 13 and in whom the nuchal translucency was normal. If women are **high risk for chromosomal aberrations or had a NT > 3.5 mm** they should follow established pathways linking with fetal medicine. If further investigations / screening are normal with a low PAPP-A MoM they should have additional screening for SGA, this should be actioned at the mid trimester ANC appointment.

Flowchart for the identification of women with low PAPP-A results:



6.3.1 The antenatal screening midwives will identify women with a low PAPP-A (Mom using a 0.4 MoM cut off as the 5th centile and 0.2 MoM the 1st centile).

1. The screening midwives will obtain the hospital notes pertaining to these results.

2. A PAPP-A MoM sticker will be placed on the alert sheet with the result documented and a written entry will be made into the hospital records.

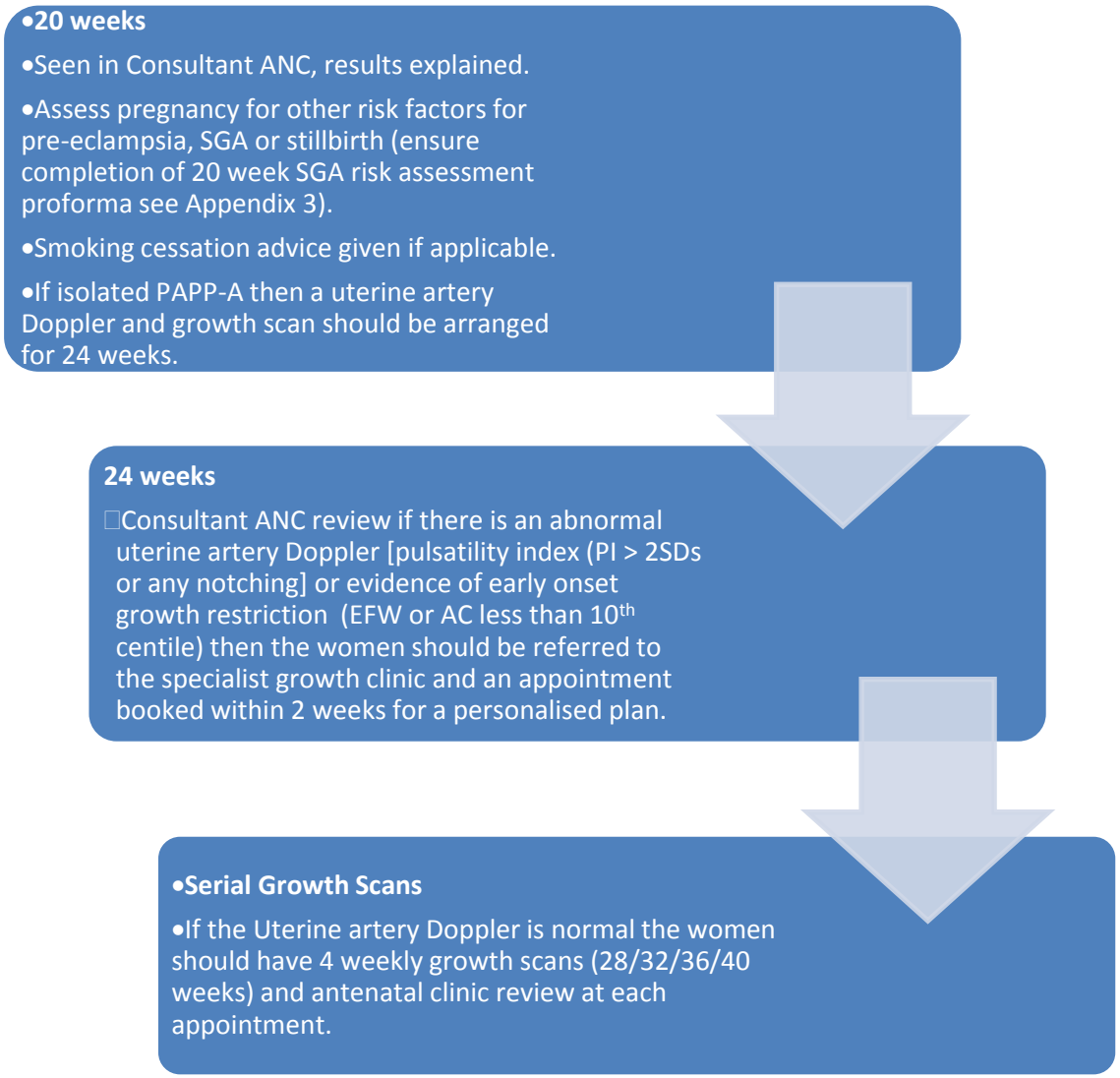
3. Women under midwifery led care will be sent a letter (Appendix 2) and a leaflet (Appendix 4) explaining the result and a clinic appointment for the specialist growth clinic at 20 weeks to coincide with the Mid Trimester scan.

4. Women with a PAPP-A MoM less than the 1st centile will also be referred to the specialist growth clinic at 20 weeks to coincide with the Mid Trimester scan. (see Appendix 3 – SGA risk assessment and referral to specialist growth clinic)

5. Women booked under consultant led care with a PAPP-A MoM result less than the 5th centile but not less than the 1st centile will be sent a letter and leaflet explaining the result and a clinic appointment with their consultant at 20 weeks to coincide with the Mid Trimester scan.

6.3.2 Antenatal clinic appointment plan schedule for women with a PAPP-A MoM less than 5th centile (not less than the 1st centile).

Flowchart for management of women with low PAPP-A results



20 weeks

- Seen in Consultant ANC, results explained (see patient information leaflet Appendix 4).
- Assess pregnancy for other risk factors for pre-eclampsia, SGA or stillbirth (ensure completion of 20 week SGA risk assessment proforma see Appendix 2).
- Smoking cessation advice given if applicable.
- If isolated PAPP-A then a uterine artery Doppler and growth scan should be arranged for 24 weeks.

24 weeks

- Consultant ANC review if there is an abnormal uterine artery Doppler [pulsatility index (PI > 2SDs or any notching] or evidence of early onset growth restriction (EFW or AC less than 10^{th} centile) then the women should be referred to the specialist growth clinic (Dr K Morris Wednesday am) and an appointment booked within 2 weeks for a personalised plan.

If the Uterine artery Doppler is normal the patient should have 4 weekly growth scans (28/32/36/40 weeks) and antenatal clinic review at each appointment.

All women with a PAPP-A MoM less than 0.2 MoM (1st centile) will be referred directly to the specialist growth clinic for a personalised plan.

6.4 Implementation and Audit

This process will be implemented from the 1st November 2016.

A database will be kept by the screening midwives and an audit of the process will be conducted 6 months following the implementation of this pathway to assess compliance.

6.5 Conclusion

Above pathway for SGA is one of the many ways to perform surveillance for SGA in women with low PAPP-A. There is a need for an accurate prediction model before performing additional surveillance for other adverse pregnancy outcomes as individual predictive values of PAPP-A are poor.

Once a model is worked out, the additional surveillance for multiple adverse pregnancy outcomes like pre eclampsia, stillbirth could include more frequent blood pressure measurements, Women may have frequent ‘day assessment ‘appointments to check blood pressure, urine dipstick test for protein and fetal surveillance in the form of cardiotocogram and checking with fetal movements. Antenatal low dose aspirin from the beginning of second trimester could be a potential intervention which needs to be explored further to prove or disprove its benefit in the context of low PAPP-A.

CHAPTER 7: CONCLUSION

7.1 Introduction

This thesis achieves the main objectives set out in chapter 3 - in that it reports:

1. systematic review and meta-analysis on Association of serum PAPP-A levels in first trimester with small-for-gestational-age and other adverse pregnancy outcomes.
2. 2.Retrospective cohort study examining the association of maternal serum PAPP-A levels, in first trimester with adverse pregnancy outcomes.

In the previous chapters, detailed discussion of the above said topics including limitations and strengths has been presented. This chapter focuses on the main findings of the work undertaken and discusses its strengths and limitations leading to general recommendations for research and practice.

7.2 Summary of main findings

7.2.1 Systematic review: Key points:

- 1715 citations found in literature.
- 310 papers were read in full: 32 included in the review with 175,240 women tested.

- Low maternal serum PAPP-A in the first trimester has shown to be associated with adverse pregnancy outcome. Though the association is statistically significant the predictive values are poor which would mean that although women with a low PAPP-A are at increased risk of an adverse outcome, the clear majority of these women will have a normal pregnancy outcome and the majority of women with an adverse outcome will have a normal PAPP-A.

7.2.2 Retrospective cohort study: Key points

- Retrospective cohort study conducted at Birmingham woman's hospital NHS foundation trust between 2011 and 2015 with all necessary approvals.

- Included 12,592 pregnancies which resulted in 852 (6.8%) PTD, 352 (2.8%) PE, 1824 (14.5%) SGA, 73 (0.6%) miscarriages, 37 (0.29%) stillbirths and 38 (0.30%) neonatal deaths.

- There were statistically significant lower odds of SGA, PTD, PE and stillbirth as PAPP-A increases whereas no statistically significant association between miscarriage, perinatal or neonatal death was found with PAPP-A.

- Low PAPP-A is an independent risk factor for adverse pregnancy outcomes, especially SGA. Further work needs to consider how this predictive factor can be combined with other risk factors to give accurate prediction for an individual in a prediction model.

7.3 Strengths of the thesis

7.3.1 Strengths of systematic review

- Comprehensive and robust search strategy.

- The review was compliant with recommended techniques for quality assessment, (63) (54) performing and interpreting meta-analyses and reporting of our findings (56, 71).

- Both prognostic and predictive ability of PAPP-A was demonstrated in the review.

- This review was followed by the original research (cohort study) answering the same question.

7.3.2 Strengths of the cohort study

- Large cohort study looking at multiple pregnancy outcomes providing reproducible statistical results.

- Our cohort a true representative of the general population due to the health service framework in the UK which avoided bias caused by skewed demographics.

- Confounding factors were taken in to account which are known to affect pregnancy outcome and have been adjusted for in our analysis.

- We have made an effort to look for lesser researched possible associations such as miscarriage and neonatal/perinatal death.

7.3.3 Strengths of PAPP-A as a marker:

- PAPP-A is an established marker for aneuploidy and is part of first trimester combined screening which is offered to every pregnant lady in the UK and possibly worldwide. Therefore, if proved to be associated with adverse pregnancy outcomes, PAPP-A will be a very cost effective biomarker guiding the clinicians to risk stratify the pregnancies without any extra cost / intervention to the patient/healthcare system. We do however appreciate extra surveillance needed for pregnancies stratified as high risk would need extra cost.

7.4 Limitations of the thesis

7.4.1 Limitations of systematic review

- Limitations within the review relate in the first instance to limitations within the included studies.

- There was significant statistical heterogeneity in some analyses which could not be accounted for when examining clinical characteristics nor study design and

was thus unexplained.

- Lack of data and thus for some bivariate meta-analysis could not be performed study.

- One limitation in the methodology employed is the need to consider PAPP-A as a dichotomous variable i.e. categorization using a threshold. This has limitations statistically as it can lead to a loss of power as much of the information is lost, classifying very similar factor values as different in opposite sides of the cut-off point and the concealment of a potential non-linear relationship between the outcome and the factor of interest (105-107).

7.4.2 Limitations of Cohort Study

- The data for potential confounding factors was limited to that which is routinely collected in our electronic maternity record as this was a retrospective study.

- Although the databases used were not designed for this study they are populated by qualified health professionals and data was obtained from multiple sources to allow cross-referencing and checking of outcomes.

- Our sample was determined by the number of patients available with an electronic record and outcome data and thus not determined by a sample size calculation. However, the size of the cohort and the number of events for each outcome were sufficient to test the prediction of the three pre-specified markers under consideration (127).

7.4.3 Limitations of PAPP-A as a marker

One of the limitations of PAPP-A as a marker would be that it can be used in antenatal period rather than prenatal period. Thus, it really does not help in 'preventing' the adverse event as the under lying pathophysiology leading to adverse pregnancy event would have already established. Hence the window of golden opportunity to prevent the underlying mechanism leading to the adverse pregnancy outcome is lost. However, it does give us a chance to be more vigilant in the 'high risk' pregnancies where there is scope to change the outcome of the pregnancy by being more vigilant and look out for early signs of adverse pregnancy outcomes thus identifying and to some extent slowing down the process of pathology leading to adverse pregnancy events. Currently it is attempted by starting women on low dose aspirin before 16 weeks of pregnancy, more frequent blood pressure monitoring and regular growth scans under the care of a consultant obstetrician.

7.5 Recommendations for practice

- The association could be mentioned during the counselling for screening for Down's syndrome (20) which is done universally in the UK. This needs to be done in a balanced way considering the poor predictive value of the test.

- The association between PAPP-A and SGA has been identified and there are national guidelines suggesting increased surveillance for impaired fetal growth.

Our systematic review and the cohort study confirm the association between low first trimester maternal serum PAPP-A and SGA.

- Systematic review and cohort study explained in this thesis also demonstrates the association of first trimester low maternal serum PAPP-A in the first trimester has an association with other adverse pregnancy namely pre term birth, pre- eclampsia. At present, there are no recommendations/guidelines for surveillance for other adverse outcomes e.g. miscarriage, preterm birth, pre-eclampsia nor for interventions due to the increased risk of stillbirth (e.g. induction at term). We do appreciate that the possible extra surveillance comes with extra cost and increases maternal anxiety.

- Perhaps a national guideline for the management of pregnancies with low PAPP-A suggesting the surveillance (e.g. regular growth scans, glucose tolerance test) and possible interventions for SGA and other adverse pregnancy outcomes (e.g. Aspirin, induction of labor at term) could guide clinicians in looking after this cohort of pregnant women.

- It must be recognized that for the individual predictive value are poor and thus the majority of adverse outcomes will occur in the group without an abnormally low PAPP-A and thus outside any guidelines for increased surveillance or intervention.

7.6 Recommendations for research

- There is further need to develop robust and accurate prediction models that can allow modern day obstetrics to practice truly stratified medicine.

- Other possible predictors (e.g. previous obstetric history, CRL: B HCG, uterine artery Doppler) could be studied and work could be done on creating a possible prediction model to stratify the risk assignment for the pregnancy.

- There is scope to look out for prenatal markers along with antenatal markers to identify women with high risk pregnancies making it possible to prevent them by treating them even before

- **Publications from our study**

Morris RK, Bilagi A, Devani P, Kilby MD. Association of serum PAPP-A levels in first trimester with small for gestational age and adverse pregnancy outcomes: systematic review and meta-analysis. Prenat Diagn. 2017;37(3):253-65.

Ashwini Bilagi, Danielle L Burke, Richard D Riley, Ian Mills, Mark D Kilby, R. Katie Morris. Association of maternal serum PAPP-A levels, nuchal translucency and crown rump length in first trimester with adverse pregnancy outcomes: Retrospective cohort study. Accepted on 11/5/2017 by Prenat Diagn.

ASSOCIATION OF MATERNAL SERUM PAPP-A LEVELS IN FIRST TRIMESTER WITH SMALL-FOR-GESTATIONAL-AGE AND OTHER ADVERSE PREGNANCY OUTCOMES: SYSTEMATIC REVIEW AND RETROSPECTIVE COHORT STUDY

By Ashwini Bilagi

A thesis submitted to the University of Birmingham

For the degree of

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APPENDICES AND REFERENCES

Appendix 1 Search strategy for electronic database identification of Association of serum PAPP-A levels in first trimester with small-for-gestational-age and adverse pregnancy outcomes

A) PAPP-A and Stillbirth

1. EMBASE; exp PREGNANCY DIABETES MELLITUS

2. EMBASE; (impaired AND blood AND sugar AND in AND pregnancy).ti,ab;

4. EMBASE; (diabetes AND mellitus AND in AND pregnancy).ti,ab;

5. EMBASE; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

6. EMBASE; PAPP-A.ti,ab;

7. EMBASE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

8. EMBASE; 5 OR 6 OR 7;

9. EMBASE; 1 OR 2 OR 3 OR 4;

10. EMBASE; 8 AND 9;

11. EMBASE; (impaired AND glucouse AND tolerance AND in AND pregnancy).ti,ab;

12. EMBASE; (insulin AND resistance AND in AND pregnancy).ti,ab;

13. EMBASE; 8 AND 12;

14. MEDLINE; exp PREGNANCY DIABETES MELLITUS/;

15. MEDLINE; (impaired AND blood AND sugar AND in AND pregnancy).ti,ab;

16. MEDLINE; (gestational AND diabetes AND mellitus).ti,ab;

17. MEDLINE; (diabetes AND mellitus AND in AND pregnancy).ti,ab;

18. MEDLINE; (impaired AND glucose AND tolerance AND in AND pregnancy).ti,ab;

19. MEDLINE; (insulin AND resistance AND in AND pregnancy).ti,ab;

20. MEDLINE; exp DIABETES, GESTATIONAL/ OR exp GLUCOSE TOLERANCE TEST/;

21. MEDLINE; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

22. MEDLINE; PAPP-A.ti,ab;

23. MEDLINE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

24. MEDLINE; 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20;

25. MEDLINE; 21 OR 22 OR 23;

26. MEDLINE; 24 AND 25;

27. CINAHL; exp PREGNANCY DIABETES MELLITUS/;

28. CINAHL; (impaired AND blood AND sugar AND in AND pregnancy).ti,ab;

29. CINAHL; (gestational AND diabetes AND mellitus).ti,ab;

30. CINAHL; (diabetes AND mellitus AND in AND pregnancy).ti,ab;

31. CINAHL; (impaired AND glucose AND tolerance AND in AND pregnancy).ti,ab;

32. CINAHL; (insulin AND resistance AND in AND pregnancy).ti,ab;

33. CINAHL; exp DIABETES, GESTATIONAL/ OR exp GLUCOSE TOLERANCE TEST/;

34. CINAHL; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

35. CINAHL; PAPP-A.ti,ab;

36. CINAHL; (Pregnancy AND associated AND plasma AND protein).ti,ab;

37. CINAHL; 34 OR 35 OR 36;

38. CINAHL; 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33;

39. CINAHL; 37 AND 38;

B) PAPP-A & Prematurity

4. EMBASE; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

5. EMBASE; PAPP-A.ti,ab;

6. EMBASE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

7. EMBASE; 4 OR 5 OR 6;

8. EMBASE; exp PREMATURE LABOR/;

9. EMBASE; exp PREMATURITY/;

10. EMBASE; (pre AND term AND delivery).ti,ab;

11. EMBASE; (pre AND term AND labour).ti,ab;

12. EMBASE; prematurity.ti,ab;

13. EMBASE; (pre AND term AND birth).ti,ab;

14. EMBASE; (premature AND birth).ti,ab;

17. EMBASE; (premature AND delivery).ti,ab;

18. EMBASE; 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 17;

19. EMBASE; 7 AND 18;

20. MEDLINE; PAPP-A.ti,ab;

21. MEDLINE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

22. MEDLINE; 4 OR 20 OR 21;

23. MEDLINE; exp PREMATURE LABOR/;

24. MEDLINE; exp PREMATURITY/;

25. MEDLINE; (pre AND term AND delivery).ti,ab;

26. MEDLINE; (pre AND term AND labour).ti,ab;

27. MEDLINE; prematurity.ti,ab;

28. MEDLINE; (pre AND term AND birth).ti,ab;

29. MEDLINE; (premature AND birth).ti,ab;

30. MEDLINE; (premature AND delivery).ti,ab;

31. MEDLINE; 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 15 OR 30;

32. MEDLINE; 22 AND 31;

33. MEDLINE; exp PREGNANCY-ASSOCIATED PLASMA PROTEIN-A/;

35. MEDLINE; 20 OR 21 OR 33;

36. MEDLINE; 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30;

39. MEDLINE; 35 AND 36;

40. CINAHL; PAPP-A.ti,ab;

41. CINAHL; (Pregnancy AND associated AND plasma AND protein).ti,ab;

50. CINAHL; 40 OR 41;

52. CINAHL; exp PREMATURE LABOR/;

53. CINAHL; exp PREMATURITY/;

54. CINAHL; (pre AND term AND delivery).ti,ab;

55. CINAHL; (pre AND term AND labour).ti,ab;

56. CINAHL; prematurity.ti,ab;

57. CINAHL; (pre AND term AND birth).ti,ab;

58. CINAHL; (premature AND birth).ti,ab;

59. CINAHL; (premature AND delivery).ti,ab;

60. CINAHL; 52 OR 53 OR 54 OR 55 OR 56 OR 57 OR 58 OR 59;

61. CINAHL; 50 AND 60;

C) PAPP-A & Small for gestation

1. EMBASE; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

2. EMBASE; (pregnancy AND associated AND plasma AND protein AND A).ti,ab;

3. EMBASE; PAPP-A.ti,ab

4. EMBASE; 1 OR 2 OR 3;

5. EMBASE; exp INTRAUTERINE GROWTH RETARDATION/ OR exp FETUS GROWTH/ OR exp BIRTH WEIGHT/;

6. EMBASE; (intrauterine AND growth AND retardation).ti,ab;

7. EMBASE; (intrauterine AND growth AND restriction).ti,ab;

8. EMBASE; (birth AND weight).ti,ab;

9. EMBASE; (fetus AND growth).ti,ab;

10. EMBASE; (fetal AND growth AND anomaly).ti,ab;

11. EMBASE; (prenatal AND growth).ti,ab;

12. EMBASE; (small AND for AND gestational AND age).ti,ab;

13. EMBASE; (small AND for AND gestation AND age).ti,ab;

14. EMBASE; 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13;

15. EMBASE; 4 AND 14;

16. MEDLINE; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

17. MEDLINE; (pregnancy AND associated AND plasma AND protein AND A).ti,ab;

18. MEDLINE; PAPP-A.ti,ab;

19. MEDLINE; 16 OR 17 OR 18;

20. MEDLINE; exp INTRAUTERINE GROWTH RETARDATION/ OR exp FETUS GROWTH/ OR exp BIRTH WEIGHT/;

21. MEDLINE; (intrauterine AND growth AND retardation).ti,ab;

22. MEDLINE; (intrauterine AND growth AND restriction).ti,ab;

23. MEDLINE; (birth AND weight).ti,ab;

24. MEDLINE; (fetus AND growth).ti,ab;

25. MEDLINE; (fetal AND growth AND anomaly).ti,ab;

26. MEDLINE; (prenatal AND growth).ti,ab;

27. MEDLINE; (small AND for AND gestational AND age).ti,ab;

28. MEDLINE; (small AND for AND gestation AND age).ti,ab;

29. MEDLINE; 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28;

30. MEDLINE; 19 AND 29;

31. MEDLINE; 19 AND 29;

32. CINAHL; exp PREGNANCY ASSOCIATED PLASMA PROTEIN A/;

33. CINAHL; (pregnancy AND associated AND plasma AND protein AND A).ti,ab;

34. CINAHL; PAPP-A.ti,ab;

35. CINAHL; 32 OR 33 OR 34;

36. CINAHL; exp INTRAUTERINE GROWTH RETARDATION/ OR exp FETUS GROWTH/ OR exp BIRTH WEIGHT/;

37. CINAHL; (intrauterine AND growth AND retardation).ti,ab;

38. CINAHL; (intrauterine AND growth AND restriction).ti,ab;

39. CINAHL; (birth AND weight).ti,ab;

40. CINAHL; (fetus AND growth).ti,ab;

41. CINAHL; (fetal AND growth AND anomaly).ti,ab;

42. CINAHL; (prenatal AND growth).ti,ab;

43. CINAHL; (small AND for AND gestational AND age).ti,ab;

44. CINAHL; (small AND for AND gestation AND age).ti,ab;

45. CINAHL; 36 OR 37 OR 38 OR 39 OR 40 OR 41 OR 42 OR 43 OR 44;

46. CINAHL; 35 AND 45;

D) PAPP-A & PET

1. MEDLINE; exp PREGNANCY-ASSOCIATED PLASMA PROTEIN-A/;

2. MEDLINE; PAPP-A.ti,ab;

3. MEDLINE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

4. MEDLINE; 1 OR 2 OR 3;

5. MEDLINE; exp PRE-ECLAMPSIA/;

6. MEDLINE; Pre-eclampsia. Ti,ab;

7. MEDLINE; (toxaemia AND of AND pregnancy).ti,ab;

8. MEDLINE; exp HYPERTENSION, PREGNANCY-INDUCED/;

9. MEDLINE; exp PREGNANCY COMPLICATIONS, CARDIOVASCULAR/ OR exp HYPERTENSION,
PREGNANCY-INDUCED/;

10. MEDLINE; (pregnancy AND induced AND hypertension).ti,ab;

11. MEDLINE; 5 OR 6 OR 7 OR 8 OR 9 OR 10;

12. MEDLINE; 4 AND 11;

13. MEDLINE; exp PREGNANCY-ASSOCIATED PLASMA PROTEIN-A/;

14. MEDLINE; PAPP-A.ti,ab;

15. MEDLINE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

16. MEDLINE; 13 OR 14 OR 15

17. MEDLINE; exp PRE-ECLAMPSIA/

18. MEDLINE; Pre eclampsia.ti,ab;

19. MEDLINE; (toxaemia AND of AND pregnancy).ti,ab;

20. MEDLINE; exp HYPERTENSION, PREGNANCY-INDUCED/;

21. MEDLINE; exp PREGNANCY COMPLICATIONS, CARDIOVASCULAR/ OR exp HYPERTENSION,PREGNANCY-INDUCED/;

22. MEDLINE; (pregnancy AND induced AND hypertension).ti,ab;

23. MEDLINE; 17 OR 18 OR 19 OR 20 OR 21 OR 22;

24. MEDLINE; 16 AND 23;

25. EMBASE; exp PREGNANCY-ASSOCIATED PLASMA PROTEIN-A/;

26. EMBASE; (Pregnancy AND associated AND plasma AND protein).ti,ab;

27. EMBASE; 25 OR 2 OR 26;

28. EMBASE; (toxaemia AND of AND pregnancy).ti,ab;

29. EMBASE; exp HYPERTENSION, PREGNANCY-INDUCED/;

30. EMBASE; exp PREGNANCY COMPLICATIONS, CARDIOVASCULAR/ OR exp HYPERTENSION,PREGNANCY-INDUCED/;

31. EMBASE; (pregnancy AND induced AND hypertension).ti,ab;

32. EMBASE; exp MATERNAL HYPERTENSION/;

33. EMBASE; (gestational AND hypertension).ti,ab;

34. EMBASE; 28 OR 29 OR 30 OR 31 OR 32 OR 33; 110567 results.

35. EMBASE; 27 AND 34;

13. CINAHL; exp PREGNANCY-ASSOCIATED PLASMA PROTEIN-A/;

14. CINAHL; PAPP-A.ti,ab;

15. CINAHL; (Pregnancy AND associated AND plasma AND protein).ti,ab;

16. CINAHL; 13 OR 14 OR 15;

17. CINAHL; exp PRE-ECLAMPSIA/;

18. CINAHL; (toxaemia AND of AND pregnancy).ti,ab;

19. CINAHL; exp HYPERTENSION, PREGNANCY-INDUCED/;

20. CINAHL; exp PREGNANCY COMPLICATIONS, CARDIOVASCULAR/ OR exp HYPERTENSION,

PREGNANCY-INDUCED/;

21. CINAHL; (pregnancy AND induced AND hypertension).ti,ab;

22. CINAHL; 17 OR 18 OR 19 OR 20 0R 21;

23. CINAHL; 16 AND 22

Appendix 2: Data Collection Form

Data Collection Sheet

Date: _____

Reviewer ID: _____ **Paper No:** _____

Year Of Publication: _____ **Language:** _____

Region study performed: _____

Section A: Study Selection

Population:

1) Low Risk ☐ 2) High Risk ☐ 3) Unselected ☐ 4) Unreported ☐

Index test:

Reference number _____

Other _____

Reference Test / Outcome Measure:

Fetal growth

1) SGA ☐ 4) Pre term birth ☐

2) Pre eclampsia ☐ 5)Stillbirth ☐

3) Gestational diabetes ☐

2 x 2 Table Possible:

Yes ☐ No ☐

Study Selected:

Yes ☐ No ☐ Give reason if NO _____

Appendix 3: The Standards of Reporting in Diagnostic Accuracy (STARD) checklist.

Section and Topic	Item		Code 1 2 3 4
TITLE, ABSTRACT AND KEYWORDS			
	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading " sensitivity and specificity")	**Yes □ No □ Unclear □ N/A □**
INTRODUCTION			
	2	State the research questions or aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups	**Yes □ No □ Unclear □ N/A □**
METHODS			
Participants	**3**	Describe the **study population**: the inclusion and exclusion criteria and the settings and locations where the data were collected.	**Yes □ No □ Unclear □ N/A □**
	4	Describe **participant recruitment**: was this based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	**Yes □ No □ Unclear □ N/A □**

	5	Describe **participant sampling**: was this a consecutive series of participants defined by selection criteria in items 3 and 4? If not, specify how participants were further selected.	**1= consecutive 2=random** **3= unclear 4=N/a**
	6	Describe data collection: was data collection planned before the index tests and reference standard were performed (prospective study) or after (retrospective study)?	**1= prospective 2= retrospective** **3= unclear 4= N/a**
Test Methods	**7**	Describe the reference standards and its rationale.	**Yes □ No □ Unclear □ N/A □**
	8	Describe technical specifications of material and methods involved, including how and when measurements were taken, or cite references for a) index test or b) reference test	**Yes □ No □ Unclear □ N/A □**
	9	Describe definition of and rationale for the units, cut-off points, or categories of the results of the a) index test and b) reference standard.	**Yes □ No □ Unclear □ N/A □**
	10	Describe the number, training and expertise of the persons executing and reading the a) index tests and b) reference standards.	**Yes □ No □ Unclear □ N/A □**
	11	Were the readers of the a) index test and b) reference standards blind (masked) to the results of the other test?	**Yes □ No □ Unclear □ N/A □**

		Describe any other clinical information available to the readers.	
Statistical Methods	**12**	Describe methods for calculating or comparing methods of a) diagnostic accuracy and the statistical methods used to b) quantify uncertainty (e.g. 95% CI)	**Yes □ No □ Unclear □ N/A □**
	13	Describe methods for calculating test reproducibility, if done.	**Yes □ No □ Unclear □ N/A □**
RESULTS			
Participants	**14**	Report when study was done, including beginning and ending dates of recruitment	**Yes □ No □ Unclear □ N/A □**
	15	Report clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, co morbidity, current treatments, recruitment centres)	**Yes □ No □ Unclear □ N/A □**
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test.	**Yes □ No □ Unclear □ N/A □**
Test results	**17**	Report time interval from the index tests to the reference standard, and any treatment administered between.	**Yes □ No □ Unclear □ N/A □**
	18	Report distribution of severity of disease (define criteria) in	**Yes □ No □ Unclear □ N/A □**

		those with the target condition; other diagnoses in participants without the target condition.	
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	**Yes** □ **No** □ **Unclear** □ **N/A** □
	20	Report any adverse events form performing the index tests or the reference standard.	**Yes** □ **No** □ **Unclear** □ **N/A** □
Estimates	**21**	Report estimates of a) diagnostic accuracy and b) measures of statistical uncertainty (e.g. 95% CI)	**Yes** □ **No** □ **Unclear** □ **N/A** □
	22	Report how indeterminate results, missing responses and outliers of the index tests were handled.	**Yes** □ **No** □ **Unclear** □ **N/A** □
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centres, if done.	**Yes** □ **No** □ **Unclear** □ **N/A** □
	24	Report estimates of test reproducibility, if done.	**Yes** □ **No** □ **Unclear** □ **N/A** □
DISCUSSION			
	25	Discuss the clinical applicability of the study findings.	**Yes** □ **No** □ **Unclear** □ **N/A** □

Appendix 4: QUADAS-2 tool: Risk of bias and applicability judgments

Domain 1: Patient selection	
A. Risk of bias	
Describe methods of patient selection:	
• **Was a consecutive or random sample of patients enrolled?**	Yes/No/Unclear
• **Was a case-control design avoided?**	Yes/No/Unclear
• **Did the study avoid inappropriate exclusions?**	Yes/No/Unclear
Could the selection of patients have introduced bias?	RISK: LOW/HIGH/UNCLEAR
B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that the included patients do not match the review question?	CONCERN: LOW/HIGH/UNCLEAR
Domain 2: Index test(s) ***(if more than 1 index test was used, please complete for each test)***	
A. Risk of bias	
Describe the index test and how it was conducted and interpreted:	
• **Were the index test results interpreted without knowledge of the results of the reference standard?**	Yes/No/Unclear
• **If a threshold was used, was it pre-specified?**	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	RISK: LOW/HIGH/UNCLEAR
B. Concerns regarding applicability	

Is there concern that the index test, its conduct, or interpretation differ from the review question?	CONCERN: LOW/HIGH/UNCLEAR
Domain 3: Reference standard	
A. Risk of bias	
Describe the reference standard and how it was conducted and interpreted:	
• **Is the reference standard likely to correctly classify the target condition?**	Yes/No/Unclear
• **Were the reference standard results interpreted without knowledge of the results of the index test?**	Yes/No/Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	RISK: LOW/HIGH/UNCLEAR
B. Concerns regarding applicability	
Is there concern that the target condition as defined by the reference standard does not match the review question?	CONCERN: LOW/HIGH/UNCLEAR
Domain 4: Flow and timing	
A. Risk of bias	
Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):	
Describe the time interval and any interventions between index test(s) and reference standard:	
• **Was there an appropriate interval between index test(s) and reference standard?**	Yes/No/Unclear
• **Did all patients receive a reference standard?**	Yes/No/Unclear
• **Did patients receive the same reference standard?**	Yes/No/Unclear
• **Were all patients included in the analysis?**	Yes/No/Unclear
Could the patient flow have introduced bias?	RISK: LOW/HIGH/UNCLEAR

Appendix 5: References of included papers for systematic review on Association of serum PAPP-A levels in first trimester with SGA AND other adverse pregnancy outcomes

1. Barrett SL, Bower C, Hadlow NC. Use of the combined first-trimester screen result and low PAPP-A to predict risk of adverse fetal outcomes. Prenat Diagn. 2008;28(1):28-35.

2. She B-Q, Chen S-C, Lee F-K, Cheong M-L, Tsai M-S. Low Maternal Serum Levels of Pregnancy-associated Plasma Protein-A During the First Trimester are Associated with Subsequent Preterm Delivery with Preterm Premature Rupture of Membranes. Taiwanese Journal of Obstetrics and Gynecology. 2007;46(3):242-7.

3. Carbone JF, Tuuli MG, Bradshaw R, Liebsch J, Odibo AO. Efficiency of first-trimester growth restriction and low pregnancy-associated plasma protein-A in predicting small for gestational age at delivery. Prenat Diagn. 2012;32(8):724-9.

4. Mei-Leng Cheong B-QS, Su-Chee Chen1, Fa-Kung Lee2, Ming-Song Tsai*. CAN FIRST-TRIMESTER MATERNAL SERUM LEVEL OF

PREGNANCY-ASSOCIATED PLASMA PROTEIN-A

PREDICT SUBSEQUENT FETAL GROWTH RESTRICTION? Taiwanese J Obstet Gynecol JUNE 2005;44(2):148-52.

5. Conserva V, Signaroldi M, Mastroianni C, Stampalija T, Ghisoni L, Ferrazzi E. Distinction between fetal growth restriction and small for gestational age newborn weight enhances the prognostic value of low PAPP-A in the first trimester. Prenat Diagn. 2010;30(10):1007-9.

6. Dane B, Dane C, Kiray M, Cetin A, Koldas M, Erginbas M. Correlation between first-trimester maternal serum markers, second-trimester uterine artery doppler indices and pregnancy outcome. Gynecol Obstet Invest. 2010;70(2):126-31.

7. Dane B, Dane C, Batmaz G, Ates S, Dansuk R. First trimester maternal serum pregnancy-associated plasma protein-A is a predictive factor for early preterm delivery in normotensive pregnancies. Gynecol Endocrinol. 2013;29(6):592-5.

8. D'Antonio F, Rijo C, Thilaganathan B, Akolekar R, Khalil A, Papageourgiou A, et al. Association between first-trimester maternal serum pregnancy-associated plasma protein-A and obstetric complications. Prenat Diagn. 2013;33(9):839-47.

9. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol. 2004;191(4):1446-51.

10. Fox NS, Chasen ST. First trimester pregnancy associated plasma protein-A as a marker for poor pregnancy outcome in patients with early-onset fetal growth restriction. Prenat Diagn. 2009;29(13):1244-8.

11. Goetzinger KR, Singla A, Gerkowicz S, Dicke JM, Gray DL, Odibo AO. The efficiency of first-trimester serum analytes and maternal characteristics in predicting fetal growth disorders. Am J Obstet Gynecol. 2009;201(4):412 e1-6.

12. Goetzinger KR, Cahill AG, Macones GA, Odibo AO. Association of first-trimester low PAPP-A levels with preterm birth. Prenat Diagn. 2010;30(4):309-13.

13. Cervino Gomez GRL. Association of first trimester PAPP-A with small for gestational age infant and other adverse pregnancy outcomes. J Matern Fetal Neonatal Med. 2014;27 (S1).

14. Jelliffe-Pawlowski LL, Shaw GM, Currier RJ, Stevenson DK, Baer RJ, O'Brodovich HM, et al. Association of early-preterm birth with abnormal levels of routinely collected first- and second-trimester biomarkers. Am J Obstet Gynecol. 2013;208(6):492 e1-11.

15. Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther. 2011;29(2):148-54.

16. Kavak ZN, Basgul A, Elter K, Uygur M, Gokaslan H. The efficacy of first-trimester PAPP-A and free beta hCG levels for predicting adverse pregnancy outcome. J Perinat Med. 2006;34(2):145-8.

17. Kirkegaard I, Henriksen TB, Torring N, Uldbjerg N. PAPP-A and free beta-hCG measured prior to 10 weeks is associated with preterm delivery and small-for-gestational-age infants. Prenat Diagn. 2011;31(2):171-5.

18. Krantz D, Goetzl L, Simpson JL, Thom E, Zachary J, Hallahan TW, et al. Association of extreme
first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. Am J Obstet Gynecol. 2004;191(4):1452-8.

19. Kwik M, Morris J. Association between first trimester maternal serum pregnancy associated plasma protein-A and adverse pregnancy outcome. Aust N Z J Obstet Gynaecol. 2003;43(6):438-42.

20. Leung TY, Sahota DS, Chan LW, Law LW, Fung TY, Leung TN, et al. Prediction of birth weight by fetal crown-rump length and maternal serum levels of pregnancy-associated plasma protein-A in the first trimester. Ultrasound Obstet Gynecol. 2008;31(1):10-4.

21. Marttala J, Peuhkurinen S, Laitinen P, Gissler M, Nieminen P, Ryynanen M. Low maternal PAPP-A is associated with small-for-gestational age newborns and stillbirths. Acta Obstet Gynecol Scand. 2010;89(9):1226-8.

22. Montanari L, Alfei A, Albonico G, Moratti R, Arossa A, Beneventi F, et al. The impact of first-trimester serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A on the diagnosis of fetal growth restriction and small for gestational age infant. Fetal Diagn Ther. 2009;25(1):130-5.

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24. Patil M, Panchanadikar TM, Wagh G. Variation of papp-a level in the first trimester of pregnancy and its clinical outcome. Journal of obstetrics and gynaecology of India. 2014;64(2):116-9.

25. Pilalis A, Souka AP, Antsaklis P, Daskalakis G, Papantoniou N, Mesogitis S, et al. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11-14 weeks' gestation. Ultrasound Obstet Gynecol. 2007;29(2):135-40.

26. Viorica Radoi M, L.C. Bohiltea, MD. Pregnancy-Associated Plasma Protein A and Pregnancy Outcomes. gineco ro maternal fetal medicine. 2009;5(1).

27. Ranta JK, Raatikainen K, Romppanen J, Pulkki K, Heinonen S. Decreased PAPP-A is associated with preeclampsia, premature delivery and small for gestational age infants but not with placental abruption. Eur J Obstet Gynecol Reprod Biol. 2011;157(1):48-52.

28. Saruhan Z, Ozekinci M, Simsek M, Mendilcioglu I. Association of first trimester low PAPP-A levels with adverse pregnancy outcomes. Clinical and experimental obstetrics & gynecology. 2012;39(2):225-8.

29. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. The Journal of clinical endocrinology and metabolism. 2002;87(4):1762-7.

30. Spencer K, Yu CK, Cowans NJ, Otigbah C, Nicolaides KH. Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free beta-hCG and with second-trimester uterine artery Doppler. Prenat Diagn. 2005;25(10):949-53.

31. Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin-A. Prenat Diagn. 2003;23(12):990-6.

32. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn. 2002;22(9):778-82.

Appendix 6: Characteristics of included studies for systematic review of association and prediction of first trimester maternal serum pregnancy associated plasma protein a (PAPP-A) and adverse pregnancy outcome.

First Author, year	Population (including risk), Study design, Setting (country).	Number included in analyses	Time of test (weeks)	Details of index test (timing, method, threshold)	Details of reference standard (method, timing, threshold)
Barrett et al 2008	Risk: unselected Incl :Singleton pregnancy Excl: fetal demise, multiple Pregnancies, chromosomal or structural abnormalities Recruitment: Unreported, over 2 years, Australia	10273	11-13+6	Kryptor analyser (Brahms AG, Berlin, Germany), PAPP-A ≤ 0.3MoM	Pregnancy loss (miscarriage, stillbirth and neonatal death) Low birth weight < 2500 g Preterm birth <37, 34 or 28 completed weeks
Bo-Quing She et al 2007	Risk: Unselected Incl: Singleton	2561	10-13	Alpha software (Logical Medical Systems) for MoM	Preterm delivery <37 weeks

	Excl : multiple pregnancies, spontaneous abortion, abnormal fetal karyotype, fetal structural anomalies, intrauterine fetal death. Recruitment: Retrospective, case control Taiwan				
Carbone et al 2012	Risk: Unselected Incl: Singleton pregnancy with first trimester combined screening Excl: Multiple pregnancy, structural and chromosomal anomaly Recruitment: Retrospective cohort study, July 2003-February 2009 USA	3329	10-14	Not reported PAPP-A<5th centile for gestational age	Small for gestational age: birth weight<5th centile ,<10th centile for gestational age*

Cheong et al 2005	Risk: women undergoing Down's syndrome screening Incl: non-smoking women with a singleton Excl : incomplete or incorrect chart record , chromosomal/major structural abnormalities, abortion at less than 24/40, and major maternal disease Recruitment: April 1999-December 2003, retrospective, case control Taiwan	3085	10-13	Enzyme linked immunosorbet assay (Genemed Biotechnologies Inc, South San Francisco, California, USA) PAPP-A <5th centile, <0.3 MoM and < 0.5 MoM	Small for gestational age: birth weight<10th, 5th, or 3rd centile Low birth weight <2,500 g
Conserva et al 2010	Risk: unselected Incl: All women undergoing Downs syndrome screening Excl: chromosomal and structural anomalies Recruitment: retrospective,	1687	11+2 -13+6	PAPP-A values corrected for maternal weight and smoking PAPP-A ≤ 0.4MoM (5th centile)	Small for gestational age: birth weight < 10th centile Preterm delivery : <37 weeks Gestational Hypertension and pre-eclampsia :Davy and MacGillivray 1988∞

	cohort, January 2007-January 2008, Italy				Placental abruption Intra-uterine death: Fetal demise after 22 weeks
Dane et al 2010	Risk: unselected Incl: patients attending for first trimester combined screening Excl: History of recurrent miscarriage using aspirin, heparin or low molecular weight heparin, fetal anomaly, type 1 diabetes mellitus Recruitment: prospective, cohort, January 2008-March 2009 Turkey	404	11-14	Chemiluminescent enzyme immunoassay methods (DPC, Los Angeles, California, USA) Corrected for maternal weight, smoking and ethnicity PAPP-A < 0.4 MoM	Low birth weight <2500g Pregnancy induced hypertension: diastolic blood pressure >90mmHg after 20 weeks with or without proteinuria Preterm delivery < 37 weeks
Dane et al 2013	Risk: unselected Incl: All women with first trimester screening	868	11-14	Chemiluminescent enzyme immunoassay (DPC, Los	Small for gestational age: birth weight < 10^{th} centile

	Excl: structural and chromosomal anomalies, multiple pregnancies Recruitment: prospective, consecutive, cohort Turkey			Angeles,California, USA) PAPP-A < 0.35 MoM; 0.35 – 2.42 MoM; < 2.42 MoM	
D'Antonio et al 2013	Risk: Unselected risk INC: All pregnancies delivered at or beyond 24 weeks EXC: Multiple pregnancies Recruitment: retrospective observational study consecutive enrolment; January 2008-October 2011, UK	12355	10-11+4	Certified by FMF correction done for FMF factors PAPP-A adjusted for gestational age, maternal weight, parity, ethnicity, smoking, maternal diabetes, assisted conception and expressed as MoM. PAPP-A <5th (0.39 MoM), 3rd (0.34 MoM) or 1st (0.25 MoM) centiles	Pre-eclampsia as per ISSHP (Early requiring delivery < 34 weeks Premature delivery: birth before 37 completed weeks. Small for gestational age: birth weight <5th percentile for gestational age (≠ Mikolajczyk et al) Composite adverse outcome: no definition

Dugoff et al 2004	Risk: unselected Incl : women enrolled in FASTER trial, viable singleton pregnancy Excl: anencephaly or septated cystic hygroma, chromosomal or structural anomaly, IDDM. Recruitment: prospective, cohort USA	33395	10+3 – 13+6 days	Enzyme linked two site immunosorbent assay and 2-site immunoradioactove assay (Diagnostic Systems Labs, Webster, Texas) PAPP-A $\leq 10^{th}$ (0.52MoM), $\leq 5^{th}$ (0.42MoM) or $\leq 1^{st}$ centile (0.28MoM)	Spontaneous loss $\leq$ 24 weeks of gestation Fetal loss >24 weeks of gestation Preterm delivery <37 weeks and $\leq$ 32 weeks Gestational hypertension – blood pressure >140/90 mmHg at least two occasions at least 6 hours apart with no chronic hypertension or significant proteinuria Pre-eclampsia – gestational hypertension in the setting of significant proteinuria (300mg/24 hours or 0.1g/l on two random samples $\geq$ 6 hours apart) SGA: birth weight<10^{th} and $\leq 5^{th}$ centile* Macrosomia – birth weight >4500g
Fox et al 2009	Risk: High risk INC: singleton pregnancy with an 18- to 24-week ultrasound showing evidence of FGR	198	First trimester	Not reported Converted to MoM	Gestational hypertension and pre-eclampsia defined according to American College of Obstetricians and Gynecologists, 2002.

	(EFW<25th percentile according to Hadlock) EXC: multiple pregnancies, fetuses with major anomalies, infections or aneuploidy, pregnancies complicated by second trimester premature rupture of membranes, patients did not delivered at the institution. Data collection: unreported. Enrolment unreported. USA			PAPP-A ≤5th centile (0.37 MoM) and ≤10th centile (0.47 MoM)	Birth weight percentile (Oken et al., 2003). Intra-uterine death – no definition Preterm birth – no definition Composite adverse neonatal outcome: intrauterine or neonatal death, indicated preterm birth or NICU admission
Goetzinger et al 2009	Risk: Unselected Incl: singleton pregnancy Excl: Multiple gestations And aneuploidy. Recruitment: Retrospective cohort study, 2003-2008 USA	2150¥	11-13+6	Genzyme (Cambridge, MA)or GeneCare (Chapel Hill, NC) PAPP-A<5th centile (0.46 MoM), <10th centile (0.59MoM), adjusted for gestational age,	Small for gestational age: birth weight <10th percentile* Large for gestational age: birth weight >90th percentile*

				ethnicity, body mass index.	
Goetzinger et al 2010	Risk Unselected consecutive patients Incl singleton gestations with first-trimester screening Excl: Aneuploidy Retrospective Consecutive enrolment, cohort USA	2231[¥]	11-13+6	Genzyme (Cambridge, MA) from 2003 to2007 using the DPC Immulite immunoassay system GeneCare (Chapel Hill, NC) from 2007 to 2008 using ELISA.	Pre-term birth :delivery before 35 weeks gestation and, secondarily as before 32 weeks
Gomez et al 2014	Risk :low risk Incl :Singleton, spontaneous conception, maternal age ≤ 38 years, having first trimester combined test Excl :Twin pregnancy, maternal illness, IVF, fetal malformation, aneuploidy	512	11-13+6	Not reported PAPP-A ≤ 5th centile (0.4 MOM)	Small for gestational age: birth weight<10^{th} and 5^{th} centile Pre-term birth: definition unreported

	Recruitment: Case control, retrospective, January 2012-June 2013 Spain				
Jelliffe-Pawlowski et al 2013	Risk: Unselected Incl: Participants in California Prenatal Screening Program EXCL: Missing data, diabetes mellitus, chromosomal or neural tube defects Recruitment: September 2009-December 2010. Retrospective, case-control USA	2070	10-13+6	Auto DELFIA; Perkin Elmer Life Sciences, Waltham, MA Converted to MoM adjusted for gestation, maternal weight, ethnicity PAPP-A $\leq 5^{th}$ centile	Early Preterm delivery between 22+0 and 29+6 weeks subgroups of medically indicated and spontaneous. Controls were term delivery $\geq$ 37 weeks
Karagiannis et al 2011	Risk: Unselected Incl: Singleton Excl: major fetal anomalies, TOP, miscarriage of fetal death before 24 weeks, pre-eclampsia and lost to follow-up	32850	11-13+6	Not reported	Small for gestational age: birth weight<5th centile for gestation at delivery (reference range local population)

	Recruitment: Prospective cohort, March 2006-September 2009 UK				
Kavak et al 2006	Risk: low risk Incl: singleton pregnancy attending for first trimester screening Excl: Women with multiple pregnancy, chronic hypertension, previously diagnosed diabetes, or pregnancy with a prenatal or postnatal diagnosis of a chromosomal or structural abnormality. Women with a history of previous pre-eclamptic pregnancy, previous gestational hypertension, gestational diabetes or IUGR Recruitment: July 2001- July 2004, enrolment not reported	476	First trimester	Rapid random access immunoassay analyser, Kryptor analyzer (Brahms AG,Berlin) PAPP-A various cut offs MoM, <5th centile (0.4 MoM)	Small for gestational age :Birth weight < 10th centile for gestational age Gestational hypertension and preeclampsia were diagnosed according to the criteria of the 2000 Working Group$^{\$}$.

	Turkey				
Kirkegaard et al 2011	Risk: unselected Incl: Singleton pregnancy Excl :Pregnancies with abnormal karyotype and major abnormalities Recruitment: cohort study, prospective January 2005-December 2007 Denmark	9450	8 -13 + 6	Brahm's Kryptor method Converted to MoM and corrected for maternal weight PAPP-A threshold of < 0.4 MoM	Small for gestational age: birth weight < 5th centile # Pre-term delivery <37 weeks
Krantz et al 2004	Risk: unselected Incl: singleton pregnancy Excl: multiple gestation, recent vaginal bleeding equivalent to a menstrual period, pre-gestational diabetes mellitus, pregnancy resulting from a donor oocyte unknown cytogenetic or phenotypic status, previous pregnancy affected with Trisomy 21 or 18, Trisomy 21or 18 this	8012	10+4-13+6	NTD Laboratories in Huntington Station, New York PAPP-A <1st,5th and >95th and 99th centile	Preterm delivery < 34 weeks. Small for gestational age: birth weight < 10th centile for gestational age *

	pregnancy, without delivery information, TOP ,fetal loss. Recruitment: Prospective cohort study. USA				
Kwik et al 2003	Risk: unselected Incl: Singleton pregnancy, 11-13+6 weeks Excl: Aneuploidy Recruitment: Retrospective, Cohort study, June 2000-November 2001 Australia	827	11-13+6	enzyme-linked immunosorbent assays (Diagnostic Service Laboratories Webster,Tx USA) PAPP-A <0.3 MoM and <0.5 MoM	Small for gestational age: birth weight <10th centile ,<3rd centiles for gestational age New South Wales population Pre-term delivery <37 weeks
Leung et al 2008	Risk: unselected Incl: Chinese women with singleton pregnancy Excl: miscarriage, IUD, structural and chromosomal anomaly	2760	11-13+6	Kryptor analyser (Brahms Diagnostica GmbH, Berlin, Germany), Converted to MoM corrected for maternal weight	Small for gestational age: birth weight <10th Centile for corresponding gestational age

	Recruitment: Prospective Observational study, June 2003-November 2004 China			PAPP-A<5th centile (0.43 MoM)	
Marttala et al 2010	Risk: unselected undergoing first trimester Downs syndrome screening Incl: singleton Excl: None reported Recruitment: cohort, prospective, 1/1/2005 – 31/12/2008 Finland	19536	9-13+6	Perkin Elmer AutoDELFIA time resolved fluoroimmunoassay kit (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) Converted to MoM corrected for maternal weight, diabetic status and smoking. PAPP-A <5th centile (<0.3 MoM)	Small for gestational age: birth weight < 2 standard deveiations related to gestational age, (national sex-specific standards) Stillbirth defined as fetal death during or after the 22nd gestational week or birth weight under 500g.
Montanari	Risk: Unselected	2134	11-14	Fluorimetric immunoassays (Kryptor; Brahms,	Small for gestational age: birth weight <10th centile for gestational age Italian population, sex of infant

et al 2009	Incl: Singleton pregnancy undergoing first trimester combined screening, 11-14 weeks Excl: lost follow up, miscarriage, TOP Recruitment: prospective longitudinal study, 2003-2005 Italy			Berlin, Germany), Converted to MoM corrected for maternal weight and smoking.	Fetal growth restriction was defined as: 1) fetal abdominal circumference was <10^{th} centile on at least 2 ultrasound examinations, 2-4 weeks apart, 2) a Doppler pulsatility index of umbilical artery >90^{th} centile; 3) birth weight <10^{th} centile for gestational age adjusted for sex of infant
Ong et al 2000	Risk :unselected Incl: All singleton pregnancies undergoing Downs syndrome screening Excl :multiple pregnancies Recruitment: Cohort, retrospective, May 1998-July 1999, UK	5297	10-14	Kryptor analyser PAPP-A threshold <5^{th}, < 10^{th} centile and < median	Miscarriage – spontaneous delivery <24 completed weeks Preterm delivery – spontaneous delivery <37 weeks and < 34 completed weeks Gestational diabetes mellitus: Glucose tolerance test as per WHO ^ Pregnancy induced hypertension: diastolic blood pressure 110mmHg or more on any one occasion or a diastolic blood pressure of 90mmHg or more on two consecutive occasions four hours apart in women with no pre-existing hypertension or renal disease;

					proteinuric and non proteinuric, depending on the presence or absence of either > 300 mg of total protein in a 24-hour urine collection or an 1+ on dipstick Small for gestational age: Birth weight below the 10th, 5th or 3rd centiles for gestational age (Yudkin)[¥].
Patil et al 2014	Risk: unselected Incl: Singleton pregnancy delivered at Bharati hospital Pune Excl :Multiple pregnancy Recruitment :Case control India	524	11-13	PerkinElmer lab PAPP-A<0.5 MoM	Preterm delivery: <37 weeks IUGR no definition PIH no definition Stillbirth no definition
Pilalis et al 2007	Risk: unselected Incl :Singleton, uterine artery Doppler and known outcome Excl: 4 with miscarriage, 11 TOP Recruitment: Cohort, prospective,	878	11-14	Kryptor analyser (Brahms AG, Berlin, Germany),	Pre-eclampsia: blood pressure systolic ≥ 140mmHg or diastolic ≥ 90mmHg on two recordings 6 hrs apart and with proteinuria ≥.300mg in 24 hours or ≥ 2_ on dipstick Small for gestational age: birth weight

	consecutive, September 2002 and March 2004 Greece			PAPP-A $\leq 5^{th}$ (0.41 MoM) and $\leq 10^{th}$ centile (0.52 MoM)	$<5^{th}$ or <10th centile (Alexander et al 1996)* Placental abruption : vaginal bleeding after mid gestation from a normally situated placenta Combined adverse outcome: any or one of combination of pre-eclampsia, Small for gestational age $\leq 5^{th}$ centile and placental abruption
Radoi et al 2009	Risk: unselected Incl : Women booking at Life Memorial Hospital undergoing combined screening Excl: records with missing values for birth weight, perinatal outcome, gestational age at delivery was outside 24-43 weeks, abnormal or missing karyotype.	456	10-13+6	Method not reported Corrected for maternal weight PAPP-A $<5^{th}$ centile (0.4MoM)	Small for gestational age: birth weight < 5thcentile (G.C.S. Smith Scottish data 1992-1998). Preterm birth <37 completed weeks Very preterm birth: between 24 and 32 weeks. gestation Moderately preterm delivery between 33 and 36 wk. gestation Spontaneous preterm birth was defined as vaginal delivery of a live-born baby

	Recruitment: prospective non-intervention cohort study Romania				between 24-36 weeks where labour had not been induced. Stillbirth was defined as delivery of a dead baby at or after 24 weeks gestational age and the denominator was all births at or after 24 weeks. Pre-eclampsia :pregnancy-induced hypertension with proteinuria
Ranta Et al 2011	Risk: Unselected Incl: spontaneous conceived structurally normal singleton pregnancy, living in Kuopio catchment area Excl: Multiple pregnancies ,major structural abnormality, miscarriages and induced abortions Recruitment: Retrospective cohort, Finland, January 2005 – December 2007	2844	9-13	Auto- DELFIA kit fluoroimmunoassay (PerkinElmer Wallac, Turku,Finland) Converted to MoM and corrected for maternal weight and diabetes PAPP-A <0.4, < 0.6, < 0.8, < 1.0 MoM	Pre-eclampsia: BP>140/90 mmHg with proteinuria >0.5g/day Pre term delivery: birth before 37 completed weeks Small for gestational age: sex and age adjusted birth weight <10th centile compared to local records Placental abruption

Saruhan et al 2012	Risk: unselected Incl: Singleton pregnancy Excl: chromosomal abnormality, spontaneous abortions, multiple pregnancies, no record. Collection :cohort, retrospective, December 2008-September 2009 Turkey	318	First trimester	Immunosassays Roche diagnostics GmbH Manheim PAPP-A adjusted for maternal weight, smoking status, ethnicity and diabetes. PAPP-A $\leq 10^{th}$ centile	Gestational hypertension: blood pressure>140/90 mmHg 6 hrs apart with no chronic hypertension and no significant sign of proteinuria. Pre-eclampsia :gestational hypertensions and proteinuria (0.1 g/l i.e. >2+ on dipstick 6 hrs apart or >300 mg in 24 hrs on 24 hour collection) Preterm birth <37 weeks Small for gestational age : birth weight $\leq 5^{th}$ and 10^{th} percentile

Smith et al 2002	Risk Unselected Inclusion :All singleton pregnancies Excl: missing values for birth weight ,missing values for perinatal outcome ,records where the gestational age at delivery was outside 24–43 wk, abnormal or missing karyotype ,multiple pregnancy Recruitment: Prospective consecutive, non-interventional, multicenter enrolment	8839	8-14 weeks	Kryptor immunoassay analyzer (Brahms, Berlin, Germany; formerly supplied by CIS-Bio International, Burgess Hill, UK). Converted to multiples of the median (MOMs) and corrected for maternal weight and smoking. PAPP-A $\leq 5^{th}$ centile	Small for gestational age: birth weight $<5^{th}$ percentile of birth weight for the given week of gestation, using percentiles derived from 409,541 live births in Scotland between 1992–1998 (G. C. S. Smith, unpublished data). Very pre-term delivery :24-32 weeks Pre-term delivery <37 weeks Stillbirth was defined as delivery of a dead baby at or after 24 weeks. Pre-eclampsia: Pregnancy- induced hypertension with proteinuria.
Spencer et al 2005	Risk: unselected (all women			Kryptor analyser	Pre-term delivery and early preterm

	offered Trisomy 21 screening) Incl: singleton pregnancies, 11-13+6 weeks Excl: Structural or chromosomal anomalies Recruitment: Prospective, consecutive, cohort, October 1999-August 2002 UK	4171	11-13+6	(Brahms AG, Berlin, Germany), PAPP-A ≤ 5th centile (0.422MoM) adjusted for maternal; weight, smoking and ethnicity	delivery: spontaneous delivery <37/40 early pre-term <34 weeks Pre-eclampsia was defined according to ISSHP (i.e. two recordings of diastolic blood pressure >90mmHg at least 4h apart in previously normotensive women and proteinuria ≥300mg or more in 24h, or two readings of ++ on dipstick analysis of midstream or catheter specimen) (Brown et al., 2001). Small for gestational age: birth weight < 5th centile for gestational age (Yudkin et al., 1987).
Tul et al 2003	Risk: unselected Incl: singleton pregnancy Excl: multiple pregnancy	1136	10-14	Kryptor analyser rapid random access immunoassay (Brahms AG, Berlin, Germany), Converted to MoM	Small for gestational age: birth weight<10th centile (Slovene reference standards) (Verdenik, 2000) †.

	Recruitment: Retrospective, cohort February 1999 – August 2001, Slovenia			PAPP-A ≤0.5 MoM	
Yaron et al 2002	Risk: unselected Incl :Singleton Excl: chromosomal or structural anomalies. Recruitment: July 1998 – June 2000, Prospective, study design unreported Israel.	1622	10-13	Fluoro immunoassay kit (Wallac, CR61-105) PAPP-A≤ 0.25 MoM and ≤ 0.50 MoM	Spontaneous preterm delivery: delivery prior to 37 completed weeks Fetal growth restriction: birth weight <5th percentile for gestational age Pregnancy induced hypertension: Diastolic blood pressure of 110 mmHg on one occasion or greater than 90 mmHg on at least two consecutive occasions, 4 h apart with no history of pre-existing hypertension or renal disease; further subdivided into proteinuric (presence of >300 mg total protein on a 24 h urine collection or >1 + albumin on a single dip-stick examination) and non-proteinuric. Intra-uterine fetal demise was defined as fetal death before delivery and after 23 completed weeks of gestation. Spontaneous miscarriage was defined

					weeks of gestation. Placental abruption: Grade 2 or 3 placental abruption. Oligohydramnios: amniotic fluid index <5 cm. Adverse outcome – no definition

Footnote: Incl: inclusion; excl: exclusion; PAPP-A pregnancy associated plasma protein A; MoM multiples of the median; g grams; mmHg millimetres of mercury; FMF Fetal Medicine Foundation; ISSHP International Society for Study of Hypertension in Pregnancy; IDDM insulin dependent diabetes mellitus; FGR fetal growth restriction; EFW estimated fetal weight; NICU neonatal intensive care unit; termination of pregnancy; IUGR intrauterine growth restriction; IUD intrauterine death; WHO World Health Organisation; PIH pregnancy induced hypertension; BP blood pressure; UK United Kingdom; USA United States of America.

* Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996;87:163-8.

$ Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 183 (2000)

Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996; 85: 843–848.

^ World Health Organization Expert Committee on Diabetes Mellitus (1980) Technical Report Series 646. Geneva: WHO, p.8.

¥ Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. New birthweight and head circumference centiles for gestational ages 24 to 42 weeks. Early Hum Dev 1987; 15: 45-52.

† Verdenik I. 2000. Slovenski referenˇcni standardi za teˇzo, dolˇzino in obseg glavice ob rojstvu za doloˇceno gestacijsko starost populacije, rojene v letih 1987–96. Zdrav Vestn 69: 153–156.

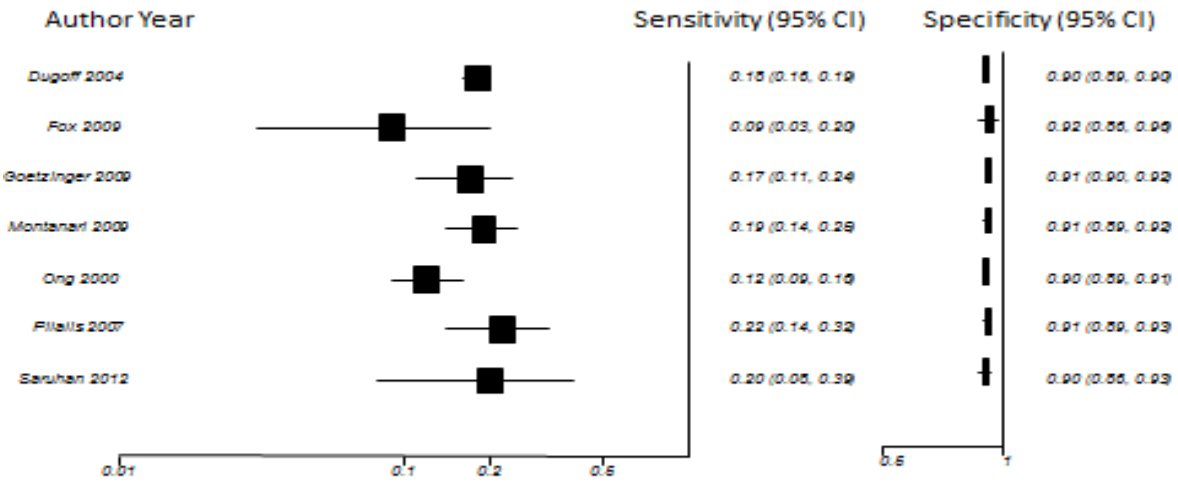
≠ Mikolajczyk RT, Zhang J, Betran AP, et al. A global reference for fetal weight and birthweight percentiles. Lancet 2011;37:1855–61.

∞ Davey DA, MacGillivray I. 1988. The classification and definition of the hypertensive disorders of pregnancy. Am J Obstet Gynecol 158: 892–898.

¥ Note the two papers by Goetzinger are separate publications with different outcomes but include the same cohort of women.

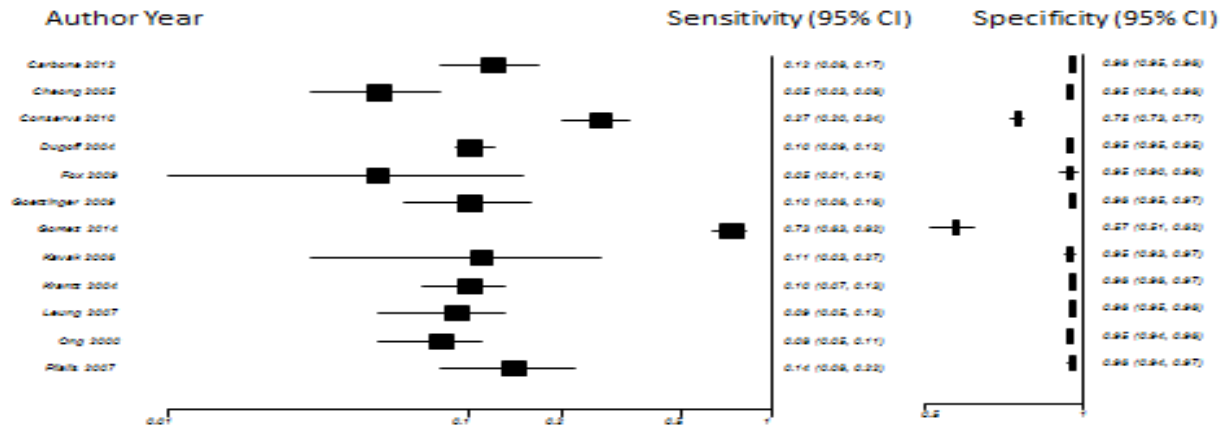
Appendix 7: Forest plots for Pregnancy associated plasma protein A and association with birth weight <10th centile (sensitivity and specificity)

Figure S1A: Forest plots for analysis of pregnancy associated plasma protein A <10th centile and birth weight <10th centile



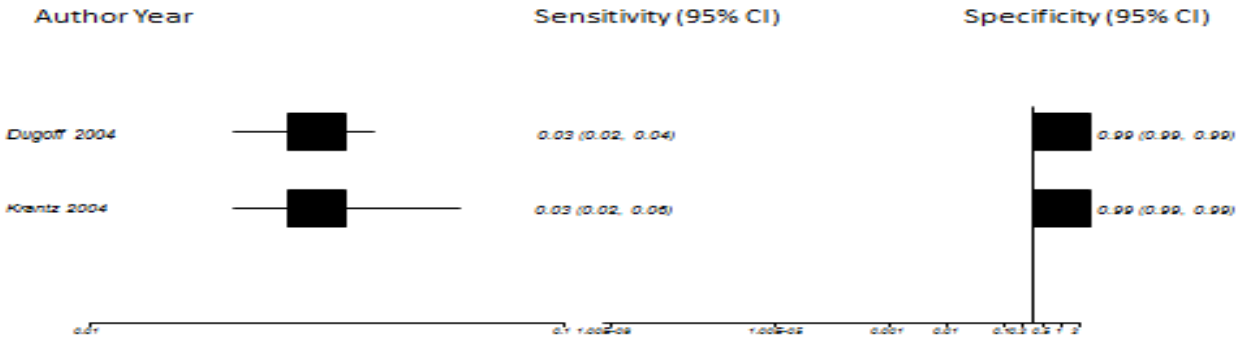
Peter's test p=0.387 ; Sensitivity I^2 = 54% χ^2 = 0.04; Specificity I^2 =0% χ^2 = 0.57

Figure S1B: Forest plots for analysis of pregnancy associated plasma protein A <5th centile and birth weight <10th centile



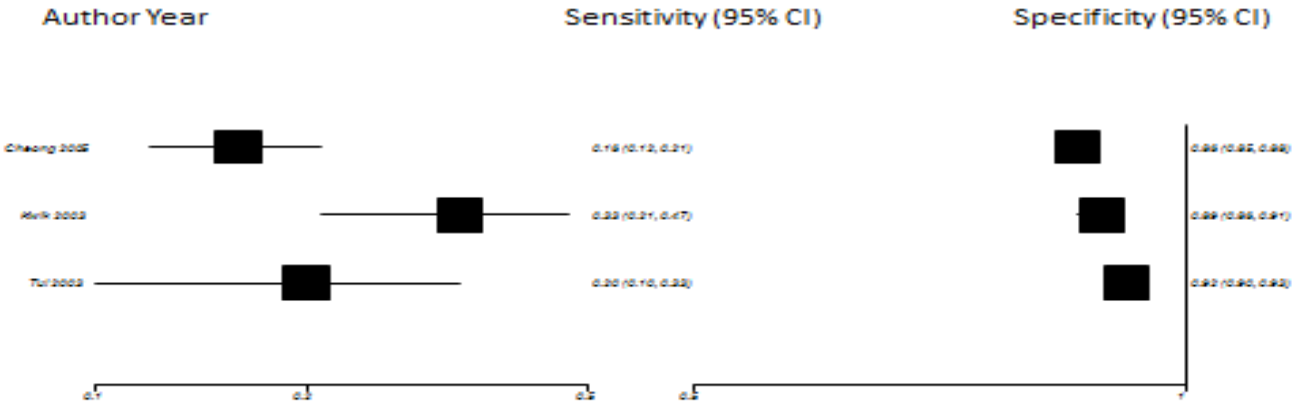
Peter's test p=0.424 ; Sensitivity I^2 = 96% χ^2 = 0.00; Specificity I^2 =99% χ^2 = 0.00

Figure S1C: Forest plots for analysis of pregnancy associated plasma protein A <1st centile and birth weight <10th centile



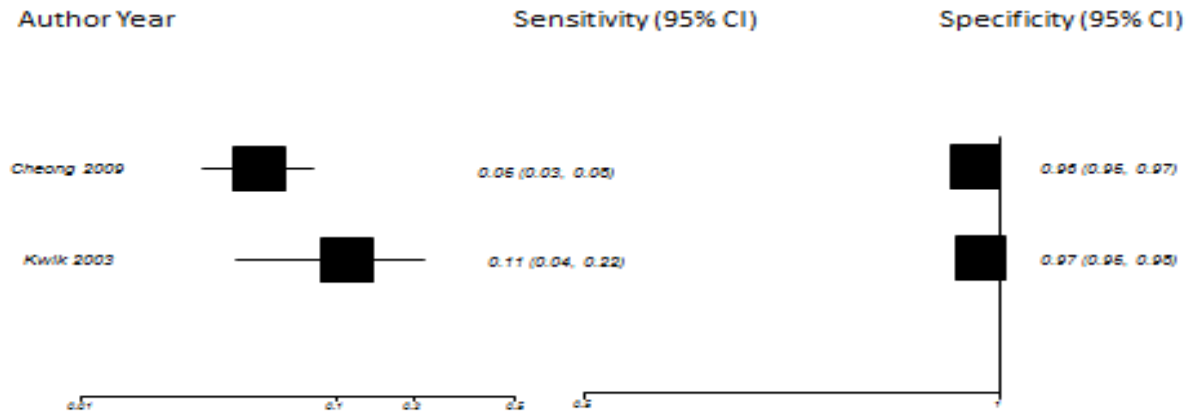
Peter's test not possible n=2 ; Sensitivity I^2 = 0% χ^2 = 0.69; Specificity I^2 =68% χ^2 = 0.08

Figure S1D: Forest plots for analysis of pregnancy associated plasma protein A < 0.5 multiples of median and birth weight <10th centile



Peter's test p=0.622 ; Sensitivity I^2 = 74% χ^2 = 0.02; Specificity I^2 =90.3% χ^2 = 0.00

Figure S1E: Forest plots for analysis of pregnancy associated plasma protein A < 0.3 multiples of median and birth weight <10th centile



Peter's test not possible n=2 ; Sensitivity I^2 = 63% χ^2 = 0.01; Specificity I^2 =0% χ^2 = 0.39

Appendix 8: National Research Ethics Services Declaration of a end of the study

DECLARATION OF THE END OF A STUDY

(For all studies except clinical trials of investigational medicinal products)

To be completed in typescript by the Chief Investigator and submitted to the Research Ethics Committee that gave a favourable opinion of the research ("the main REC") within 90 days of the conclusion of the study or within 15 days of early termination. For questions with Yes/No options please indicate answer in bold type.

1. Details of Chief Investigator

Name:	Professor Mark Kilby
Address:	University of Birmingham Birmingham B15 2TT
Telephone:	0121 627 2778
Email:	m.d.kilby@bham.ac.uk
Fax:	0121 623 6875

2. Details of study

Full title of study:	Association of serum PAPP-A levels in first trimester with small for gestational age and other adverse Pregnancy outcomes: systematic review and retrospective cohort study.
Research sponsor:	Birmingham Women's Hospital
Name of main REC:	NRES Committee North West – Preston
Main REC reference number:	14/NW/1394

3. Study duration

Date study commenced:	1/11/14
Date study ended:	1/3/2016
Did this study terminate prematurely?	*No* *If yes please complete sections 4, 5 & 6, if no please go direct to section 7.*

4. Circumstances of early termination

What is the justification for this early termination?	

5. Temporary halt

Is this a temporary halt to the study?	*Yes / No*
If yes, what is the justification for temporarily halting the study? When do you expect the study to re-start?	*e.g. Safety, difficulties recruiting participants, trial has not commenced, other reasons.*

6. Potential implications for research participants

Are there any potential implications for research participants as a result of terminating/halting the study prematurely? Please describe the steps taken to address them.	

7. Final report on the research

Is a summary of the final report on the research enclosed with this form?	*yes* *If no, please forward within 12 months of the end of the study.*

8. Declaration

Signature of Chief Investigator:	
Print name:	Professor Mark Kilby
Date of submission:	3/3/2016

Appendix 9: National Research Ethics Services letter of final approval for the retrospective cohort study for association of first trimeser maternal serum PAPP-A levels with small for gestation age and other adverse pregnancy outcomes

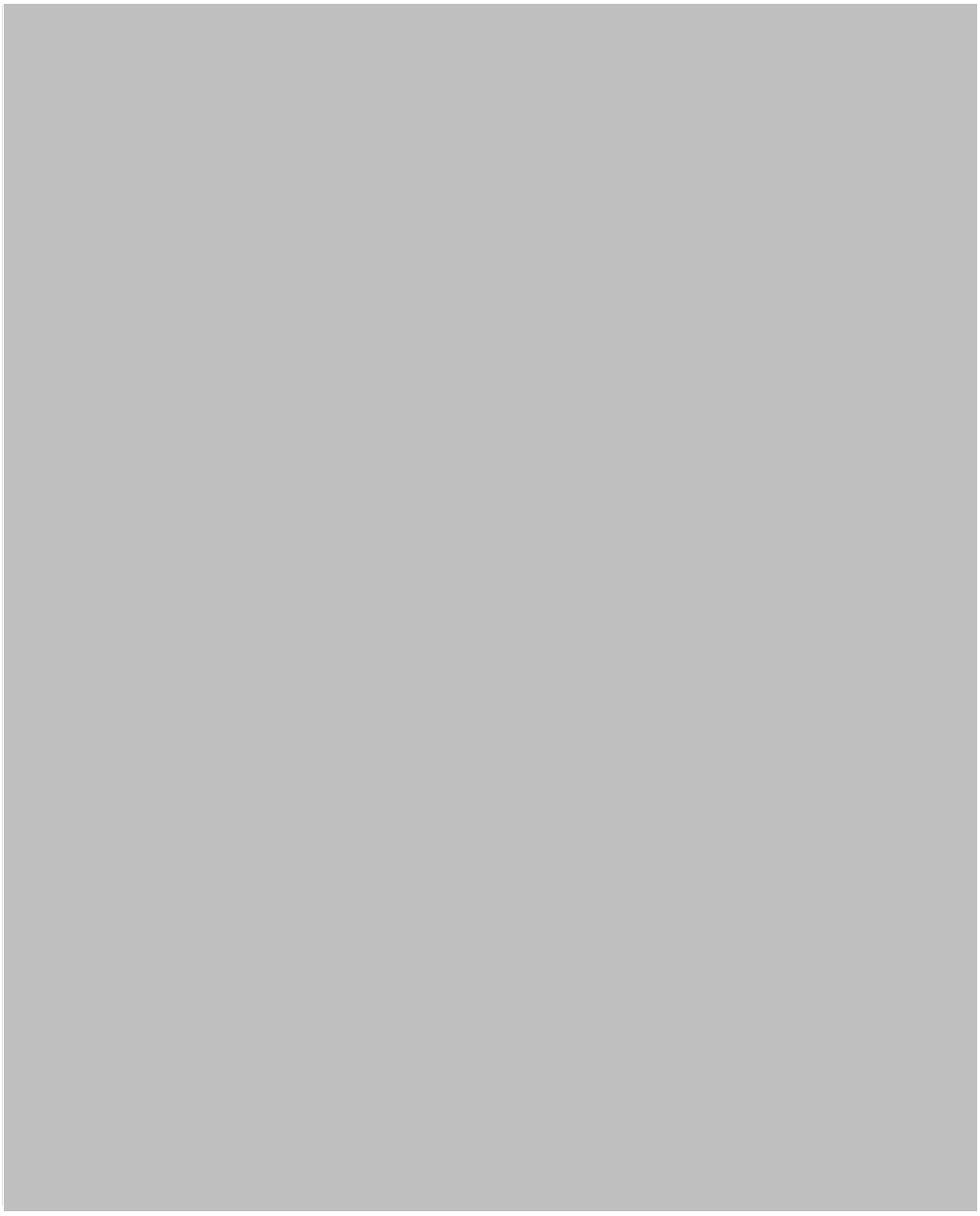
[The ethics approval letter was redacted to protect confidentail information.]

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Preston
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

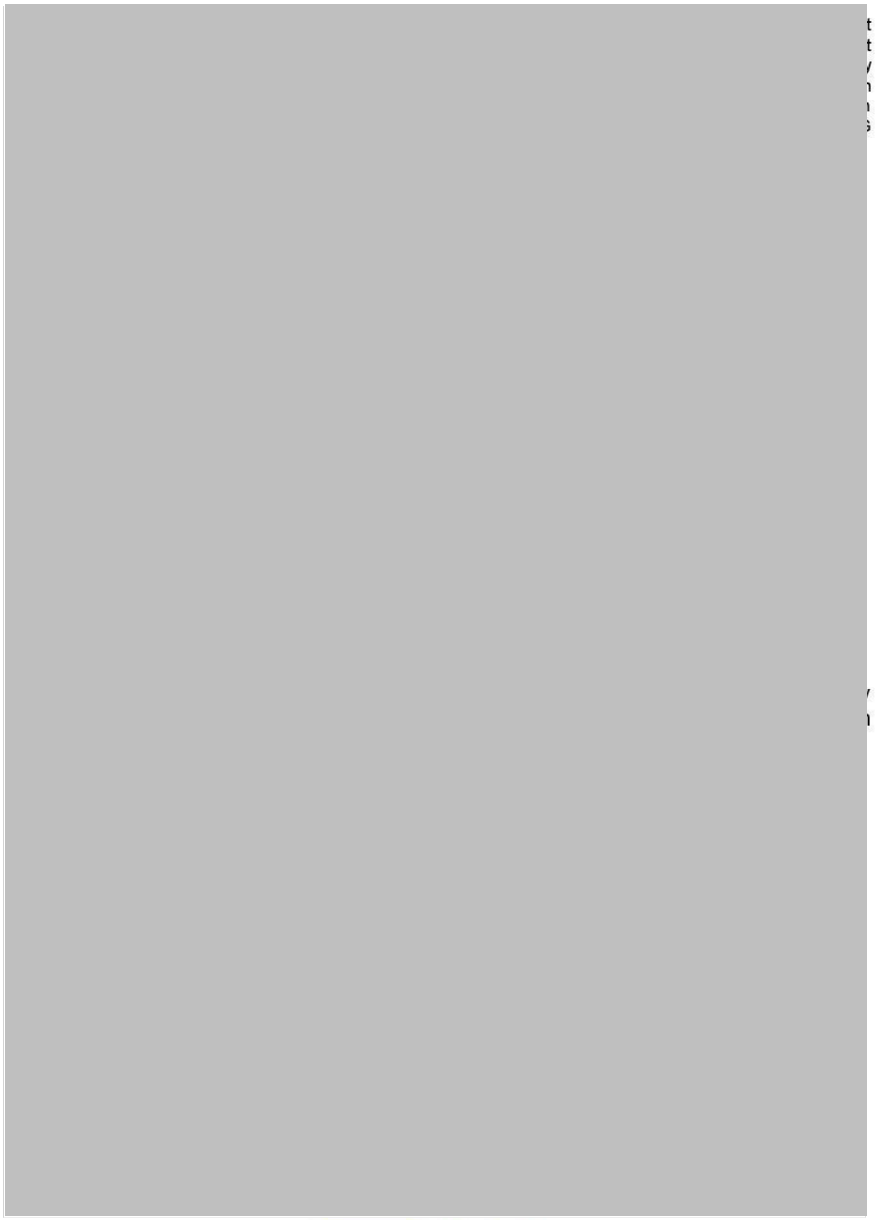
Telephone: 0161 625 7109
Fax: 0161 625 7919

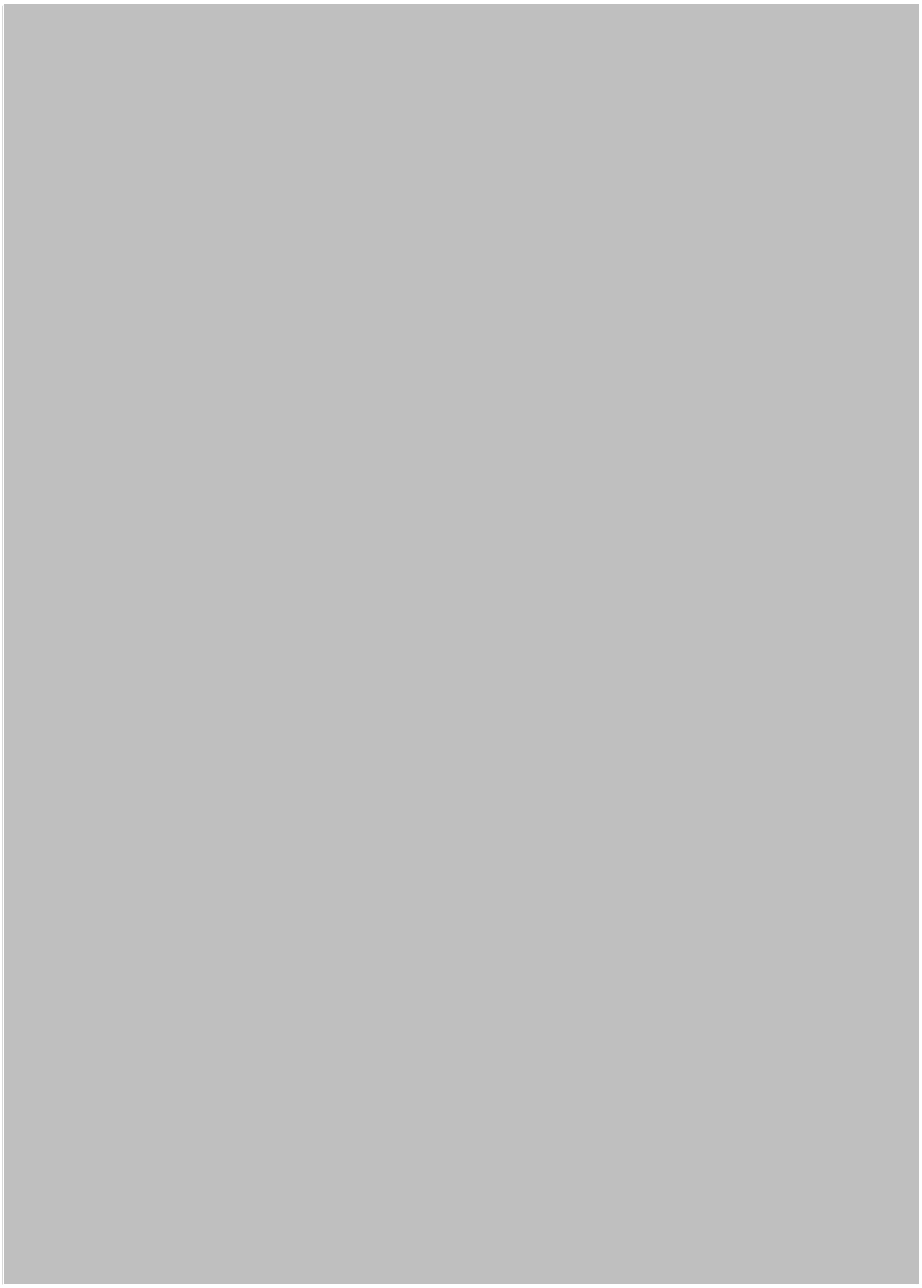
06 January 2015



Appendix 10 :BWNFT research and developmen letter of approval for the retrospective cohort study forstudy of association of serum PAPP -A levels in first trimester with small for gestational age and other adverse Pregnancy outcomes

[The research and development approval letter was redacted to protect confidentail information.]





Appendix 11: Nuchal translucency and adverse pregnancy outcomes

Table 14a: Nuchal translucency (mm): results from unadjusted logistic regression (singleton pregnancies)

Outcome	Odds Ratio	95% Confidence Interval OR	p-value
SGA	0.811	0.721 to 0.912	<0.0001
Pre-term labour	1.149	0.998 to 1.322	0.053
Pre-eclampsia	0.897	0.703 to 1.143	0.378
Miscarriage	1.942	1.538 to 2.452	<0.0001
Stillbirth	0.765	0.350 to 1.675	0.503
Perinatal death	0.696	0.394 to 1.231	0.213
Neonatal death	0.568	0.250 to 1.288	0.176

For a one unit increase in nuchal translucency (NT), the estimated OR of 0.81 for IUGR suggests that there are 19% lower odds of SGA, with a 95% CI of between 8.8% and 17.9% lower odds ($p<0.0001$).

There is some evidence of an association between NT and pre-term labour with an odds ratio estimate of 1.15 (95% CI: 0.99 to 1.32; $p=0.053$), which suggests that the odds of pre-term labour increase as NT increases. Similarly, there is highly statistically significant evidence that the odds of miscarriage are higher as NT increases with an odds ratio estimate of 1.94 (95% CI: 1.54 to 2.45; $p<0.0001$). There is no evidence of a statistically significant association between NT and pre-eclampsia, stillbirth, perinatal death or neonatal death.

Table 14b: Nuchal translucency: Results from adjusted logistic regression for SGA (singleton pregnancies, M=35 imputed datasets)

SGA (<10th customised centile)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		0.794	0.704 to 0.894	<0.0001
Mother's age (years)		1.014	1.004 to 1.025	0.006
BMI		1.014	1.002 to 1.025	0.018
Parity	0	1.141	1.022 to 1.275	0.019
	>4	1.034	0.640 to 1.770	0.893
Ethnicity	South-Asian	0.896	0.726 to 1.106	0.306
	Oriental	0.816	0.567 to 1.174	0.274
	Other Mixed	0.778	0.560 to 1.011	0.060
	White	0.786	0.650 to 0.951	0.013
Deprivation score categories	2	1.097	0.774 to 1.554	0.604
	3	1.009	0.741 to 1.373	0.956
	4	1.020	0.750 to 1.385	0.902
	5	1.173	0.868 to 1.584	0.299
Smoking status	Yes	2.694	2.347 to 3.092	<0.0001
	Stopped	1.198	0.901 to 1.593	0.213
IVF		0.682	0.446 to 1.042	0.077
Gestational diabetes		1.003	0.812 to 1.240	0.976

N=12,299

In this adjusted analysis, there is evidence of lower odds of SGA as NT increases with an odds ratio estimate of 0.80. The estimate is statistically significant with a 95% confidence interval of 0.71 to 0.90, suggesting between 10% and 29% lower odds of SGA (p=0.001) for a one unit increase in NT, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 14c: Nuchal translucency: Results from adjusted logistic regression for preterm birth (singleton pregnancies, M=35 imputed datasets)

Preterm birth (<37 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		1.085	0.926 to 1.272	0.313
Mother's age (years)		1.007	0.993 to 1.022	0.291
BMI		1.002	0.986 to 1.018	0.764
Parity	0	1.012	0.859 to 1.193	0.886
	>4	0.989	0.492 to 1.985	0.974
Ethnicity	South-Asian	0.755	0.565 to 1.009	0.058
	Oriental	0.439	0.239 to 0.806	0.008
	Other Mixed	0.719	0.499 to 1.033	0.075
	White	0.670	0.540 to 0.907	0.007
Deprivation score categories	2	0.855	0.520 to 1.407	0.538
	3	0.861	0.562 to 1.320	0.493
	4	0.914	0.599 to 1.394	0.675
	5	1.053	0.697 to 1.592	0.807
Smoking status	Yes	1.687	1.371 to 2.076	<0.0001
	Stopped	0.921	0.587 to 1.445	0.721
IVF		1.091	0.647 to 1.840	0.744
Gestational diabetes		1.316	0.994 to 1.744	0.055

N=12,453

In this adjusted analysis, there is no evidence of an association between NT and preterm birth with an odds ratio estimate of 1.09 (95% CI: 0.93 to 1.28; p=0.294) After adjusting for the other predictors.

Table 14d: Nuchal translucency: Results from adjusted logistic regression for pre-eclampsia toxaemia (singleton pregnancies, M=35 imputed datasets)

Pre-eclampsia toxaemia		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		0.870	0.674 to 1.123	0.287
Mother's age (years)		1.014	0.992 to 1.035	0.218
BMI		1.056	1.034 to 1.078	<0.0001
Parity	0	2.575	2.012 to 3.293	<0.0001
	>4	1.000	-	-
Ethnicity	South-Asian	0.641	0.429 to 0.958	0.030
	Oriental	0.319	0.124 to 0.823	0.018
	Other Mixed	0.486	0.279 to 0.849	0.011
	White	0.598	0.420 to 0.853	0.004
Deprivation score categories	2	0.688	0.365 to 1.297	0.247
	3	0.703	0.412 to 1.199	0.196
	4	0.616	0.360 to 1.053	0.077
	5	0.811	0.483 to 1.362	0.428
Smoking status	Yes	0.601	0.389 to 0.928	0.022
	Stopped	0.969	0.536 to 1.753	0.918
IVF		1.697	0.974 to 2.956	0.062
Gestational diabetes		0.921	0.597 to 1.422	0.712

N=12,321

In this adjusted analysis, there is no evidence of an association between NT and pre-eclampsia with an odds ratio estimate of 0.88 (95% CI: 0.68 to 1.13; p=0.314) after adjusting for the other predictors

Table 14e4: Nuchal translucency: Results from adjusted logistic regression for miscarriage (singleton pregnancies, M=35 imputed datasets)

Miscarriage (death <24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		1.748	1.123 to 2.721	0.013
Mother's age (years)		0.964	0.901 to 1.031	0.285
BMI		1.040	0.984 to 1.104	0.157
Parity	0	0.546	0.257 to 1.163	0.117
	>4	1.000	-	-
Ethnicity	South-Asian	1.362	0.362 to 5.121	0.647
	Oriental	1.000	-	-
	Other Mixed	1.529	0.337 to 6.942	0.582
	White	0.846	0.243 to 2.942	0.792
Deprivation score categories	2	1.000	-	-
	3	0.869	0.100 to 7.525	0.899
	4	1.765	0.225 to 13.839	0.589
	5	1.188	0.151 to 9.313	0.870
Smoking status	Yes	0.743	0.249 to 2.221	0.595
	Stopped	0.785	0.105 to 5.864	0.813
IVF		2.188	0.281 to 17.028	0.454
Gestational diabetes		1.000	-	-

N=10,404

In this adjusted analysis, there is evidence of higher odds of miscarriage as NT increases with an odds ratio estimate of 1.75. The estimate is statistically significant with a 95% confidence interval of 1.12 to 2.72, suggesting between 12% and 272% higher odds of miscarriage (p=0.001) for a one unit increase in NT, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 14f: Nuchal translucency: Results from adjusted logistic regression for stillbirth (singleton pregnancies, M=35 imputed datasets)

Stillbirth (death >24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		0.694	0.249 to 1.935	0.485
Mother's age (years)		0.980	0.9032to 1.066	0.637
BMI		1.033	0.959 to 1.113	0.394
Parity	0	1.832	0.761 to 4.408	0.177
	>4	1.000	-	-
Ethnicity	South-Asian	2.783	0.337 to 22.954	0.342
	Oriental	1.000	-	-
	Other Mixed	1.122	0.069 to 18.129	0.936
	White	1.820	0.233 to 14.198	0.568
Deprivation score categories	2	0.546	0.068 to 4.396	0.569
	3	1.079	0.375 to 3.106	0.888
	4	0.976	0.347 to 2.744	0.963
	5	1.000	-	
Smoking status	Yes	1.000	-	-
	Stopped	1.051	0.139 to 7.968	0.961
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=9,753

In this adjusted analysis, there is no evidence of an association between NT and stillbirth with an odds ratio estimate of 0.68 (95% CI: 0.24 to 1.90; p=0.460) after adjusting for the other predictors.

Table 14g: Nuchal translucency: Results from adjusted logistic regression for perinatal death (singleton pregnancies, M=35 imputed datasets)

Perinatal death (death between 24 weeks gestation and 7 days after birth)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		0.888	0.431 to 1.828	0.746
Mother's age (years)		0.960	0.903 to 1.022	0.199
BMI		1.075	1.025 to 1.128	0.003
Parity	0	2.173	1.128 to 4.187	0.020
	>4	1.000	-	-
Ethnicity	South-Asian	0.657	0.260 to 1.657	0.373
	Oriental	0.428	0.052 to 3.547	0.432
	Other Mixed	0.384	0.100 to 1.478	0.164
	White	0.261	0.109 to 0.624	0.003
Deprivation score categories	2	2.255	0.804 to 6.319	0.122
	3	0.961	0.392 to 2.356	0.931
	4	1.176	0.553 to 2.500	0.674
	5	1.000	-	-
Smoking status	Yes	0.879	0.298 to 2.598	0.816
	Stopped	1.439	0.339 to 6.117	0.622
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,898

In this adjusted analysis, there is no evidence of an association between NT and perinatal death with an odds ratio estimate of 0.88(i5%CI:0.42 to 1.82,p=0.730) after adjusting for the other predictors.

Table 14h: Nuchal translucency: Results from adjusted logistic regression for neonatal death (singleton pregnancies, M=35 imputed datasets)

Neonatal death (death between birth and 28 days)		Odds Ratio	95% Confidence Interval OR	p-value
Nuchal translucency (mm)		1.069	0.391 to 2.926	0.896
Mother's age (years)		0.949	0.866 to 1.040	0.262
BMI		1.117	1.051 to 1.188	<0.0001
Parity	0	2.387	0.921 to 6.185	0.073
	>4	1.000	-	-
Ethnicity	South-Asian	0.437	0.136 to 1.410	0.166
	Oriental	0.617	0.069 to 5.596	0.665
	Other Mixed	0.257	0.051 to 1.308	0.102
	White	0.064	0.018 to 0.221	0.000
Deprivation score categories	2	5.690	1.618 to 20.015	0.007
	3	0.385	0.048 to 3.080	0.368
	4	1.247	0.422 to 3.684	0.689
	5	1.000	-	-
Smoking status	Yes	2.964	0.881 to 9.975	0.079
	Stopped	2.204	0.277 to 17.522	0.455
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,876

In this adjusted analysis, there is no evidence of an association between NT and neonatal death with an odds ratio estimate of 1.064 (95% CI: 0.38 to 2.99; p=0.907) after adjusting for the other predictors.

Appendix 12 Crown rump length and adverse pregnancy outcomes

Table 55a: Crown rump length (mm): results from unadjusted logistic regression (singleton pregnancies)

Outcome	Odds Ratio	95% Confidence Interval OR	p-value
SGA	0.994	0.988 to 1.000	0.065
Pre-term labour	0.999	0.990 to 1.007	0.730
Pre-eclampsia	1.010	0.997 to 1.023	0.123
Miscarriage	1.021	0.993 to 1.049	0.147
Stillbirth	0.971	0.932 to 1.011	0.150
Perinatal death	0.980	0.952 to 1.008	0.160
Neonatal death	0.984	0.946 to 1.024	0.432

There is some evidence of lower odds of SGA crown rump length (CRL) increases, but this is not statistically significant at the 5% significance level (0.065). There is no statistical evidence of an association between CRL and any of the other six outcomes. For interpretation, for example, for a one unit increase in CRL (mm?), the estimated OR of 0.984 for neonatal death suggests that the odds of SGA are lower by 1.6%, with a 95% CI of between 5.4% lower and 2.4% higher (p=0.432) odds of SGA.

Table 15b: Crown rump length: Results from adjusted logistic regression for SGA (singleton pregnancies, M=35 imputed datasets)

SGA (<10th customised centile)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		0.994	0.988 to 1.000	0.057
Mother's age (years)		1.014	1.003 to 1.024	0.006
BMI		1.013	1.001 to 1.025	0.025
Parity	0	1.144	1.025 to 1.278	0.016
	>4	1.024	0.634 to 1.655	0.922
Ethnicity	South-Asian	0.890	0.721 to 1.100	0.280
	Oriental	0.791	0.550 to 1.138	0.208
	Other Mixed	0.776	0.598 to 1.007	0.056
	White	0.777	0.642 to 0.940	0.009
Deprivation score categories	2	1.096	0.773 to 1.552	0.608
	3	1.015	0.746 to 1.381	0.925
	4	1.024	0.754 to 1.391	0.879
	5	1.176	0.870 to 1.579	0.235
Smoking status	Yes	2.680	2.335 to 3.075	<0.0001
	Stopped	1.188	0.894 to 1.579	0.235
IVF		0.680	0.445 to 1.040	0.075
Gestational diabetes		1.001	0.811 to 1.238	0.986

N=12,299

In this adjusted analysis, there is evidence of lower odds of SGA as CRL increases with an odds ratio estimate of 0.99. The estimate is just statistically significant with a 95% confidence interval of 0.99 to 1.00, suggesting between 0% and 1% lower odds of SGA (p=0.051) for a one unit increase in CRL, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table15c: Crown rump length: Results from adjusted logistic regression for preterm birth (singleton pregnancies, M=35 imputed datasets)

Preterm birth (<37 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		0.998	0.989 to 1.007	0.624
Mother's age (years)		1.008	0.994 to 1.023	0.259
BMI		1.000	0.987 to 1.019	0.731
Parity	0	1.011	0.858 to 1.192	0.894
	>4	0.991	0.493 to 1.989	0.979
Ethnicity	South-Asian	0.754	0.564 to 1.008	0.057
	Oriental	0.444	0.242 to 0.814	0.009
	Other Mixed	0.719	0.500 to 1.035	0.076
	White	0.699	0.539 to 0.907	0.007
Deprivation score categories	2	0.855	0.520 to 1.406	0.536
	3	0.860	0.562 to 1.318	0.490
	4	0.914	0.599 to 1.394	0.675
	5	1.055	0.698 to 1.595	0.798
Smoking status	Yes	1.689	1.373 to 2.078	<0.0001
	Stopped	0.926	0.590 to 1.451	0.736
IVF		1.100	0.652 to 1.857	0.720
Gestational diabetes		1.318	0.995 to 1.746	0.055

N=12,453

In this adjusted analysis, there is no evidence of an association between CRL and preterm birth with an odds ratio estimate of 0.998 (95% CI: 0.989 to 1.007;

Table 15d: Crown rump length: Results from adjusted logistic regression for pre-eclampsia (singleton pregnancies, M=35 imputed datasets)

Pre-eclampsia		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		1.006	0.993 to 1.020	0.325
Mother's age (years)		1.012	0.991 to 1.034	0.257
BMI		1.055	1.033 to 1.078	<0.0001
Parity	0	2.573	2.012 to 3.292	<0.0001
	>4	1.000	-	-
Ethnicity	South-Asian	0.647	0.433 to 0.967	0.034
	Oriental	0.317	0.123 to 0.817	0.017
	Other Mixed	0.489	0.280 to 0.853	0.012
	White	0.603	0.426 to 0.859	0.005
Deprivation score categories	2	0.692	0.368 to 1.304	0.255
	3	0.706	0.415 to 1.204	0.202
	4	0.618	0.361 to 1.057	0.079
	5	0.809	0.482 to 1.359	0.424
Smoking status	Yes	0.601	0.390 to 0.928	0.022
	Stopped	0.959	0.530 to 1.734	0.890
IVF		1.660	0.953 to 2.892	0.073
Gestational diabetes		0.920	0.595 to 1.420	0.707

N=12,321

In this adjusted analysis, there is no evidence of an association between CRL and pre eclampsia with an odds ratio estimate of 1.01 (95% CI: 0.99 to 1.02; p=0.357) after adjusting for the other predictors .

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Table 15e: Crown rump length: Results from adjusted logistic regression for miscarriage (singleton pregnancies, M=35 imputed datasets)

Miscarriage (death <24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		1.031	0.991 to 1.073	0.130
Mother's age (years)		0.963	0.901 to 1.031	0.279
BMI		1.044	0.986 to 1.106	0.141
Parity	0	0.540	0.255 to 1.147	0.109
	>4	1.000	-	-
Ethnicity	South-Asian	1.427	0.379 to 5.367	0.599
	Oriental	1.000	-	-
	Other Mixed	1.596	0.352 to 7.237	0.544
	White	0.914	0.263 to 3.176	0.887
Deprivation score categories	2	1.000	-	-
	3	0.840	0.097 to 7.265	0.874
	4	1.717	0.219 to 13.447	0.607
	5	1.185	0.151 to 9.275	0.872
Smoking status	Yes	0.755	0.253 to 2.254	0.615
	Stopped	0.804	0.108 to 5.991	0.831
IVF		2.055	0.265 to 15.953	0.491
Gestational diabetes		1.000	-	-

N=10,404

In this adjusted analysis, there is no evidence of an association between CRL and miscarriage with an odds ratio estimate of 1.03 (95% CI: 0.99 to 1.07; p=0.130) after adjusting for the other predictors.

Table 15f: Crown rump length: Results from adjusted logistic regression for stillbirth (M=35 imputed datasets)

Stillbirth (death >24 weeks gestation)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		0.940	0.890 to 0.993	0.027
Mother's age (years)		0.987	0.908 to 1.074	0.769
BMI		1.033	0.958 to 1.113	0.401
Parity	0	1.867	0.775 to 4.499	0.164
	>4	1.000	-	-
Ethnicity	South-Asian	2.542	0.308 to 21.009	0.387
	Oriental	1.000	-	-
	Other Mixed	1.078	0.067 to 17.411	0.958
	White	1.643	0.210 to 12.857	0.636
Deprivation score categories	2	0.513	0.064 to 4.136	0.531
	3	1.076	0.374 to 3.092	0.892
	4	0.967	0.344 to 2.718	0.949
	5	1.000	-	-
Smoking status	Yes	1.000	-	-
	Stopped	1.071	0.141 to 8.134	0.946
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=9,753

In this adjusted analysis, there is evidence of lower odds of stillbirth as CRL increases with an odds ratio estimate of 0.94. The estimate is just statistically significant with a 95% confidence interval of 0.89 to 0.99, suggesting between 1% and 11% lower odds of stillbirth (p=0.027) for a one unit increase in CRL, having adjusted for mother's age, BMI, parity, ethnicity, deprivation score, smoking status, IVF status, and gestational diabetes.

Table 15g: Crown rump length: Results from adjusted logistic regression for perinatal death (singleton pregnancies, M=35 imputed datasets)

Perinatal death (death between 24 weeks gestation and 7 days after birth)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		0.970	0.934 to 1.007	0.110
Mother's age (years)		0.964	0.906 to 1.026	0.245
BMI		1.075	1.025 to 1.128	0.003
Parity	0	2.190	1.136 to 4.223	0.019
	>4	1.000	-	-
Ethnicity	South-Asian	0.631	0.249 to 1.560	0.330
	Oriental	0.408	0.049 to 3.376	0.405
	Other Mixed	0.379	0.098 to 1.459	0.158
	White	0.250	0.105 to 0.598	0.001
Deprivation score categories	2	2.192	0.781 to 6.147	0.136
	3	0.953	0.389 to 2.333	0.916
	4	1.161	0.546 to 2.466	0.698
	5	1.000	-	-
Smoking status	Yes	0.870	0.295 to 2.570	0.802
	Stopped	1.454	0.342 to 6.182	0.612
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,898

In this adjusted analysis, there is no evidence of an association between CRL and perinatal death with an odds ratio estimate of 0.97 (95% CI: 0.93 to 1.01; p=0.108) after adjusting for the other predictors.

Table 15h: Crown rump length: Results from adjusted logistic regression for neonatal death (singleton pregnancies, M=35 imputed datasets)

Neonatal death (death between birth and 28 days)		Odds Ratio	95% Confidence Interval OR	p-value
Crown rump length (mm)		0.997	0.947 to 1.050	0.919
Mother's age (years)		0.949	0.866 to 1.040	0.268
BMI		1.118	1.051 to 1.188	<0.0001
Parity	0	2.389	0.921 to 6.192	0.073
	>4	1.000	-	-
Ethnicity	South-Asian	0.436	0.135 to 1.406	0.165
	Oriental	0.621	0.070 to 5.515	0.669
	Other Mixed	0.258	0.051 to 1.308	0.102
	White	0.064	0.019 to 0.221	<0.0001
Deprivation score categories	2	5.676	1.612 to 19.983	0.007
	3	0.383	0.048 to 3.065	0.366
	4	1.241	0.420 to 3.667	0.697
	5	1.000	-	-
Smoking status	Yes	2.952	0.876 to 9.945	0.081
	Stopped	2.213	0.278 to 17.590	0.453
IVF		1.000	-	-
Gestational diabetes		1.000	-	-

N=10,876

In this adjusted analysis, there is no evidence of an association between CRL and neonatal death with an odds ratio estimate of 0.997 (95% CI: 0.95 to 1.05; p=0.916) after adjusting for the other predictors.

Appendix 13:

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

Outcome	PAPP-A (U/L) OR (95% CI), p-value	NT (mm) OR (95% CI), p-value	CRL (mm) OR (95% CI), p-value
SGA (<10th customised centile)	0.87 (0.85 to 0.90), <0.0001	0.80 (0.71 to 0.90), <0.0001	0.99 (0.99 to 1.00), 0.051
Preterm birth (<37 weeks)	0.92 (0.90 to 0.96), 0.001	1.09 (0.93 to 1.28), 0.294	1.00 (0.99 to 1.01), 0.614
Pre-eclampsia toxaemia	0.91 (0.85 to 0.97), 0.004	0.88 (0.68 to 1.13), 0.314	1.01 (0.99 to 1.02), 0.357
Miscarriage (death <24 weeks gestation)	1.01 (0.84 to 1.21), 0.912	1.75 (1.12 to 2.72), 0.013	1.03 (0.99 to 1.07), 0.130
Stillbirth (death >24 weeks gestation)	0.72 (0.53 to 0.99), 0.044	0.68 (0.24 to 1.90), 0.460	0.94 (0.89 to 0.99), 0.027
Perinatal death (death between 24 weeks gestation and 7 days after birth)	0.88 (0.73 to 1.05), 0.164	0.88 (0.42 to 1.82), 0.730	0.97 (0.93 to 1.01), 0.108
Neonatal death (death between birth and 28 days)	1.03 (0.83 to 1.27), 0.786	1.06 (0.38 to 2.99), 0.907	1.00 (0.95 to 1.05), 0.916

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

Appendix 14: Patient letter informing them of low PAPP-A result

Birmingham Women's NHS
NHS Foundation Trust

Patient Details Date

Dear

Following your recent blood tests we have noted that one of your hormone levels is a little lower than we would expect and for that reason we would like to invite you to attend for a consultant appointment at the time of your 20 week scan to discuss this further. A low level of hormone is sometimes associated with smaller babies, so we take the precaution of offering you extra surveillance during the pregnancy to monitor this; there is no cause for alarm.

Please do not hesitate to contact us if you would like further information before your ultrasound scan on 0121 623 6959 between the hours of 9-5pm Monday to Friday.

Yours sincerely,

Antenatal Screening Midwives

Appendix 15: **SGA risk assessment proforma and referral to Growth clinic**

Birmingham Women's NHS
NHS Foundation Trust

I.D label

Small for Gestational Age screening risk assessement.

Please complete for all women at booking, 20 weeks and third trimester.

	Risk assigned.	Clinician	Signature
Booking			
Risk assessment at booking.	High/Low		
20 weeks assessment			
Customised antenatal growth chart in notes (please sign)			
Reassess risk.	High/Low		
28 weeks assessment			
Reassess risk.	High/Low		
Antenatal admission - reassess risk	High/Low		

Risk assessment and algorithm: **Algorithm of care**

Low Risk
□ No known risk factors

High Risk: one or more of the following:
Maternal Risk Factors
□ Maternal age ≥40 years at term
□ Smoker (ongoing at booking)
□ Drug misuse
Previous Pregnancy History
□ Previous SGA baby (<10th centile)
□ Previous stillbirth
Maternal Medical History
□ Chronic hypertension
□ Diabetes
□ Renal impairment
□ Antiphospholipid syndrome
Unsuitable for monitoring by fundal height- e.g.
□ Large fibroids
□ BMI >35
Current Pregnancy Complications
Early Pregnancy
□ PAPP-A <0.4 MoM (5th centile)
□ Fetal echogenic bowel
Late Pregnancy
□ Severe pregnancy induced hypertension or pre-eclampsia (PIH and proteinuria)
□ Unexplained antepartum haemorrhage

Low Risk Care
Serial assessment (2-3 weekly) of fundal height (FH) from 26-28 weeks until birth
FH measurements plotted on customised chart

Suspected abnormal growth: FH <10th centile or not following curve or 'crossing centile lines'

Normal

Direct referral for assessment within 72 hours for estimated fetal weight (EFW), liquor volume and umbilical artery Doppler

Abnormal growth:
- cust EFW <10th centile and/or
- Serial measurements not following curve and/or
- abnormal umbilical artery Doppler

Transfer to consultant led care.

Management as per trust SGA guideline.

High Risk Care
Serial assessment of referral weight and umbilical Doppler from 26-28 weeks until birth; Scheduled scans 28/32/36/40 weeks.
EFWs plotted on customised chart.

Review (overleaf) at booking, 20 and 28-32 weeks to assess if requires referral to the specialist SGA clinic.

Appendix 16: **Patient information leaflet for low PAPP-A results.**

Information for parents:

Low Pregnancy associated plasma protein (PAPP-A)

You have been given this leaflet as you have a low PAPP-A on the combined screening test.

What is PAPP-A?

Pregnancy associated plasma protein A (PAPP-A) is a hormone that is produced by the placenta in pregnancy. It is one of two hormones that are measured during the 12 week combined screening test.

Low levels of PAPP-A can be associated with Down's Syndrome (an extra chromosome 21), Edward's (extra chromosome 18) and Patau's syndrome (extra chromosome 13). If your baby has an increased risk for these chromosome differences, one of the antenatal screening midwives will have already contacted you before sending you this leaflet.

Studies have also shown that low PAPP-A may also be associated with small babies, early deliveries and pre-eclampsia (high blood pressure and protein in your urine). An unborn baby is small if, at that stage of pregnancy, his or her size or estimated weight on scan is in the lowest 10% of babies. This means the smallest ten out of every 100 babies. Because of this, national guidelines suggest that extra scans should be considered to check the growth of babies when a low PAPP-A level has been found.

What would being small mean for my baby?

If your baby is small but healthy, he or she is not at increased risk of complications. If your baby is growth restricted, there is an increased risk of stillbirth (the baby dying in the womb). The extra scans help us to identify those babies that are small and allow us to put in place extra monitoring as required and consider earlier delivery.

When will I have the extra scans?

We will ask you to come to the antenatal clinic after your 20-week anomaly scan to discuss the low PAPP-A result and make a personalised plan.

At 24 weeks we will measure the blood flow to the uterus and check the growth of the baby.

We will then check your baby's growth, your baby's fluid levels and the blood flow in the placenta at least every 4 weeks until delivery.

When we see you will depend on your individual circumstances and be tailored to your specific

needs. All women and their babies will have a personalised plan made with the doctors looking after them.
Sometimes you will be asked to attend our specialist growth clinic this is nothing to worry about.

Is there anything I can do help my baby to grow well?

If you smoke, it is extremely important that you stop. Smoking can affect the placenta and the baby's growth. Your midwife can refer you for help to stop smoking.

Who can I speak to if I need further information?

You are welcome to phone one of the antenatal screening midwives if you have any queries or concerns. Receiving the news that you have low PAPP-A levels may cause anxiety but please be assured that the majority of babies will have normal growth and the pregnancy will progress normally.

Contact details

Antenatal Screening Midwives 0121 623 6959 Mon-Fri 9:00 – 17:00

Appendix 17: References:

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