SCREENING FOR PREECLAMPSIA

ANDREAS TSIAKKAS

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Faculty of Health, Psychology and Social Care

Manchester Metropolitan University

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ABSTRACT

Background: Preeclampsia (PE) affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. It is thought to occur due to abnormal placentation characterised by poor trophoblastic invasion resulting in oxidative stress and release of factors that promote endothelial dysfunction and inflammation. The current approach of screening for PE is to identify risk factors from maternal demographic characteristics and medical history. In the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) has issued guidelines recommending that women should be considered to be at high risk of developing PE if they have any 1 high-risk factor or any 2 moderate-risk factors. With this approach, defined by NICE, at a screen positive rate of 11%, the detection rate (DR) for total PE is 35%. Such a screening approach has two main limitations. Firstly, it does not provide individualised, patient specific results and secondly, it does not allow the integration of biomarkers for improving the performance of the screening test. However, the integration of such biomarkers is essential in achieving an effective screening strategy for PE.

Objectives: The aims of the papers included in this thesis are firstly, to identify and quantify the effects of variables from maternal characteristics and medical history on specific biochemical markers, secondly to present a model for standardising biochemical marker measurements in all three trimesters of pregnancy into multiples of the normal median (MoM) values, thirdly to summarize the distribution of MoM values in pregnancies with normal outcomes and those that subsequently develop PE and fourthly, to examine the potential improvement in performance of screening for PE at 30-34 weeks' gestation by maternal factors alone with the addition of biophysical and biochemical markers.

Methods: The data for this thesis were derived from prospective screening of women with singleton pregnancies attending for three routine hospital visits at 12, 22 and 32 or 36 weeks' gestation. We have recorded a series of maternal characteristics and history, measured the maternal weight and height as well as the uterine artery pulsatility index (UTPI), mean arterial pressure (MAP), serum concentration of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFLIT-1). The pregnancy outcomes were obtained from the hospital maternity records or the general medical practitioners of the women. Bayes theorem was used

to combine the *a priori* risk from maternal factors with various combinations of biomarker MoM values. The potential value of biophysical and biochemical markers in improving the performance in screening for PE were evaluated.

Results: Firstly, in pregnancies that developed PE, serum PlGF is decreased, while sFLIT-1, MAP and UTPI were increased, secondly, the separation in MoM values from normal is greater with earlier than later gestational age at which delivery for PE is necessary and thirdly, the slope of the regression lines of PlGF MoM with gestational age at delivery in pregnancies that develop PE increases with gestational age at screening. Combined screening at 30-34 weeks' gestation by maternal factors, MAP, UTPI, PlGF, and sFLIT-1 predicted 98% (95% confidence interval, 88-100%) of preterm PE and 49% (95% confidence interval, 42-57%) of term PE, at a false positive rate of 5%.

Conclusions: This thesis has demonstrated that biophysical and biochemical markers increase significantly the performance of screening for PE and as a result the timing and content of clinical visits can be defined by the patient-specific risk of developing the disease. The vast majority of women would be screened low risk and these can follow the routine antenatal care, whereas those few who are high risk could be directed to a more specialized pathway, where early therapeutic interventions prophylactically may lead to the prevention of the disease and close follow-up will reduce the adverse consequences of PE.

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ABBREVIATIONS

PE:	Preeclampsia
BP:	Blood pressure
UTPI:	Uterine artery pulsatility index
MoM:	Multiples of the median
ISSHP:	International Society for the Study of Hypertension in Pregnancy
MAP:	Mean arterial pressure
VEGF:	Vascular endothelial growth factor
s-FLT:	Soluble fms-like tyrosine kinase-1
PlGF:	Placental growth factor
TNF-a:	Tissue necrosis factor-a
NICE:	National Institute for health and Clinical Excellence
DR:	Detection rate
FPR:	False positive rate
SPREE:	Screening Programme for Preeclampsia

CONTENTS

Publications

Tsiakkas A, Cazacu R, Wright A, Wright D, Nicolaides KH. Serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015;doi: 10.1002/uog.15816.

Tsiakkas A, Mendez O, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015;doi: 10.1002/uog.15817.

Publication

Tsiakkas A, Saiid Y, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 30-34 weeks' gestation. Am J Obstet Gynecol 2016;doi:10.1016/j.ajog.2016.02.016.

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

Preeclampsia (PE) remains a leading cause of maternal perinatal morbidity and mortality complicating around 2-3% of pregnancies (World Health Organisation, 2005; Cantwell *et al*., 2011; Duley, 2009; Confidential Enquiry into Maternal and Child Health; 2008). Maternal organ dysfunction is a known complication of PE involving the kidneys with increased serum creatinine concentration, the liver with elevated transaminases, neurological complications such as hyperreflexia, severe headache accompanied with visual scotoma, blindness, stroke or eclampsia, and haematological complications characterised by low platelet counts.

The aetiology of PE is still unclear but it has been linked to abnormal placentation due to impaired trophoblastic invasion of the spiral arteries (Khong *et al.,* 1986; Lyall, 2002.) In pregnancies which are not complicated by PE there is an increased capacitance of the spiral arteries mainly due to the replacement of endothelial smooth muscle by trophoblasts. This physiological process results in a high utero-placental vascular capacitance while at the same time impedance to blood flow is greatly decreased (Sagol *et al.,* 1999).

One of the most important features of PE is elevated blood pressure (BP) as well as proteinuria after 20 weeks' of gestation. Thus the BP should be measured at the booking visit, as early as possible, and during the course of pregnancy. In addition, a detailed maternal medical history should be obtained in order to identify risk factors for the development of PE.

All women, in the hospitals where I am a research fellow, are attending for three routine visits. In the first visit, at 11+0-13+6 weeks' gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies. The second visit, at 19+0-24+6 weeks' gestation, and third visit, initially at 30+0-34+6 weeks and subsequently at 35+0-37+6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size. Biophysical markers such as uterine artery pulsatility index (UTPI) are routinely measured. Maternal blood samples were also taken for analysis for various biochemical markers. These assessments are carried out by several research fellows including myself.

I have been prospectively collecting all these data including pregnancy outcomes as well as ensuring quality controls of these collections. I was involved in training of junior research fellows in obtaining ultrasound measurements and individual operator distributions were produced. Further training was provided for operators whose distributions were inappropriate. In cases where hypertensive disorders were reported, maternal notes have been evaluated to ensure data quality. I liaised with the National Ethics Committee for ethical approval and worked closely with the Research and Development Department to keep study documents up to date. I was responsible for maintaining the storage of samples in freezers, arranging the medical transfer agreements and I also maintained the database for samples stored for research.

1.2 OVERALL AIMS OF THE THESIS

This thesis aims to develop a new method of screening for PE during the course of pregnancy that will identify the majority of women that will develop PE. The rationale for such early identification of high-risk pregnancies is that treatment at this stage, with such drugs as low-dose aspirin, can potentially prevent the development of the disease. Screening in the second trimester will essentially re-categorise women into high risk or low risk groups. Some of the previous high risk women will be classified at this point as low risk maybe because the pharmacological intervention has managed to produce healthy placentation, and these women will follow the routine antenatal care path. Previously low risk women might now be classified as high risk and thus be closely monitored for early identification of the signs of the disease. In the third trimester, and in particular at 30-34 week's gestation, we will screen women and depending on their result we will aim to decide when, where and by which method we will deliver the baby.

The aims of the papers included in this thesis are:

- effectively use Bayes theorem to combine maternal characteristics with biophysical and biochemical markers
- produce patient specific risks
- identify and quantify the effects of variables from maternal characteristics and medical history on specific biochemical markers

- present a model for standardising biochemical marker measurements in all three trimesters of pregnancy into multiples of the normal median (MoM)
- summarize the distribution of MoM values in pregnancies with normal outcomes and those that subsequently develop PE
- investigate the performance of our screening method throughout the course of pregnancy
- examine the potential improvement in performance of screening for PE at 30-34 weeks' gestation by maternal factors alone with the addition of biophysical and biochemical markers.

1.3 DEFINITION OF PREECLAMPSIA

PE is a disorder in pregnancy characterised by hypertension and proteinuria in a previously normotensive woman. There are several definitions for the diagnosis of PE but the one that is widely used is that of the International Society for the study of Hypertension in Pregnancy (ISSHP) (Davey and MacGillivray, 1988; Brown *et al.*, 2001, Tranquilli *et al.,* 2014). Thus according to the ISSHP, PE is defined as a systolic blood pressure of ≥140mm Hg and/or diastolic blood pressure of ≥90mm Hg on at least 2 occasions 4 hours apart that develops after 20 weeks' gestation in a previous normotensive woman and proteinuria of ≥300mg in 24 hours or 2 readings of at least ++ on dipstick analysis of midstream or catheter urine specimens, if no 24-hour collection is available.

In PE, hypertension develops as a result of vasoconstriction and reduced peripheral vascular compliance. It is an important sign of PE, since it is an early indication of the disease. This highlights the importance of monitoring BP during antenatal care. Usually the first BP measurement is the highest while the subsequent measurements become lower since the patient becomes more familiar to the procedure (Poon *et al.,* 2012). Thus it is recommended by professional bodies that a series of blood pressure measurements should be made until a level of stability is achieved (National Heart Foundation of Australia, 2004; Pickering *et al.,* 2005). Accurate assessment of BP has also been hindered by the considerable variability that BP exhibits within each individual operator. The gold standard in clinical practice is the use of sphygmomanometers but there are concerns for their clinical performance

(Markandu *et al.,* 2000). In these studies, mean arterial pressure (MAP) was measured at each visit. The use of validated automated devices allows simple, standardised, and repeated measurements to be taken. It also addresses many of the errors associated with the conventional sphygmomanometer but their use still requires the selection of the correct cuff size, proper patient positioning and at least two measurements should be taken from each arm simultaneously.

1.4 PATHOGENESIS OF PREECLAMPSIA

The pathogenesis of PE is still unclear but it is believed to occur due to the impaired invasion of trophoblasts in the spiral arteries. These impairment results in an endothelial dysfunction which manifests itself in clinical signs and symptoms frequently encountered in preeclamptic women.

The placenta is mainly formed by the trophoblasts. The trophoblasts themselves can be divided into syncytiotrophoblasts and cytotrophoblasts. At around 11-12 days of gestation the syncytiotrophoblasts erode in the endothelial level of the maternal capillaries to establish the uteroplacental circulation. Later on, the cytotrophoblasts invade the syncytiotrophoblasts forming primary villi. During the third week of pregnancy the cytotrophoblasts perforate the endometrium while having an outer covering by the syncytiotrophoblasts. Extraembryonic mesoderm which passes through the cytotrophoblasts forms the secondary villi. Finally, tertiary villi are produced by the differentiation of their vessels at the level of the extraembryonic mesoderm. The trophoblastic invasion takes place in two distinct stages of pregnancy, the first very early as described above while the second at around 14 to 16 weeks' gestation (Robillard, 2002). The result of this physiological mechanism is to convert these high resistance muscular vessels to low resistance vessels, ensuring sufficient blood supply to the developing fetus (Brosens *et al.,* 1972). Failure of the physiological invasion and remodelling of the spiral vessels result in vessels of smaller diameter producing placental ischemia and poor perfusion.

Placental, endothelial and genetic factors as well as immunological maladaptation are believed to be involved in the pathogenesis and subsequent development of PE.

1.4.1 PLACENTAL FACTORS

It has been demonstrated that trophoblastic invasion of spiral arteries might play a role in the pathogenesis of PE. Moreover, the degree of invasion seems to be of interest since in cases with PE only the decidual portion of the vessels has been invaded while in cases unaffected by PE the trophoblastic invasion had reached the myometrial portion of the spiral arteries (Brosens et al., 1972; Meekins et al., 1994a). There is an association between the severity of histological changes in spiral arteries, the impedance to uterine artery blood flow and the severity of PE. It has been documented that the more advanced the histological changes in the utero-placental circulation the higher the UTPI, the more severe and the earlier the onset of the disease suggesting that there is an increased resistance in the blood flow in the uterine arteries suppling the placenta (Olofsson *et al.,* 1993; Meekins *et al.,* 1994a).

Another interesting element in the pathogenesis of PE is the observation of the presence of acute atheromata in the utero-placental circulation (Sheppard and Bonnar, 1981).
Upon histological examination of the placenta and its vascular bed, it has been observed the presence of fibrinoid necrosis which in turn stimulated the chemotaxis of macrophages which appeared to be lipid laden as well as mononuclear cellular infiltrates (Pijnenborg *et al.,* 1991). In addition, there has been an increased deposition of lipoprotein in arterial walls as well as micro-thrombi formation which resulted in placental infarctions. These histological findings were not only observed in cases with PE but also in cases with recurrent miscarriages, pregnancies complicated with fetal growth restriction as well as pregnancies of women with antiphospholipid syndrome (Meekins *et al.,* 1994b; Salafia *et al.,* 1995).

1.4.2 ENDOTHELIAL DYSFUNCTION

It has been suggested that endothelial dysfunction plays a major role in the development of PE. This is essentially the result of a cascade involving primarily an increased vascular resistance which leads to placental hypoxia and ischemia. These in turn favour the release of reactive oxygen species increasing substantially the oxidative stress on the endothelium. These mechanisms result in a profound

intravascular inflammatory response and a consequent activation or repression of endothelial cell function (Redman and Sargent, 2003; Myatt and Webster, 2009; Saito and Nakashima, 2014).

These series of changes on the endothelial level cause altered levels of angiogenic and anti-angiogenic proteins. Vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1 also known as sFLT-1 are examples of such proteins. This protein imbalance leads to further dysfunction of the endothelium (Luttun *et al.,* 2002; Maynard *et al.,* 2003; Widmer *et al.,* 2007; Murphy *et al.,* 2013).

PlGF, a glycosylated dimeric glycoprotein, is a member of the vascular endothelial growth factor subfamily. It binds to vascular endothelial growth factor receptor-1 which has been shown to rise in pregnancy. PlGF is synthesized in villous and extravillous cytotrophoblasts and has both vasculogenetic and angiogenetic functions. It is believed to contribute a change in angiogenesis from a branching to a non-branching phenotype controlling the expansion of the capillary network. Its angiogenetic abilities have been speculated to play a role in normal pregnancy and changes in the levels of PlGF or its inhibitory receptor have been implicated in the development of PE (Maynard et al., 2003; Ahmad and Ahmed, 2004; Levine et al., 2004; Stepan et al., 2007). PE is associated with reduced placental production of PlGF and these reduced levels of serum PlGF precede the clinical onset of the disease and are evident from both the first and second trimesters of pregnancy (Su et al., 2001; Tidwell et al., 2001; Tjoa et al., 2001; Polliotti et al., 2003; Krauss et al., 2004; Thadhani et al., 2004; Akolekar et al., 2008; Crispi et al., 2008; Erez et al., 2008).

sFLT-1, is a circulating anti-angiogenic protein implicated in the pathogenesis of PE. It is expressed at very high levels in the trophoblast and its production is highly increased in hypoxic conditions. The concentration of sFLT-1 is increased in the placenta and serum of women with PE and exogenous sFLT-1 administered to pregnant rats induces hypertension, proteinuria and glomerular endotheliosis (Maynard et al., 2003). There is also evidence that the levels of serum sFLT-1 are increased in the few weeks preceding the clinical onset of PE and consequently sFLT-1 may be a useful biochemical marker in screening for PE (Levine *et al* 2004;

Chaiworapongsa *et al* 2011; Verlohren *et al*., 2012; Rana *et al*.,2012; Lai *et al*., 2014).

Another important characteristic of endothelial dysfunction is the resulting vasospasm which induces platelet activation with a subsequent dysfunction and thrombocytopenia (Romero *et al.,* 1988). In addition, there is a profound decrease in platelet size, life span and imbalance in the proteins produced and released by the platelets such as thromboxane and prostacyclin (Walsh*,* 1985). More specifically thromboxane levels, which is a known vasoconstrictor increase, while prostacyclin levels, responsible for vasodilation decrease. This is further demonstrated by the decreased metabolite levels of prostacyclin in maternal urine and blood.

The overall result of endothelial dysfunction is an alteration of angiogenic and anti-angiogenic proteins which leads to platelet dysfunction and a subsequent vasoconstriction which ultimately favours the formation of micro-thrombi (Romero and Duffy, 1980).

1.4.3 GENETIC AND IMMUNOLOGICAL FACTORS

It has been suggested that a genetic predisposition might be involved in the development of PE. Several studies have shown that women with a positive family history have a threefold risk for developing the condition compared to those without a family history of PE (Arngrimsson *et al*., 1990; Duckitt and Harrington 2005).

One or more genes or alleles may increase the risk of PE by either regulating blood pressure (BP) and/or modifying placental function (Cooper and Liston 1979; Chesley and Cooper, 1986). It is also believed that there is a linkage between PE and genes coding for angiotensinogen, tumour necrosis factor-a (TNF-a) and factor V Leiden (Morgan *et al*., 1999; Benedetto *et al*., 2002).

Two meta-analysis studies have suggested that there is a role for thrombophilia genes in severe PE as well as a 2-fold increase for developing the disease in the presence of a prothrombin polymorphism (Wang *et al*., 2014; Human Genome Epidemiology Review). Despite the fact that associations between gene

polymorphisms and PE has been documented by these studies, the need for larger studies is evident.

The placenta is formed from fetal tissues thus it has both maternal and paternal derived antigens. In pregnancies which are unaffected by PE it seems that the maternal immune system is tolerant to fetal antigens and any immune response opposes the rejection of the semi-allogenic fetus. According to some studies, in pregnancies with PE an immunological activation produces a reaction towards the fetus and the placenta and in particular against the paternal portion of the trophoblasts (Dekker and Robillard, 2005; Dekker et al., 1998). It has not been established yet if an immune reaction is a result of an abnormal placentation or if it is the causative mechanism of this.

Epidemiological observations have shown that firstly, PE is more common in a first pregnancy, secondly the risk for PE decreases with subsequent pregnancies provided the partner is the same, thirdly changing partners increases the risk for developing PE and fourthly donor insemination techniques as well as oocyte donation increase the likelihood for the development of the condition (Robillard *et al.,* 1999; Li and Wi, 2000).

1.5 SCREENING FOR PREECLAMPSIA

The current approach of screening for PE is to identify risk factors from maternal demographic characteristics and medical history. In the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) has issued guidelines recommending that women should be considered to be at high risk of developing PE if they have any 1 high-risk factor or any 2 moderate-risk factors. The high-risk factors are a history of hypertensive disease in a previous pregnancy, chronic kidney disease, autoimmune disease, diabetes mellitus, or chronic hypertension; the moderate-risk factors are first pregnancy, maternal age of >40 years, inter-pregnancy interval of >10 years, body mass index at first visit of $\geq$35 kg/m^2 or family history of PE. With this approach, defined by NICE, at a screen positive rate of 11%, the detection rate (DR) for total PE is 35%, for PE requiring delivery at <37 weeks' is 40% and for PE requiring delivery <34 weeks' is 44% (Wright *et al*., 2015). Such a

screening approach has three main limitations. Firstly, it does not provide individualised, patient specific results. Secondly, it does not allow the integration of biochemical and biophysical markers for improving the performance of the screening test, but the integration of such biomarkers is essential in achieving an effective screening strategy for PE. Thirdly, this screening model treats each variable as a separate screening test with additive detection and false positive rates thus a large proportion of the population is screened positive, requiring a more frequent antenatal care, creating an increased strain on the healthcare system.

1.5.1 BACKGROUND

An alternative approach to risk assessment and screening for PE is to apply Bayes' theorem to combine the *a priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy, which allows estimation of individual patient-specific risks of PE. A fundamental component of this approach is a previous distribution and a survival-time model for the gestational age at delivery with PE (Wright *et al*., 2012; Akolekar *et al*., 2013). This approach assumes that if the pregnancy was to continue indefinitely, all women would experience PE, and whether they do so or not before a specified gestational age depends on competition between delivery before or after the development of the disease. In pregnancies at low risk for PE, the gestational age distribution is shifted to the right with the implication that, in most pregnancies, delivery actually will occur before the development of PE. In high risk pregnancies, the distribution is shifted to the left, and the smaller the mean gestational age the higher is the risk for PE. However, in the application of Bayes theorem in combined screening for PE, it is essential to standardize the measured values of biomarkers for any variables included in the *prior* model into MoM values.

1.5.2 BIOCHEMICAL FACTORS

A large number of biochemical markers were investigated, since they represent a measureable manifestation of improper placentation and the subsequent release of inflammatory markers. In the literature, most studies have investigated such

biomarkers in the 2nd trimester of pregnancy as well as in pregnancies which have already developed PE.

It is imperative that adjustments need to be made in the maternal serum metabolite concentration to correct for certain maternal and pregnancy characteristics as well as machines and reagents used for the analysis of these markers. As a result, the values of biochemical markers should be expressed in MoM of the normal median. (Kagan *et al.,* 2008). In the studies included in this thesis two biochemical markers were investigated which include PlGF and sFLIT-1.

PlGF is a pro-angiogenic protein released by different cells including cytotrophoblasts. It is involved in the processes of angiogenesis, vasculogenesis and vascular permeability and it is transcribed from the PLGF gene found on chromosome 6 (Romero *et al.,* 2008b).
sFLIT-1 is produced by syncytiotrophoblasts by alternative splicing of the FLIT-1 gene resulting in a protein which is unable to bind PLGF inside cells but instead remains on the cell surface resulting in an antagonistic effect to PLGF (Maynard *et al.,*2003; Levine *et al.,*2004; Tache *et al.,* 2011). sFLIT-1 also compromises endothelial function at the level of the brain, liver and kidneys (Esser *et al.,* 1998). By blocking the effects of VEGF and PLGF, sFLIT-1 leads to hypertension and proteinuria (Eremina *et al.*, 2003).

1.5.3 BIOPHYSICAL FACTORS

Biophysical markers can be used in screening for PE since they can be correlated to the disease. UTPI is essentially the opposition exerted to blood flow and as described earlier can be a measurable manifestation of histological alteration of the utero-placental system. Under physiological conditions the distal branches of uterine arteries show a decrease in resistance as the pregnancy progresses and this can be demonstrated using Doppler studies (Carbillon *et al*., 2001). Two mechanisms have been suggested for the low resistance in these arteries. The first one is the replacement of the muscular wall of the arteries with trophoblastic cells and the second one is the hormonal effect exerted on the compliance of the vascular wall. The use of specific techniques for obtaining the UTPI measurements ensures

reliability of the collected data. In the studies included in this thesis UTPI was obtained transabdominally. The uterine arteries were identified using colour Doppler at the level of the internal cervical os. Then pulsed-wave Doppler was applied with the sample gate set at 2mm to cover the whole vessel. The angle of insonation was less than 30° and the peak systolic velocity was greater than 60cm/s, to ensure that the uterine artery, rather than the arcuate artery, was being examined. When three similar waveforms had been obtained consecutively the UTPI was measured and the mean pulsatility index of the left and right arteries was calculated (Papageorghiou *et al.*, 2001).

There is evidence showing that in women who will eventually develop PE, BP might be increased from the beginning of the pregnancy (Poon *et al*., 2008). A meta-analysis showed that MAP is significantly better at predicting PE compared to systolic or diastolic BP. Another contradictory conclusion of this meta-analysis is that BP measurement is not effective for screening for PE. It is important to note that there have been major differences in between the studies in terms of design, sample size and machines used to measure the BP (Cnossen *et al.,* 2008).

This highlights the necessity of a standardised method of BP measurement. In the studies included in this thesis MAP was measured for each visit by validated automated machines. The machines were calibrated before and at regular intervals during the studies. For the measurements the patients were in sitting position, their arms were supported at the level of the heart and a small (22cm), medium (22-32cm) or large (33-42cm) adult cuff was used depending on the mid-arm circumference. After resting for 5 minutes, two recordings of BP were made in both arms simultaneously. The final MAP was calculated as the average of all four measurements (Poon *et al.,* 2012).

The next chapter will address and define the contribution of maternal variables which influence the measured levels of PLGF and sFLIT-1 and express them in MoM values.

CHAPTER 2

EFFECT OF MATERNAL FACTORS ON MARKERS OF PLACENTATION

This chapter is based on two publications. Our approach to screening for pregnancy complications, such as preeclampsia (PE), is to apply Bayes theorem to combine the *a priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy (Wright *et al.*, 2012; Akolekar *et al.*, 2013, Nicolaides *et al*., 2011). In normal pregnancies serum biochemical marker concentrations are affected by the gestational age as well as maternal history and characteristics, including weight, racial origin and outcome of previous pregnancies (Pandya et al.,2012., Lai et al., 2014). Consequently, the aims of the first 2 publications are to standardise the measured levels of soluble fms-like tyrosine kinase-1 (sFLT-1) and placenta growth factor (PlGF) into multiples of the median (MoM) values by taking into account these variables for their effective use in risk assessment.

In the first paper maternal characteristics and medical history were recorded and serum PLGF was measured in women with singleton pregnancies attending for three routine hospital visits at 12, 22 and 32 or 36 weeks' gestation. We measured in 38,002 cases in the first trimester, 10,281 in the second trimester and 12,398 in the third trimester. Significant independent contributions to serum PLGF were provided by gestational age, maternal age, weight and racial origin, cigarette smoking, diabetes mellitus, and gestational age at delivery and birth-weight Z-score of the neonate in the previous pregnancy. The machine used to measure serum PlGF was also found to have a significant effect. Allowing for other factors, the effect of maternal age on PlGF changed over the three trimesters, whereas other variables had constant effects over the three trimesters. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum PlGF and express the values as MoMs. The model was shown to provide an adequate fit of MoM values for all covariates, both in pregnancies that developed PE and in those without this complication. Previous studies have investigated the value of PlGF in screening for trisomies in the 1st trimester as well as small for gestational age foetuses and PE in all three trimesters (Zaragoza et al., 2009; Pandya et al., 2012., Akolekar et al., 2008, Poon et al., 2008; Akolekar et al., 2011; Karagiannis et al., 2011; Lai et al., 2014). In this study a larger population was used, developing a model which identifies variables which have effects on specific

gestational age and variables which have a common effect across trimesters. Furthermore, the effects of Diabetes Mellitus type 1 and 2 as well the outcome of the previous pregnancies on the measured concentrations of PLGF have been documented by this study.

In the second publication a similar methodology was used in which maternal characteristics and medical history were recorded and serum sFLT-1 was measured in women with singleton pregnancies attending for three routine hospital visits at 12, 22 and 32 or 36 weeks' gestation. Serum sFLT-1 was measured in 7,066 cases in the first-trimester, 8,078 in the second-trimester and 10,464 in the third-trimester. Median levels of serum sFLT-1 showed an increasing curvilinear relationship with gestational age, the increase was much steeper in the third trimester than in the first and second trimesters, while the effects on serum sFLT-1 of variables were similar in all three trimesters. Serum sFLT-1 decreased with maternal weight and parity but it was higher in cigarette smokers and in women of Afro-Caribbean racial origin than in Caucasian women. In addition, the serum levels of sFLT-1 increased with the inter-pregnancy interval and birth weight Z-score of the neonate in the previous pregnancy. In this paper also the model was shown to provide an adequate fit of MoM values for all covariates both in pregnancies that developed PE and in those without this pregnancy complication. Previous studies investigating sFLT-1 for the prediction of PE did not adjust the measured values according to maternal characteristics and medical history (Karumanchi 2004; Chaiworapongsa et al., 2011; Verlohren et al., 2012; Rana et al., 2012). The observation that sFLT-1 decreases with increasing maternal weight, is higher in Afro-Caribbean women than in Caucasian women and is lower in parous than nulliparous women has been made in previous studies (Lai et al., 2014). In this study though, it was also identified that sFLT-1 is lower with smoking, is affected by the outcome of the previous pregnancy in parous women and the effects of variables on sFLT-1 are similar in all trimesters.

Strengths and limitations

The main strength of these studies is the large number of women that were examined. Also women were examined on specific gestational ages which are widely used for routine screening for chromosomal abnormalities in the first trimester and for

the assessment of fetal anatomy and wellbeing in the second and third trimesters. The assessment and counselling can be done on the same visit since results can be readily available by the use of automated machines. Furthermore, the application of multiple regression analysis resulted in determining which maternal variables affect the measured levels of PlGF and sFLT-1 across all three trimesters of pregnancy.

An alternative approach was to include all gestational weeks from the beginning to the end of the pregnancy but a more practical approach was to collect data from the gestational-age ranges used in routine clinical practice.

Publications

https://www.ncbi.nlm.nih.gov/pubmed/25653039 Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol 2015; 45:591-598.

https://www.ncbi.nlm.nih.gov/pubmed/25678265 Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol 2015; 45:584-590.

The next chapter will address the distribution of biochemical markers across all trimesters of pregnancies which develop PE and examine the performance of these biomarkers in screening for PE.

CHAPTER 3

EFFECT OF PLACENTATION MARKERS IN SCREENING FOR PREECLAMPSIA

This chapter is based on two publications. The incorporation of biochemical markers in preeclampsia (PE) screening could potentially improve the prediction of PE provided by maternal factors alone and in addition could provide patient specific risks within 40 minutes of sampling so that complete assessment and counselling can be undertaken in the same hospital visit.

In the third publication serum PlGF was measured in 40,212 cases at 11-13 weeks, in 10,282 cases at 19-24 weeks, in 10,400 at 30-34 weeks and 4,043 at 35-37 weeks; Bayes theorem was used to combine the *a priori* risk from maternal factors with serum PlGF. To provide model based estimates for the performance of screening, we obtained the dataset of 123,406 pregnancies including 2,748 (2.2%) cases of PE. This dataset was also used previously to develop a model for PE based on maternal characteristics and history (Wright et al., 2015; Andrietti et al., 2015). For the empirical performance of screening five-fold cross validation was used by models combining maternal characteristics and history with PlGF. This large prospective screening study has demonstrated firstly, that in pregnancies that developed PE, serum PLGF is decreased, secondly, the separation in multiples of the median (MoM) values from normal is greater with earlier than later gestational age at which delivery for PE is necessary and thirdly, the slope of the regression lines of PlGF MoM with gestational age at delivery in pregnancies that develop PE increases with gestational age at screening. The detection rate (DR) at a false positive rate (FPR) of 10%, for PE at <32 weeks was 79% and 96% with screening at 12 and 22 weeks, respectively, the DR for PE at 32^{+0}-36^{+6} weeks was 57%, 69% and 90% with screening at 12, 22 and 32 weeks and the DR for PE at ≥37 weeks was 40%, 39%, 54% and 64% with screening at 12, 22, 32 and 36 weeks, respectively. The measurement of serum PlGF improved substantially the performance of screening of PE provided by maternal factors alone. While several studies have shown that PlGF is decreased in both the 1^{st} and 3^{rd} trimesters of pregnancies that develop PE (O'Gorman *et al*., 2015; Akolekar *et al.,* 2008; Akolekar *et al* 2011; Lai *et al*.,2014), in this study we have compared the performance of screening for early, intermediate and late PE by maternal factors alone and in combination with PlGF. Furthermore, the relationship between gestational age at screening and performance of the test was established.

The same methodology was used in the fourth paper in which the distribution and performance in screening for PE of maternal sFLT-1 was examined. The levels of this biochemical marker were determined in 7,066 cases at 11-13 weeks, 8,079 cases at 19-24 weeks, 8,472 at 30-34 weeks and 4,043 at 35-37 weeks. The performance of screening for PE in women requiring delivery <32, between 32 and 36^{+6} weeks and >37 weeks' gestation were estimated. In pregnancies that developed PE, serum soluble fms-like tyrosine kinase-1 (sFLT-1) was increased and the separation in MoM values from normal was greater with earlier, compared to later, gestational age at which delivery for PE became necessary. In pregnancies that developed PE, the slope of the regression lines of sFLT-1 MoM with gestational age at delivery increased with advancing gestational age at screening. Measurement of sFLT-1 at 11-13 weeks did not improve the prediction of PE achieved by maternal factors alone, sFLT-1 at 19-24 weeks improved the prediction of PE delivering <37 weeks but not for PE delivering >37 weeks, sFLT-1 at 30-34 weeks improved the prediction of PE delivering <37 weeks and PE delivering >37 weeks and sFLT-1 at 35-37 weeks improved the prediction of PE delivering >37 weeks. The DRs, at a FPR of 10%, of PE delivering <32 weeks were 52% and 65% with screening at 12 and 22 weeks, respectively. The DRs for PE delivering between 32 and 36^{+6} weeks were 44%, 44% and 93% with screening at 12,22 and 32 weeks. The DRs for PE delivering >37 weeks were 37%, 37%, 52% and 69% with screening at 12, 22, 32 and 36 weeks, respectively. Thus the performance of combined screening with maternal factors and serum sFLT-1 is superior for detection of early, compared to late, PE and improves with advancing gestational age at screening. A few studies have shown that sFLT-1 concentration is increased a few weeks before the development of the disease (Levine et al., 2004; Dong *et al*., 2011; Verlohren *et al.*, 2012; Rana *et al.,* 2012; Lai *et al.,* 2014). Moreover, case–control studies have shown that in women with PE serum sFLT-1 is elevated from the 1^{st} trimester, improving the performance of screening at this stage of the pregnancy (Crovetto *et al.,* 2014; Crovetto *et al*., 2015). In this study we have documented the performance of screening using maternal factors alone and in combination with sFLT-1 for early, intermediate and late PE. Opposite to what has been shown by the studies mentioned above, incorporation of sFLT-1 at 11-13 weeks' gestation did not improve the prediction of PE compared to maternal factors alone.

Strengths and limitations

Potential strengths for these studies include firstly, the examination of a large population of pregnant women attending for routine care secondly, data on maternal characteristics and history with known associations with PE have been recorded thirdly, since biochemical marker measurements can be performed by automated machines which are currently used in routine screening for aneuploidies, results can be available in less than one hour allowing for counselling of patients in the same hospital visit fourthly, the measured values of PlGF and sFLT-1 have been converted to MoM's allowing for adjustment for factors that affect the measurements and fifthly, patient specific risks can be produced at different stages of pregnancy since Bayes' theorem was used to combine the a priori risk derived from maternal factors with biochemical markers. A possible limitation of these studies is the potential overestimation of the performance of screening since the same dataset was used to develop the screening model. A cross validation was used to reduce this effect, demonstrating a good agreement between the modelled and empirical performance.

Publications

https://www.ncbi.nlm.nih.gov/pubmed/26582455 Tsiakkas A, Cazacu R, Wright A, Wright D, Nicolaides KH. Serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015; 47:472-477.

https://www.ncbi.nlm.nih.gov/pubmed/26582564 Tsiakkas A, Mendez O, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015; 47:478-483.

The next chapter will address the performance of screening for PE by a combination of maternal factors with early third-trimester biochemical and biophysical markers.

CHAPTER 4

COMBINED SCREENING FOR PREECLAMPSIA AT 30-34 WEEKS' GESTATION

This chapter is based on one publication. The objectives of third trimester screening for preeclampsia (PE), is to improve perinatal outcome through close monitoring of the high-risk population for earlier diagnosis of the clinical signs of the disease and selection of the appropriate, time, place and method of delivery. Thus the objective of the fifth publication was to develop a model of screening for PE based on a combination of maternal factors with early third-trimester biomarkers and in the estimates of performance of screening, empirical results are compared with model-based rates. Data were collected for mean arterial pressure (MAP), uterine artery pulsatility index (UTPI), placenta growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFLT-1) from 29,042, 30,935, 10,123 and 8,264 women respectively, attending for their routine hospital visits at 30-34 weeks' gestation. Bayes theorem was used to combine the *a priori* risk from maternal factors with various combinations of biomarkers (Wright *et al.,* 2015; O'Gorman *et al.,*2016; Gallo *et al.,* 2015). The measured levels of biomarkers are affected by variables from maternal characteristics and history and thus their levels were standardised into multiples of the median (MoM) values (Wright *et al.,*2015; Tayyar *et al.,* 2015; Tsiakkas *et al.,* 2015a; Tsiakkas *et al.,* 2015b). The performance of screening for PE requiring delivery at <37 weeks and >37 weeks' gestation and within 4 and 6 weeks of assessment were determined firstly by examining the empirical results in 7927 pregnancies with complete data on MAP, UTPI, PLGF and sFLT-1, secondly by examining the empirical results using all available data for each biomarker, and thirdly by modelling, whereby values on biomarkers were simulated for our 123,406 singleton pregnancies with available data on maternal factors (Gallo *et al*., 2015).We concluded that in pregnancies that developed PE, the values of MAP, UTPI and sFLT-1 were increased and PlGF was decreased. For all the biomarkers the deviation from normal was greater for preterm than term PE and therefore the performance of screening was inversely related to the gestational age at which delivery was necessary for maternal and/or fetal indications. Screening by maternal factors alone predicted 47% and 37% of PE at <37 and >37 weeks' gestation, respectively, at a false positive rate (FPR) of 10%. The respective values for combined screening with maternal factors, MAP, UTPI, PLGF and sFLT-1 were 99% and 49%.

Strengths and limitations

A large population of women attending for routine care is a gestational age widely used for the assessment of fetal growth was used. The a *priory* risk was generated from the maternal characteristics and medical history allowing the calculation of patient-specific risks using Bayes theorem. Furthermore, the methods used in obtaining the measurements of all the biophysical markers were well established. The measurements were obtained by appropriately trained doctors thus ensuring reliable datasets. Equally important is the prompt availability of biochemical results by automated machines. Lastly, biochemical and biophysical markers have been expressed in MoM thus allowing for adjustment of factors affecting the measurements. A limitation of this study is that some findings rely on modelling. A 10-fold cross-validation was used on the empirical data, reducing bias. In addition, the empirical and modelled performance was compatible which is reassuring.

Publications

https://www.ncbi.nlm.nih.gov/pubmed/26875953 Tsiakkas A, Saiid Y, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 30-34 weeks' gestation. AmJObstet Gynecol 2016;doi:10.1016/j.ajog.2016.02.016.

The next chapter involves the discussion and the conclusions of all studies presented in previous chapters.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Implications for clinical practice

Preeclampsia (PE) is a major cause of morbidity and mortality for both the mother and the baby. The current screening guidelines in the United Kingdom issued by the National Institude for Health and Clinical Excellence (NICE), unfortunately result in poor detection of women who will develop the disease (Wright *et al* 2015; O'Gorman *et al*., 2016; Gallo *et al*., 2015). This is mainly because of the methodology used, which involves only maternal medical history and characteristics and their incorporation into a risk scoring system, which treats each risk factor as a separate screening test with additive detection rates (DR) (Wright *et al*.,2015; O'Gorman *et al*., 2016; Gallo *et al.*, 2015). Depending on this score the intensity of prenatal care is adjusted accordingly. In the late second or third trimester of pregnancy current prenatal care involves screening for PE based on the demonstration of elevated blood pressure (BP) and proteinuria during a routine clinical visit. The objective of minimizing adverse perinatal outcomes can be achieved using an alternative method of screening which will produce patient-specific risks. According to these risks the appropriate intensity in maternal and fetal monitoring, time and place of delivery and even pharmacological intervention could be determined. It has been suggested from meta-analysis studies that initiation of aspirin from early pregnancy can reduce the incidence of PE (Bujold *et al*.,2010; Roberge *et al.,* 2012a and 2012b).

Bayes' theorem states the probability of an event, based on conditions that might be related to the event. Another approach to screening which is based on Bayes' theorem and allows estimation of individual patient-specific risks involves the combination of maternal characteristics and history with various biochemical and biophysical markers (Wright *et al.,*2015; O'Gorman *et al.*, 2016; Gallo *et al.,* 2015). Such an approach is based on a survival-time model which treats the gestation at the time of delivery for PE as a continuous rather than a categorical variable. Thus PE could be considered as a single pathophysiological entity with a wide spectrum of severity manifested in gestational age at which delivery becomes necessary for maternal and/or fetal indications. Essentially this gives the option to clinicians and researchers to select their own gestational age cut-off to define the high risk group that could potentially benefit from therapeutic interventions. This thesis has shown that the screening performance of such an approach is improved compared to the

model based series of maternal characteristics alone. This concept is well accepted in screening for aneuploidies in which a combined risk cut-off is used to guide pregnancy management thus this philosophy could be adopted in screening for PE.

There is a significant effect of maternal characteristics on the concentration of biochemical markers in normal pregnancies and thus the adjustment for these characteristics before comparison with pregnancies affected with PE is needed (Kagan et al., 2008a). The first two publications included in this thesis have defined the contribution of these maternal characteristics on the measured concentrations of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFLT-1) and converted them into multiples of the median (MoM) so that they can be used in the model described before. In addition, the distribution of both PlGF and sFLT-1 in normal pregnancies and those that developed PE was examined.

In the third publication it was demonstrated that PlGF improves the prediction of PE provided by maternal factors alone. In pregnancies which developed PE serum PlGF was decreased and the performance of the test is higher for early onset PE than late onset PE. It has also been shown that the performance of screening from PE delivering before 32 weeks is superior with screening at 22 than at 12 weeks, the performance of screening at 32 to 36+6 weeks is superior with screening at 32 than at 22 or 12 weeks and the performance of screening for PE delivering after 37 weeks is superior with screening at 36 weeks than at earlier gestations. This study has essentially documented the relationship between gestational age at screening and performance of the test using PLGF and maternal factors.

In the fourth publication it was demonstrated that incorporation of sFLT-1 in the screening model greatly improves its' performance in the 2^{nd} and 3^{rd} trimesters of pregnancy compared to the prediction of PE by maternal factors alone. Serum sFLT-1 increases in pregnancies that develop PE and the performance of the test is higher for early onset PE than late onset PE. In addition, the findings of this study suggest that the performance of screening for PE delivering at 32 to 36+6 weeks is superior with screening at 32 than at 22 weeks and the performance of screening for PE delivering after 37 weeks is superior with screening at 36 weeks than at earlier gestations. Case-control studies have shown that serum sFLT-1 is increased from

the 1st trimester of pregnancy and that the incorporation of this biomarker in the screening model increases the performance of the test (Crovetto *et al.*, 2014; Crovetto *et al.,* 2015). The relationship between gestational age at screening and the performance of the screening test using sFLT-1 and maternal factors has been documented in this study.

In the fifth publication, we have shown that in pregnancies that develop PE, the early 3rd trimester values of uterine artery pulsatility index (UTPI), mean arterial pressure (MAP) and sFLT-1 are increased and PlGF is decreased and the performance of screening is better for preterm than term PE. Screening for PE at 30-34 weeks' gestation with the combination of biophysical and biochemical factors used in this study could predict at 5% false positive rate (FPR), 98% of preterm PE and 49% of term PE. These results are superior to the ones achieved by screening at 11-13 or 19-24 weeks (O'Gorman *et al*., 2015; Gallo *et al.,* 2015). This study has shown also that a screening positive result at 5% FPR is associated 20 –fold and 11-fold increase in the odd ratios of preterm PE and term PE respectively. In the same time as screen negative result for the same FPR translates to a 42-fold and 2-fold decrease in the odds ratios of preterm and term PE respectively.

PE can have different levels of severity depending especially on the gestational age it develops. Mild forms of preeclampsia will manifest through hypertention, proteinuria, heartburn, nausea or vomiting, oedema of feet, hands, face and ankles, as well as severe headaches, and vision problems. These symptoms apart from their somatic effects, have also psychological effects since they cause anxiety and concern to the pregnant woman. In cases where PE develops in the early second trimester of pregnancy it might cause severe complications to both the mother and the fetus. Eclampsia is one of a series of complications of PE and it includes convulsions. Most women recover completely but a small percentage of these women might develop permanent disability, brain damage or even death. HELLP syndrome is another complication of the disease involving haemolysis, elevated liver enzymes and low platelets, leading to liver and kidney failure as well as pulmonary oedema. PE is also accompanied by an increased risk for stroke due to the increased blood pressure during the course of the disease. In severe PE babies might need to be delivered prematurely leading to the development of neonatal

respiratory distress syndrome due to inadequate development of the lung alveoli. In addition, the incidence of stillbirths is increased in pregnancies complicated with PE.

Effective screening for PE is especially important since it can greatly improve the outcome of pregnancies complicated with this condition. By identifying the high risk population from the beginning of the pregnancy we can possibly reduce the incidence of the PE by simply offering aspirin (Bujold et al., 2014). Furthermore, we can monitor the pregnancy closer by offering ultrasound scans more frequently, and by examining the Doppler studies, reassuring the mothers. The close monitoring of fetal growth and Doppler studies will enable the physician to decide when is the most appropriate time to deliver the baby, thus lowering the incidence of emergency caesarean sections and stillbirths, as well as other fetal complications. Physicians can consult women who screened negative and reassure them that the risk for developing PE is very low. Thus mothers won't need to attend the hospital for unnecessary scans.

Conclusively, this thesis has demonstrated that biophysical and biochemical markers increase significantly the performance of screening for PE and as a result the timing and content of clinical visits can be defined by the patient-specific risk of developing the disease. The vast majority of women would be screened low risk and these can follow the routine antenatal care, whereas those few who are high risk could be directed to a more specialized pathway. The patient-specific a posteriori risk for early, intermediate and late PE were calculated by multiplying the a priori patient characteristics-derived risk with the likelihood ratio of a series of biophysical and biochemical markers after appropriate adjustments for the inter-correlations between these markers. As in the cases of maternal factors the differences in biophysical and biochemical markers of impaired placentation between the PE and unaffected groups were in general more pronounced in those developing early disease compared to intermediate or late-PE. The major advantage of the new model, is that it offers the option to clinicians and researchers to select their own gestational age cut-off to define the high-risk group that could potentially benefit from therapeutic interventions starting from the first trimester of pregnancy.

5.2 Strengths and limitations of this thesis

Strengths of this thesis include:

- Prospective cohort studies
- Large populations included
- Routine care in well-defined gestational age ranges
- Measurement of biochemical markers by automated machines currently used in screening for aneuploidies which provide results in 40 minutes thus complete assessment and counselling can take place in the same appointment
- Measurement of biophysical markers such as MAP and UTPI by specific methodologies which ensure accurate data
- Accurate recording of maternal characteristics and medical history in the first appointment

Limitations of this thesis include:

- Not using cross-sectional studies
- Not including data of each gestational week from the beginning to the end of pregnancy
- Potential overestimation of the screening performance of the model which is derived and tested using the same dataset
- Some of the results rely on modelling which introduces optimistic bias
- Need of further prospective screening studies which can potentially support our results

5.3 Future research

Future studies should define contingent strategies for the selection of patients who will benefit from assessment at different points in pregnancy and develop protocols for the effective management of the high-risk cases which will be identified through the screening programme. Finally, the determination if such management protocols will improve the perinatal outcome is something which needs to be addressed.

Future research is necessary to validate these algorithms in clinical practice and the performance of screening to be tested in different populations. If such studies demonstrate similar results to those of my publications, the use of Bayes theorem and the incorporation of maternal serum PlGF and sFLT-1 in screening for PE could substantially improve antenatal care with timely diagnosis and treatment of PE, which will potentially decrease the adverse consequences of PE for both mother and baby.

Such a multicentre study involving the use of an algorithm based on Bayes' theorem will undertake screening at 11-13 weeks' gestation in 16,500 singleton pregnancies (SPREE- Screening programme for preeclampsia). Another multicentre study which is also based on the same philosophy is the ASPRE study involving several centres in Europe. It aims to screen 33,680 singleton pregnancies and to randomise high risk patients into either placebo or aspirin. This study is ongoing and results are expected by the end of 2016.

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APPENDIX

My contribution for each publication

Reference	Collection of data	Writing of manuscript	Statistical analysis	Study design
†Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol 2015;45:591-8	**80%**	**50%**	**5%**	**50%**
†Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol 2015;45:584-90	**80%**	**50%**	**5%**	**50%**
†Tsiakkas A, Cazacu R, Wright A, Wright D, Nicolaides KH. Serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015;doi: 10.1002/uog.15816	**80%**	**50%**	**10%**	**80%**
†Tsiakkas A, Mendez O, Wright A, Wright D, Nicolaides KH. Serum soluble fms-like tyrosine kinase-1 at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. Ultrasound Obstet Gynecol 2015;doi: 10.1002/uog.15817	**80%**	**50%**	**10%**	**80%**
*Tsiakkas A, Saiid Y, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 30-34 weeks' gestation. Am J Obstet Gynecol 2016;doi:10.1016/j.ajog.2016.02.016	**80%**	**50%**	**10%**	**80%**

Scientific Journal	Impact factor for 2015
†Ultrasound in Obstetrics and Gynaecology	3.853
*American Journal Of Obstetrics and Gynecology	4.704