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# EARLY PREDICTION OF PREECLAMPSIA

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Thesis submitted for the Degree of Doctor of Medicine

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## DECLARATION

This thesis entitled 'Early prediction of preeclampsia' has been composed by me, Ranjit Akolekar, and the work in this thesis is my own. This research project was composed by me with advice from my supervisors, Dr Sarah Stock, Professor Andrew Calder and Professor Kypros Nicolaides. I liaised with the National Ethics Committee to ensure the project was ethically approved and worked closely with the Research and Development Department to keep study documents and site-file up to date with relevant paperwork and substantial amendments whenever necessary. I liaised with the Human Tissue Authority to ensure that storage of serum and plasma samples was according to their recommendations and guidelines. I was responsible for ensuring that research samples were stored appropriately in -80°C freezers and for maintaining a database for stored research samples throughout the study period. I contributed to the process of obtaining pregnancy outcomes and ensured that the outcome measures were accurately coded in the research database by reviewing obstetric records of women reported as having hypertensive disorders of pregnancy. I carried out the statistical analysis for the studies in this thesis, conducted literature searches for reviews of published studies and wrote and composed this thesis. This work has not been previously submitted, in part or whole, for consideration for any other degree or professional qualification.

Ranjit Akolekar
December 2015

**ABSTRACT**

Preeclampsia (PE) is a major cause of perinatal and maternal morbidity and mortality. In the United Kingdom, the National Institute for Clinical Excellence (NICE) has issued guidelines on routine antenatal care recommending that at the booking visit a woman's level of risk for PE should be determined and the subsequent intensity of antenatal care should be based on this risk assessment. This method relies on a risk scoring system derived from maternal characteristics and medical history; the performance of screening by this method is poor with detection of less than 50% of cases of preterm-PE and term-PE.

The objective of this thesis is to develop a method for the estimation of the patient-specific risk for PE by combining the *a priori* risk based on maternal characteristics and medical history with the results of biophysical and biochemical markers obtained at 11-13 weeks' gestation. Such early identification of high-risk pregnancies could lead to the use of pharmacological interventions, such as low-dose aspirin, which could prevent the development of the disease.

The data for the thesis were derived from two types of studies: First, prospective screening in 65,771 singleton pregnancies, which provided data for maternal factors and serum pregnancy associated plasma protein-A (PAPP-A). In an unselected sequential process we also measured uterine artery pulsatility index (PI) in 45,885 of these pregnancies, mean arterial pressure (MAP) in 35,215 cases and placental growth factor (PLGF) in 14,252 cases. Second, cases-control studies for evaluating the ten most promising biochemical markers identified from search of the literature; for these studies we used stored serum or plasma samples obtained during screening and measured PLGF, Activin-A, Inhibin-A, placental protein-13 (PP13), P-selectin, Pentraxin-3 (PTX-3), soluble Endoglin (sEng), Plasminogen activator inhibitor-2 (PAI-2), Angiopoietin-2 (Ang-2) and soluble fms-like tyrosine kinase-1 (s-Flt-1).

A competing risk model was developed which is based on Bayes theorem and combines the *prior* risk from maternal factors with the distribution of biomarkers to derive patient-specific risk for PE at different stages in pregnancy. The *prior* risk was derived by multiple regression analysis of maternal factors in the screening study. The distribution of biophysical and biochemical markers was derived from both the screening study and the case-control studies.

The *prior* risk increased with advancing maternal age, increasing weight, was higher in women of Afro-Caribbean and South-Asian racial origin, those with a previous pregnancy with PE, conception by *in vitro* fertilization and medical history of chronic hypertension, type 1 diabetes mellitus and systemic lupus erythematosus (SLE) or anti-phospholipid syndrome (APS). The estimated detection rate (DR) of PE requiring delivery at <34, <37 weeks' gestation and all PE, at false positive rate (FPR) of 10%, in screening by maternal factors were 51, 43 and 40%, respectively. The addition of biochemical markers to maternal factors, including maternal serum PLGF and PAPP-A, improved the performance of screening with respective DRs of 74, 56 and 41%. Similarly, addition of biophysical markers to maternal factors, including uterine artery PI and MAP, improved the performance of screening with respective DRs of 90, 72 and 57%. The combination of maternal factors with all the above biophysical and biochemical markers improved the respective DRs to 96, 77 and 54%.

The findings of these studies demonstrate that a combination of maternal factors, biophysical and biochemical markers can effectively identify women at high-risk of developing PE.

## LAY SUMMARY

Preeclampsia (PE), defined as high blood pressure and proteinuria developing during the second half of pregnancy, is a major cause of serious risk to the life and health of mothers and babies. In the United Kingdom, there are national recommendations to suggest that a woman's risk of developing PE should be determined by recording maternal characteristics and medical history to enable planning of her antenatal care. However, this method identifies only a minority of pregnancies that develop PE.

This thesis aims to develop a new method of screening for PE during the third month of pregnancy that will identify majority of women that will develop PE. The rationale for such early identification of high-risk pregnancies is that treatment at this stage, with drugs such as low-dose aspirin, can potentially prevent the development of the disease.

We searched the literature to identify various metabolites that can be measured in maternal blood and have been reported to have altered levels in pregnancies that develop PE (biochemical markers). We also found from previous studies that the resistance to blood flow from the mother to the uterus and the blood pressure of women that develop PE are higher than in normal pregnancies.

We measured the level of the ten most promising biochemical markers in stored blood obtained from women in the third month of pregnancy and compared the values between those that subsequently developed PE and those with normal pregnancies. This series of studies led to the conclusion that six of the ten biochemical markers would be potentially useful in identifying high-risk pregnancies.

We also conducted a large prospective study in 65,771 pregnancies which included 1,426 (2.4%) that developed PE. In this study, we measured the blood flow to the uterus in 45,885 cases and the blood pressure in 35,215 cases. The study found that

the maternal factors which place a woman at an increased risk for developing PE are advancing maternal age, increasing weight, Afro-Caribbean and Asian racial origin, previous pregnancy with PE, conception by *in vitro* fertilization and medical history of chronic hypertension, type 1 diabetes mellitus and systemic lupus erythematosus or anti-phospholipid syndrome. We also found that the resistance to blood flow from the mother to the uterus and the blood pressure of women that develop PE are higher than in normal pregnancies.

We developed a mathematical method of combining the information from maternal factors, resistance to blood flow to the uterus, blood pressure and biochemical markers measured during the third month of pregnancy. This new method could predict 96% of pregnancies that develop severe PE requiring delivery before the $34^{th}$ week of pregnancy, 77% of those with PE requiring delivery before 37 weeks and 54% of all cases that develop PE at any stage in pregnancy. This method is by far superior to previous methods that relied only on maternal factors.

## ACKNOWLEDGEMENTS

This dissertation is based on studies carried out whilst I was working at Harris Birthright Research Centre for Fetal Medicine at the King's College Hospital, London as a research fellow and subsequently as a sub-speciality trainee in Fetal Medicine. The studies presented here were carried out between March 2006 and September 2010. It would not have been possible to complete these studies and this dissertation without the support and advice of the following people.

First and foremost, I would like to extend my sincere gratitude to Professor Kypros Nicolaides, without whose vision, guidance and support, these studies would not have been possible. I feel extremely fortunate not only to have been trained in research, academic and clinical skills at one of the world's best centres for Fetal Medicine but also to have been able to work closely with Professor Nicolaides on various research projects. His passion, dedication and above all commitment have been a constant source of inspiration and motivation for me. I hope that I am able to imbibe in good measure not only academic and clinical skills but more importantly, the extraordinary work ethic that I have witnessed over the years. This study would also not have been possible without the funding support from the Fetal Medicine Foundation (Registered Charity: 1037116). The charity has a long-standing tradition of supporting high-quality research and training in Fetal Medicine and it is because of this ethos and infrastructure that it was possible to accomplish these studies.

I am also very grateful to all my research fellow colleagues who believed in the same principles of academic research and helped support these ongoing studies by not only recruiting patients for the prospective studies but by contributing to doing ultrasound scans, taking detailed medical histories and where necessary samples for research. I am particularly grateful to my friend and colleague Miss Argyro Syngelaki, who in addition to overseeing and maintaining the research database, was always there for help, support and encouragement.

Whilst these studies were possible due to the many reasons I mentioned but the manuscript of this thesis would not have come to fruition had it not been for support and timely advice from my supervisor, Dr Sarah Stock. I am indeed grateful that she helped me set pragmatic deadlines and gave prompt advice, criticism and appropriate supervision whenever needed to enable me to submit this dissertation in a timely fashion.

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## ABBREVIATIONS

| | |
|---|---|
| **AUROC:** | Area Under Receiver Operating Characteristic Curves |
| **ACOG:** | American College of Obstetricians and Gynaecologists |
| **Ang-2:** | Angiopoeitin-2 |
| **BMI:** | Body Mass Index |
| **BP:** | Blood Pressure |
| **CI:** | Confidence Interval |
| **CRL:** | Crown-Rump Length |
| **CV:** | Coefficient of Variation |
| **DELFIA:** | Dissociation-Enhanced Lanthanide Fluorescent Immunoassay |
| **DR:** | Detection Rate |
| **ELISA:** | Enzyme-Linked Immunosorbent Assay |
| **FPR:** | False Positive Rate |
| **GH:** | Gestational Hypertension |
| **hCG:** | Human Chorionic Gonadotropin |
| **IQR:** | Interquartile Range |
| **ISSHP:** | International Society for Study of Hypertension in Pregnancy |
| **IVF:** | *In-vitro* Fertilisation |
| **LMWH:** | Low Molecular Weight Heparin |
| **MAP:** | Mean Arterial Pressure |
| **MoM:** | Multiple of Median |
| **NT:** | Nuchal Translucency |
| **NICE:** | National Institute of Clinical Excellence |
| **OR:** | Odds Ratio |
| **PAPP-A:** | Pregnancy Associated Plasma Protein-A |
| **PAI-2:** | Plasminogen Activator Inhibitor-2 |
| **PE:** | Preeclampsia |
| **PI:** | Pulsatility Index |
| **PlGF:** | Placental Growth Factor |
| **PP13:** | Placental Protein 13 |

| | |
|---|---|
| **PTX-3:** | Pentraxin-3 |
| **RCT:** | Randomized Controlled Trial |
| **RR:** | Relative Risk |
| **SD:** | Standard Deviation |
| **sEng:** | Soluble Endoglin |
| **sFlt-1:** | Soluble fms-like Tyrosine Kinase-1 |
| **TGF-β1:** | Transforming Growth Factor-β1 |
| **UK:** | United Kingdom |
| **VEGF:** | Vascular Endothelial Growth Factor |
| **WHO:** | World Health Organisation |

# CHAPTER 1

## INTRODUCTION

## 1.1 OVERVIEW

Preeclampsia (PE) complicates around 2-5% of pregnancies and is an important cause of maternal and perinatal morbidity and mortality (World Health Organization, 2005; Confidential Enquiry into Maternal and Child Health Perinatal Mortality, 2009).

The aetiology of PE is quite heterogeneous but it is mainly characterised by impaired trophoblastic invasion, as documented by the findings of histological studies of the uterine arteries (Khong *et al.,* 1986; Pijnenborg *et al.,* 1991; Lyall, 2002). The diameter of spiral arteries in a normal pregnancy is greatly increased and the vascular smooth muscle is replaced by trophoblast cells. This process, which is controlled by genetic and immunological factors, is deficient in PE (Wilson *et al.,* 2003), which results in increased impedance and decreased vascular capacitance in the uteroplacental circulation (Sagol *et al.,* 1999).

The hallmark of PE is demonstration of high blood pressure (BP) and significant proteinuria during the second trimester of pregnancy. At the booking visit early in pregnancy, a detailed maternal history is obtained to identify risk factors for PE along with measurement of BP at the initial visit and later during subsequent antenatal visits, which constitutes the basis of screening for PE throughout pregnancy. There is evidence from studies that the main diagnostic feature of PE, which is raised BP, can be observed as early as in the first-trimester of pregnancy, well before the clinical onset of the disease (Moutquin *et al.,* 1985; Higgins *et al.,* 1997, Poon *et al.,* 2008a).

The use of Doppler ultrasound allows non-invasive assessment of utero-placental circulation. In normal pregnancies, impedance to flow in the uterine arteries decreases with gestation, but in pregnancies destined to develop PE this impedance is increased (Martin *et al.,* 2001; Papageorghiou *et al.,* 2002; Placensia *et al.,* 2007). The finding that impaired placental perfusion, reflected in increased uterine artery pulsatility index (PI), is associated with the development of PE is compatible with the hypothesis that PE is the consequence of impaired placentation and the results of previous first and

second-trimester Doppler studies as well as histological studies of the maternal spiral arteries (Olofsson *et al.,* 1993; Martin *et al.,* 2001; Papageorghiou *et al.,* 2002; Plasencia *et al.,* 2007).

A large number of biochemical markers have been investigated for the prediction of PE. Many such markers represent measurable manifestations of impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries and reduced placental perfusion leading to placental ischemia related damage with the release of inflammatory factors, platelet activation, endothelial dysfunction, maternal renal dysfunction, or abnormal oxidative stress (De Wolf *et al.,* 1975; Khong *et al.,* 1986; Redman 1991; Meekins *et al.,* 1994a; Pijnenborg 1996).

## 1.2 DEFINITION OF PREECLAMPSIA

PE is defined as development of hypertension and proteinuria in a previously normotensive woman. However, there is lack of consensus regarding the precise definition of PE as although hypertension and proteinuria are considered easy to measure, both are fraught with methodological problems. The traditional method of non-invasive BP monitoring is the use of mercury sphygmomanometers, but there are concerns regarding the clinical performance and safety of these instruments (Mion and Pierin, 1998; Markandu *et al.,* 2000). In addition, other sources of error which undermine clinical performance of this method are poor calibration, inappropriate cuff size, threshold avoidance and rounding up of values (Perry *et al.,* 1991). In the last few years, there is a move away from use of mercury sphygmomanometers, largely for occupational health and safety reasons, which has led to the automated devices being used more commonly.

There is also a growing recognition of potential inaccuracies in measurement of proteinuria. The dipstick analysis of a random urine sample has a poor correlation with 24-hour urine sample in the diagnosis of significant proteinuria and this leads to

inaccuracies not just in the measurement of proteinuria but also in the diagnosis of PE (Meyer *et al.,* 1994).

There are several definitions for diagnosis of PE which have been reported in published literature and proposed by various professional bodies but the accepted definition of PE 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). A summary of various definitions proposed is mentioned in Table 1.1.

**Table 1.1.** The four most commonly cited definitions of preeclampsia.

| | **Davey & MacGillivray 1988** | **WHO 1987** | **Redman & Jefferies 1988** | **NHBPEPWG 2000** |
|---|---|---|---|---|
| **Systolic/Diastolic** | Diastolic | Diastolic | Diastolic | Either |
| **Threshold** | 90 mmHg x 2 or 110 mmHg x 1 | 90 mmHg x 2 | 90 mmHg & incremental rise | 140 mmHg systolic or 90 mmHg diastolic |
| **Increment** | No | No | 25 mmHg | No |
| **Proteinuria** | Yes | Yes | No | Yes |
| **Baseline** | Normotensive < 20 weeks | Normotensive < 20 weeks | <90mmHg < 20 weeks | Normotensive < 20 weeks |
| **Endorsed by** | ISSHP | | | ACOG |

ISSHP - International Society for the Study of Hypertension in Pregnancy

WHO - World Health Organization

NHBPEPWG - National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy

ACOG - American College of Obstetrics and Gynecology

### 1.2.1 ISSHP Diagnostic criteria

The ISSHP recognised that one of the major reasons for lack of consensus and controversies was whether or not proteinuria should be an essential part of diagnosis of PE. They recommended that a broad definition, at times not including proteinuria, could be applied for the clinical definition of PE whilst the inclusion of proteinuria would ensure more specificity around the diagnosis when reporting clinical criteria for patients enrolled in scientific research (Tranquilli *et al.*, 2014).

For the purposes of this research, the ISSHP classification of hypertensive disorders of pregnancy was chosen as it represents a consensus view of an appropriate definition for research use. The ISSHP revised its classification of hypertensive disorders in pregnancy as follows:

**Table 1.2** The revised ISSHP classification of hypertensive disorders in pregnancy.

| **Revised ISSHP classification (2013) for hypertensive disorders in pregnancy** | |
|---|---|
| **1** | Chronic hypertension |
| **2** | Gestational hypertension |
| **3** | Preeclampsia – de novo or superimposed on chronic hypertension |
| **4** | White coat hypertension |

Hypertension

Hypertension is diagnosed as systolic BP of $\geq$ 140 mm Hg or diastolic BP $\geq$ 90 mm Hg. BP should be ideally measured by instruments validated for use in pregnancy either automated, liquid-crystal or mercury sphygmomanometer. Regardless of the method used, the BP should be measured at least twice.

Chronic hypertension

Chronic hypertension is hypertension ideally predating the pregnancy. As many women will not have their BP measured close to pregnancy, in practice the diagnosis of hypertension in the first trimester is considered diagnostic of chronic hypertension.

Gestational hypertension and preeclampsia

New onset of hypertension after 20 weeks' gestation is characteristic of gestational hypertension and PE. The diagnosis of PE is made by hypertension and the co-existence of one or more of the following conditions:

*Proteinuria*

The presence of significant proteinuria defined as presence of > 300 mg/day protein in a 24-hour urine sample or urine protein/creatinine ratio of > 30 mg/mmol.

*Maternal organ dysfunction*

This is characterised by:

- Renal insufficiency: characterised by serum creatinine levels of $\geq$ 90 μmol/L or 1.02 mg/dL
- Liver involvement: characterised by elevated transaminases with levels at least twice the upper limit of normal range with or without right upper quadrant or epigastric abdominal pain
- Neurological complications: characterised by hyperreflexia accompanied by clonus; severe headache accompanied by persistent visual scotoma; blindness, stroke or eclampsia
- Haematological complications: characterised by platelet count below 150,000/dL

*Utero-placental dysfunction*

This is characterised by presence of fetal growth restriction.

Gestational hypertension is diagnosed when hypertension develops *de novo* after 20 weeks' gestation and is not accompanied by any of the above features. PE superimposed on chronic hypertension is diagnosed when chronic hypertension is accompanied by one or more of the above features.

**Table 1.3** The revised ISSHP definition of preeclampsia (2014).

| **Hypertension developing after 20 weeks co-existing with new onset of one or more of the following features** | |
|---|---|
| **1** | Proteinuria |
| **2** | Maternal organ dysfunction<br>- Renal insufficiency<br>- Liver involvement<br>- Neurological complications<br>- Haematological complications |
| **3** | Utero-placental insufficiency<br>- Fetal growth restriction |

White coat hypertension

This is characterised by hypertension diagnosed in clinical setting but with normal BP measurements in home setting. This diagnosis is confirmed by taking repeated BP readings and by carrying out 24-hour ambulatory BP monitoring.

Gestational proteinuria

This is defined as finding of a significant proteinuria as defined above without accompanying hypertension or primary renal disease.

## 1.3 PATHOGENESIS OF PREECLAMPSIA

The exact pathogenesis of PE is not clear but it is thought to be the consequence of impaired trophoblastic invasion of the placenta eventually leading to endothelial dysfunction which accounts for its clinical signs and symptoms.

The placenta develops primarily from the trophoblasts, which then differentiates into two cell types, the cytotrophoblasts and syncytiotrophoblasts. The cytotrophoblasts are precursors of all trophoblast cells whereas the syncytiotrophoblasts are responsible for the invasion into the decidua, and in particular, into the maternal spiral arteries. There are two waves of trophoblastic invasion, one in early pregnancy and the second later in pregnancy, around 14 to 16 weeks' gestation (Robillard 2002). The invasion of the syncytiotrophoblasts into the spiral arteries results in converting these muscular high-resistance arteries into wide low-resistance vessels, thereby increasing the blood flow available to the developing fetus and placenta (Brosens *et al.,* 1972).

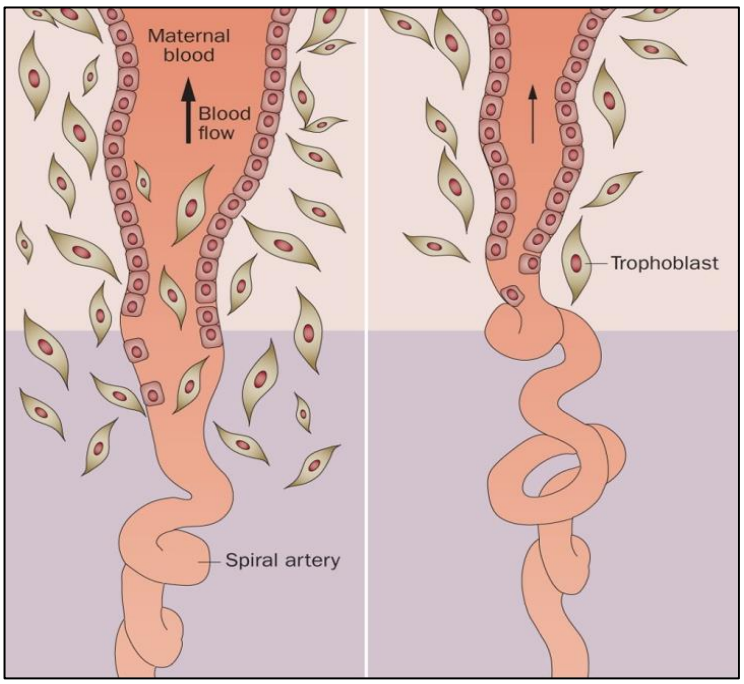



**Figure 1.1** Impaired trophoblastic proliferation of spiral arteries in preeclampsia. (Reprinted by permission from Nature Publishing group; Macmillan Publishers Ltd: [Nat Rev Immunol] (Moffett-King, A *et al.,* 2002).

In PE, trophoblastic invasion and subsequent remodeling of the spiral arteries, especially during the second wave of invasion, is deficient, resulting in spiral artery diameters that are only about 40% as wide as those in a normal pregnancy. The result is placental ischemia and poor placental perfusion in women who will eventually develop the clinical signs of PE (Wilson *et al.*, 2003).

The main factors which are thought to play an important role in aetiology and pathogenesis of PE are genetic factors, immunological maladaptation, placental factors, endothelial dysfunction and oxidative stress.

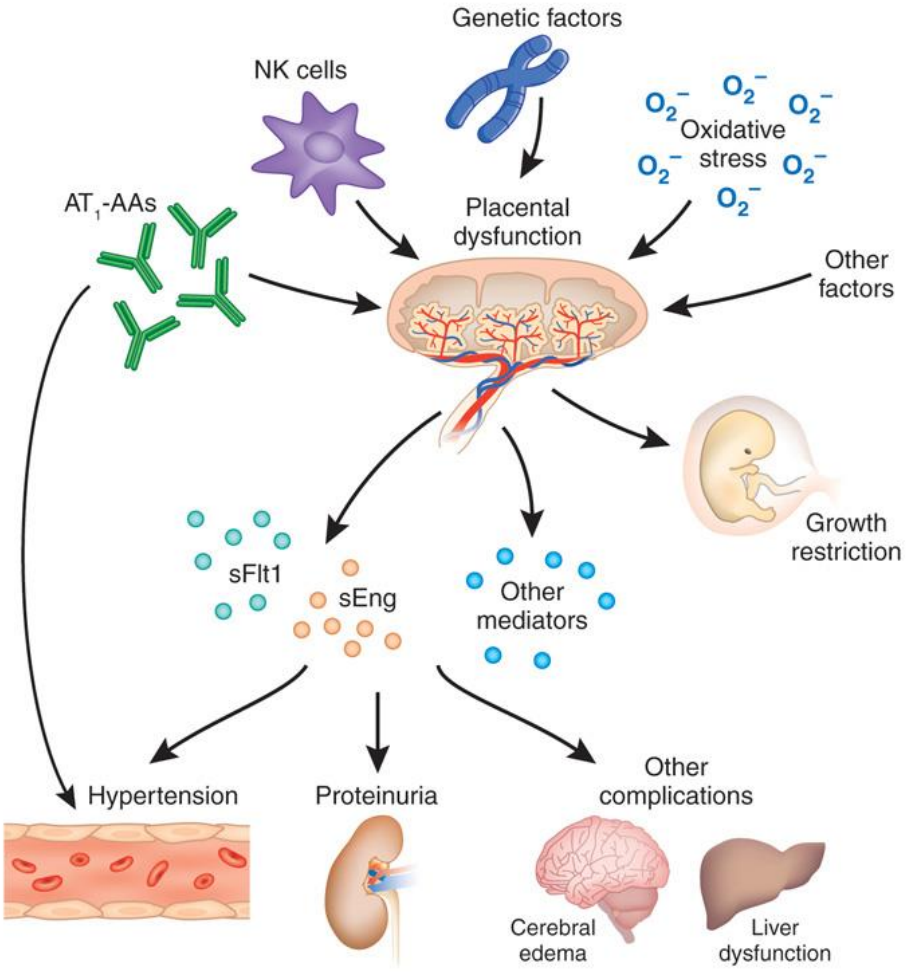



**Figure 1.2** Pathogenesis of preeclampsia (Reprinted by permission from Nature Publishing group; Macmillan Publishers Ltd: [Nat Med] (Parikh and Karumanchi, 2008).

### 1.3.1 Genetic factors

PE is thought to have a genetic bias as there is evidence that there is familial clustering for PE and in women with a positive family history, the risk of subsequent PE is increased about three times compared to those without such a family history (Arngrimsson *et al.,* 1990; Cincotta and Brennecke, 1998; Duckitt and Harrington 2005). The analysis of pedigree charts suggests that one or more genes or their alleles may act as "preeclampsia susceptibility gene" and such candidate genes may include those that are involved in regulation of BP and those that play an important role in modifying placental function. The proposed genetic models mainly include either a single recessive gene or a single dominant gene (Cooper and Liston, 1979; Chesley and Cooper, 1986). There are many reported associations between PE and polymorphisms of genes such as angiotensinogen, tumour necrosis factor-α (TNF-α), factor V Leiden and the 5,10-methylenetetrahydrofolate reductase genes (Morgan *et al.,* 1999; Lachmeijer *et al.,* 2001; Bendetto *et al.,* 2002; Heiskanen *et al.,* 2002).

In a recent systematic review of literature and meta-analysis (Human Genome Epidemiology Review [HuGE review]), the authors examined the association between maternal genotype and severe PE according to functional gene groups (immune, cell-signalling, metabolic, thrombophilic and vasoactive). They examined 57 studies which evaluated 50 genotypes in 5,049 cases and 16,989 controls and reported that a high-risk for severe PE was noted with coagulation factor V gene (proaccelerin, labile factor) polymorphism rs6025, coagulation factor II (thrombin) gene mutation G20210A, leptin receptor gene (LEPR) polymorphism rs1137100 and the thrombophilic gene group. The authors reported that although there appears to be a role for thrombophilia genes in severe PE, there is insufficient evidence to establish a causal relationship and further studies will need to be carried out to examine the thrombophilia genes and their polymorphisms (Fong *et al.,* 2014). In another meta-analysis, the authors specifically examined the relationship between the risk of PE and the two thrombophilia single-neucleotide polymorphisms (SNPs), the factor V G1691A SNP and prothrombin G20210A SNP. They examined 37 studies with 5,048

patients with PE and 6,796 controls and reported that both these polymorphisms are associated with increased risk of all types of PE, including severe PE. In this meta-analysis, the presence of a prothrombin 20210A polymorphism was associated with a 2-fold increased risk for PE and an approximately 3-fold increase for severe PE (Wang *et al.,* 2014).

Although, there are associations between these gene polymorphisms and the risk for PE, there is a need for further larger studies as the results from these meta-analyses may be affected by heterogeneity between studies and a potential for bias from poor-quality genotyping, inconsistent definition of phenotype and publication bias (Fong *et al.,* 2014; Wang *et al.,* 2014).

### 1.3.2 Immunological factors

The placental structure takes its origin from fetal tissues and it has both maternal and paternal derived antigens. During a normal pregnancy, the mother is tolerant of foreign fetal antigens and mounts a modified immune response to allow acceptance of the semi-allogenic fetus. There is evidence from studies that in PE, there is activation of a broad continuum of immune cells to induce immunity towards the fetus and placenta, rather than immune tolerance (Dekker and Robillard 2005; Robillard *et al.,* 2007; Laresgoiti-Servitje 2013). What is uncertain is whether this immune maladaptation precedes the abnormal implantation of placenta and endothelial dysfunction or follows it (Djurisic and Hviid 2014).

There is evidence from epidemiological observations that support an immunological cause for PE, which may be due to an abnormal and heightened maternal immune response to paternally derived antigens on the trophoblast. This hypothesis is supported by the epidemiological observations that, firstly, PE is more common in a first pregnancy, secondly, the risk of PE decreases after the first pregnancy due to the protection from repeated exposure to specific antigens from the same partner, thirdly,

changing partners for a subsequent pregnancy increases the risk of PE which suggests that primipaternity is important (Robillard *et al.*,1999; Li and Wi, 2000). Additional evidence to support the hypothesis is that both artificial donor insemination and oocyte donation lead to an increased risk in PE (Smith *et al.*, 1997a; Wilson *et al.*, 2003).

Human Leukocyte Antigens (HLA)

There are two classes of human leukocyte antigens (HLA): class I and II. The class HLA Ia antigens (A, B and C) and HLA II (DR, DQ and DP) are important for antigen presentation and therefore in organ transplantation and autoimmunity. The HLA Ib (E, F and G) genes on the other hand are primarily expressed in the extravillous trophoblast cells lining the placenta and by syncytiotrophoblast cells (Ishitani *et al.*, 2003; Bhalla *et al.*, 2006). HLA-G is believed to be involved in modulating immune responses in the context of vascular remodeling during pregnancy as well as in dampening potentially harmful immune attacks raised against the semi-allogeneic fetus (Djurisic and Hviid 2014). Soluble HLA-G (sHLA-G) in the maternal circulation is predominantly produced and shed from trophoblast cells during pregnancy (Kovats *et al.*, 1990).

The association between HLA-G and PE is supported by findings from immunohistochemistry and *in situ* hybridisation studies on placental samples from pregnancies with PE which demonstrate a reduced expression of HLA-G compared to normotensive pregnancies (Hara *et al.*, 1996; Goldman-Wohl *et al.*, 2000). There is evidence that circulating levels of sHLA-G are reduced in women with PE compared to normotensive women (Hackmon *et al.*, 2007; Darmochwal-Kolarz *et al.*, 2012) and that these altered levels are also noted prior to clinical onset of PE (Yie *et al.*, 2005). Although, these studies suggest a possible role for HLA-G in PE but these data are based on small case-control studies and further studies are needed to establish a precise role in the pathogenesis of PE (Djurisic and Hviid 2014).

NK cells, T-cells and B-cells

Natural killer (NK) cells are a part of the innate immune response to foreign antigens. They are present in the peripheral blood and form 5% of leukocyte population (pNK) cells whereas a unique subset of cytokine producing decidual NK cells (dNK) are identified in the placenta during the pregnancy. These dNK cells secrete vascular endothelial growth factor (VGEF), placental growth factor (PlGF), interleukin-8 (IL-8) and IFN-inducible protein-10 (IP-10) (Hanna *et al.,* 2006). These findings raise the possibility for a role for NK cells in PE but the evidence from studies is controversial with some studies showing a difference in PE and controls (Molvarec *et al.,* 2010) but others not demonstrating any significant difference (Toldi *et al.,* 2008).

There is some evidence that the release of cytokines from the Th1 subset of T-lymphocytes such as interleukin-1 (IL-1), IL-2 and interferon-γ (IFN-γ) is increased in PE whereas the production of Th2 cytokines such as IL-5 and IL-10 is decreased (Verlohren *et al.,* 2009). The presence of low IL-10 levels can be associated with development of PE as IL-10 protects the fetus from rejection during a normal pregnancy via activation of HLA-G expression at the fetal-maternal interface and lower levels may lead to immune maladaptation and eventually to PE (Hennessy *et al.,* 1999; Moreau *et al.,* 1999).

The B-cells, which produce immunoglobulins, have been implicated in the pathogenesis of PE through the identification a population of B-cells which produce autoantibodies against type I angiotensin II receptor ($AT_1$) (Wallukat *et al.,* 1999; Jensen *et al.,* 2012). These autoantibodies can activate $AT_1$ in endothelial cells, vascular smooth muscle and mesangial cells eventually leading to renal disease characterised by hypertension and proteinuria, prothrombotic and anti-angiogenic state (Siddiqui *et al.,* 2010; Parrish *et al.,* 2010).

### 1.3.3 Placental factors

The placenta is the main factor responsible for development of PE. The support for this hypothesis is from observations that the risk of PE is increased with placental mass being higher in multiple pregnancies, molar pregnancies and hydrops fetalis. In normal pregnancy, the trophoblastic cells – mainly the syncytiotrophoblast – invade the spiral arteries which become high-capacity low resistance uteroplacental vessels with a dilated tortuous lumen, complete absence of muscular and elastic tissue and no continuous endothelial lining. The development of PE is thought to be result of placental disease at two different stages: the first stage is the process that results from impaired trophoblastic proliferation which affects the spiral arteries resulting in deficient placental perfusion and the second stage involves the effects of placental ischaemia on both fetus and mother.

Placentation in normal pregnancy

The placenta is formed from cells derived from the fetus – the trophoblasts. There are two types of trophoblastic cells: the cytotrophoblasts, which are the precursors to all subsequent trophoblast cells and the syncytiotrophoblasts, which are responsible for the invasion into the decidua, and particularly, the maternal spiral arteries. At an early stage of pregnancy, approximately 5 weeks of gestation, the cytotrophoblasts differentiate from the villous Langerhans type to the invasive extravillous type and break through the syncytiotrophoblasts to invade the maternal spiral arteries resulting in what is called as the endovascular invasion.

These invasive trophoblastic cells then migrate into the decidua and through into the myometrium underlying the placental bed, a process which is termed as the interstitial invasion. The interstitial cytotrophoblasts are initially focused near the spiral arteries, but after about 8 weeks of gestation, the cytotophoblasts are more broadly distributed and invade the inner third of the myometrium (Pijnenborg *et al.*, 1991).

There is evidence suggesting that priming of the spiral artery for subsequent invasion by endovascular trophoblast may be initiated by perivascular interstitial trophoblasts which cause degenerative changes in the vessel wall structure (Kam *et al.,* 1999). The endovascular trophoblastic cells appear inside the lumen of the decidual spiral arteries from around 4 weeks of gestation, migrating towards the proximal part of the vessel (Pijnenborg *et al.,* 1991). These trophoblastic cells then replace the endothelium and invade the walls of the intradecidual portion of the spiral vessels, destroying the elastic and muscular layers of the vessel wall. As a result of this trophoblastic invasion, the narrow-diameter high resistance spiral arteries become wide-diameter, high-flow, low resistance, funnel-shaped, uteroplacental arteries. The diameter of the spiral arteries increases considerably thus allowing a ten-fold increase in uterine blood flow to meet the requirements of the fetus. This process, as alluded to before, is thought to occur in two different stages: the initial stage is the first wave of trophoblastic invasion which involves the decidual portion of the spiral arteries and starts at 8 weeks, whereas the next stage is the second wave of trophoblastic invasion which involves the myometrial segments of the spiral arteries and occurs at 14-24 weeks of gestation (Lyall, 2002).

Placentation in preeclampsia

The dominant feature in PE is failure of endovascular and interstitial invasion of the placenta that is a prerequisite for normal placentation (Khong *et al.,* 1986). The sequence of vascular modifications of the spiral arteries that are a hallmark of development of an effective uteroplacental circulation, do not occur as in the normal pregnancies. Although there is trophoblastic invasion of spiral arteries in PE, but this process is impaired and largely deficient being limited to the decidual part of the vessels without extending into the myometrial segments of the spiral arteries (Brosens *et al.,* 1972; Gerretsen *et al.,* 1981; Meekins *et al.,* 1994a).

The other characteristic histological feature of PE is endothelial dysfunction which is evidenced by finding of acute atherosis (Sheppard and Bonnar, 1981). This histological change is principally characterised by fibrinoid necrosis, lipid-laden

macrophage infiltration of the damaged vessel wall and a perivascular mononuclear cellular infiltrate (Pijnenborg *et al.,* 1991). There is increased deposition of lipoprotein within the walls of these arteries (Meekins *et al.,* 1994b) and there is evidence of both microthrombi within the vessels and macroscopic placental infarction (Salafia *et al.,* 1995). This finding of acute atherosis is not unique to PE but is also noted in other pregnancy complications such as pregnancies with recurrent miscarriages, fetal growth restriction and antiphospholipid syndrome. There is a degree of overlap and impaired trophoblastic proliferation as well as acute atherosis is noted in pregnancies with fetal growth restriction with or without PE (Frusca *et al.,* 1989).

There is an association between the severity of histological changes in spiral arteries, the impedance to blood flow in uterine arteries and the severity of PE. There is evidence from histological studies of the placenta that there is a good relationship between uterine artery Doppler and placental bed biopsies with higher resistance values of PI more related to advanced histological changes (Olofsson *et al.,* 1993; Meekins *et al.,* 1994a; Sagol *et al.,* 1999). The severity of histological changes and abnormal uterine artery values are also reflected in clinical severity of disease by an earlier clinical onset of the disease. There appears to be an inverse relationship between the severity of histological and Doppler changes and the clinical onset of PE with studies demonstrating that the likelihood of clinical diagnosis of PE before 32 weeks' gestation increases with progressive impairment of the uteroplacental circulation (Ghidini *et al.,* 1997). There is further evidence from clinical and histological studies which demonstrate that early onset disease is more likely to be associated with abnormal villous and vascular morphology while in late onset disease the placental morphology and histology is not dissimilar to those of normotensive pregnancies (Moldenhauer *et al.,* 2003; Egbor *et al.,* 2006).

There appears to be considerable overlap in the degree of placental vascular lesions between normal and PE pregnancies (Ghidini *et al.,* 1997). On the other hand, there is evidence from some studies that there can be a significant degree of uteroplacental vascular pathology with normal uterine artery Doppler flow even in normal

pregnancies (Aardema *et al.,* 2001). These studies imply that defective placentation is not universally present in all cases of PE and equally well, it is not unique to PE (Pijnenborg *et al.,* 1991; Meekins *et al.,* 1994a). There is considerable variation in the spectrum of histological changes in pregnancies with or without PE. Some investigators have demonstrated through studies that trophoblastic invasion within a vessel wall can be variable and that physiological change can be present in the whole circumference or restricted to one segment only in the same patient. Hence, the spectrum of changes in the spiral arteries may not necessarily correlate to the degree of severity of the clinical disease (Pijnenborg *et al.,* 1991).

In summary, there is evidence that impaired trophoblastic proliferation is present in pregnancies with PE. There is a significant correlation between histological and Doppler findings in pregnancies with PE with more severe changes associated with high uterine artery PI, significant histological findings and an earlier onset of the clinical disease. However, there is also evidence that in some pregnancies with PE, there is normal placentation whilst on the other hand, there are some normal pregnancies which show evidence of impaired trophoblastic proliferation. Therefore, it may be that inadequate trophoblast invasion is a predisposing factor, with the maternal response to these changes modifying the clinical symptoms of health or disease.

### 1.3.4 Endothelial dysfunction

Endothelial cell activation has been suggested as a dominant feature of PE. This is underlined by the increased vascular resistance which is a key feature of the disease. It is suggested that impaired trophoblastic proliferation leads to placental hypoxia/ischemia which in turn results in oxidative stress, intravascular inflammation and endothelial cell activation (Redman and Sargent 2003; Myatt and Webster 2009). There is evidence that impaired placentation and the resulting placental hypoxia can lead to intravascular inflammation and consequent activation or repression of

endothelial cell function (Redman, 1991; Gervasi *et al.*, 2001; Redman and Sargent, 2003; Myatt and Webster, 2009; Saito and Nakashima, 2014).

The resulting endothelial dysfunction leads to a series of changes which include alterations in level of angiogenic and anti-angiogenic proteins such as vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1 also called as soluble fms-like tyrosine kinase-1 (sFlt-1). The altered concentration of these proteins further enhances the endothelial dysfunction established by intravascular inflammation (Luttun *et al.*, 2002; Maynard *et al.*, 2003; López-Novoa, 2007; Widmer *et al.*, 2007; Zhou *et al.*, 2011; Murphy *et al.*, 2013).

VEGF is a pro-angiogenic protein released by many cell types including the cytotrophoblast and it is involved in promoting angiogenesis and vasculogenesis. The VEGF protein is transcribed from the VEGF gene which is located on chromosome 6, which also encodes for various isoforms of VEGF including placental growth factor (PlGF) (Romero *et al.*, 2008b; Cheng *et al.*, 2013). These angiogenic factors exert their role in endothelial cells by mediating increased vascular permeability, which includes angiogenesis, vasculogenesis and growth of endothelial cells (Yamazaki *et al.*, 2006; Maharaj *et al.*, 2008).

The sFlt-1 protein is primarily produced by the syncytiotrophoblast and is produced by alternative splicing of the Flt-1 gene which results in a truncated protein which cannot bind to PlGF or VEGF inside the cells but attaches to the transmembrane receptors thus acting as an antagonist to of PlGF and VEGF and preventing these angiogenic factors from interacting with their receptors (Maynard *et al.*, 2003; Levine *et al.*, 2004; Romero *et al.*, 2008b; Tache *et al.*, 2011). The sFlt-1 exerts anti-angiogenic effects by inhibiting biological activity of VEGF and PlGF (Kendall and Thomas, 1993). VEGF is important for maintaining endothelial function in fenestrated endothelium especially and in brain, liver and renal glomeruli (Esser *et al.*, 1998). The higher levels of sFlt-1 also counteract vasodilatory effects of nitric oxide induced by

VEGF thereby leading to hypertension (Maynard *et al.,* 2003). In addition, sFlt-1 can induce proteinuria by blocking effects of VEGF (Eremina *et al.,* 2003).

A second anti-angiogenic factor implicated in the pathogenesis of PE is soluble endoglin (sEng), a surface receptor of transforming growth factor-β1 (TGF- β1), which induces growth and proliferation of endothelial cells (Venkatesha *et al.,* 2006). The sEng protein has anti-angiogenic properties since it prevents binding of TGF- β1 to its receptors on endothelial cells this compromising its function and leading to impairment in production of nitric oxide (Levine *et al.,* 2006). There is evidence from animal studies that increased expression of sEng in mice leads to increased vascular permeability and hypertension. If in addition to sEng, there was also increased expression of sFlt-1, then it led to severe vascular damage, nephrotic syndrome, proteinuria and growth restriction (Venkatesha *et al.,* 2006).

Endothelial cell dysfunction leads to vasospasm and platelet activation and there is evidence to support an important role for platelet dysfunction and thrombocytopaenia prior to development of hypertension (Romero *et al.,* 1988). There are studies reporting abnormalities in platelet size, platelet life span, and increased production of thromboxane by platelets in pregnancies with PE (Kenny *et al.,* 2009). There are abnormalities in the levels of prostacyclin and thromboxane A2. Prostacyclin causes vasodilatation and inhibits platelet aggregation whereas thromboxane A2 leads to vasoconstriction and platelet aggregation (Romero *et al.,* 1988). The evidence for a central role for platelet activation in PE is supported by studies which suggest that there is reduction in levels of a metabolite of prostacyclin in maternal blood and urine in pregnancies with PE (Bussolino *et al.,* 1980). This is supported by increased production of thromboxane A2 compared to prostacyclin from placentae in pregnancies with PE (Walsh, 1985). This altered balance between these proteins along with platelet activation results in platelet aggregation and formation of thrombi in the microcirculation of different organs, which is a major contributor to pathogenesis of PE (Romero and Duffy, 1980).

### 1.3.5 Oxidative stress

The production of reactive oxygen species (ROS) leads to oxidative stress when the inherent anti-oxidant mechanisms in tissues are overwhelmed. In pregnancies with PE, impaired placentation leads to the sequence of placental hypoxia and reperfusion induced damage leading to increased production of ROS which in turn further contributes to oxidative stress (Burton and Jauniaux, 2011). There is evidence that placental anti-oxidant mechanisms are impaired in pregnancies with PE as evidenced by decreased levels of superoxide dismutase and glutathione peroxidase compared with normal pregnancies (Vaughan and Walsh, 2002). In summary, impaired placentation leads to hypoxia and reperfusion injury which lead to oxidative stress and release of proinflammatory cytokines and trophoblast debris in the circulation thus contributing to the pathophysiology of PE (Cindrova-Davies *et al.,* 2007).

There appear to be some similarities between PE and atherosclerosis with some investigators suggesting that events that lead to development of atherosclerosis may also contribute towards the pathophysiology of PE. There is evidence that altered and abnormal lipid metabolism along with oxidative stress results in changes in endothelial function leading to atherosclerosis (Witztum, 1994). Similarly, hypoxia at the maternal-fetal interface due to reduced placental perfusion results in the generation of free radicals, which in turn leads to oxidative damage to the vascular endothelium. The main mechanism of damage is suggested to be generation of ROS which interact with circulating lipids to form stable lipid peroxidation products which are capable of damaging endothelial cell structures.

Dyslipidaemia in pregnancies complicated by PE can lead to the accumulation of very low density lipoproteins (VLDL), which are easily oxidised to form highly reactive oxidised LDL. These oxidised LDL alter the membrane protein and phospholipids and increase the expression of signalling molecules which recruit monocytes. The membrane damage by oxidised LDL alters endothelial function while monocytes take

up oxidised LDL to form foam cells and eventually the fatty streak characteristic of atherosclerosis (Roberts and Cooper, 2001). In PE, the reduced reserve in net anti-oxidant activity leads to further damage by these oxygen free radicals.

## 1.4 PREDICTION OF PREECLAMPSIA

### 1.4.1 Background

The traditional method for early detection and diagnosis of PE is to undertake serial measurements of BP and assessment of proteinuria during regular scheduled antenatal visits but unfortunately this approach is not useful for early prediction or identification of a high-risk group that are likely to develop PE. Although recognition of risk factors can be useful in clinical practice, it cannot be used reliably for screening and prediction of PE (Wallenburg, 2001). However, in the UK the National Institute for Clinical Excellence (NICE, 2008) has issued guidelines for antenatal care which recommend that at the booking visit, a woman's level of risk for PE, based on factors in her history, should be determined and the subsequent intensity of antenatal care should be based on this assessment of risk (Table 1.4).

There is however, no available data on the performance of such a recommended screening strategy which treats each of the risk factors, such as age of 40 years or older, nulliparity, body mass index (BMI) of 30 Kg/m2 or above, family history or personal history of PE and pre-existing vascular disease, as separate screening tests with additive detection and false positive rates.

This approach of screening for PE is likely to result in classifying a large number of pregnant women as screen-positive and therefore in need of more frequent antenatal monitoring, which undermines the purpose of screening and creates a substantial strain on the healthcare system.

**Table 1.4** National Institute for Clinical Excellence recommendations on screening for preeclampsia.

| NICE recommendations on Screening for preeclampsia |
|---|
| At the booking appointment, the following risk factors for PE should be determined:<br>- Age 40 years or older<br>- Nulliparity<br>- Pregnancy interval of more than 10 years<br>- Family history of PE<br>- Previous history of PE<br>- Body Mass Index of 30 Kg/m2 or above<br>- Pre-existing vascular disease such as hypertension<br>- Pre-existing renal disease<br>- Multiple pregnancy<br>More frequent blood pressure measurements should be considered for pregnant women with above risk factors |

The main factors in maternal demographic characteristics and obstetric history which contribute towards the background risk for PE are discussed below. There are several observational, cohort and case studies with few studies quantifying the actual impact of an individual risk factor towards development of PE.

### 1.4.2 Maternal demographic factors and obstetric history

Maternal age

There is evidence from a considerable number of studies which suggest that there is an increase in the risk of PE with increasing maternal age. Previous studies, which

dichotomised maternal age with cut-offs at either 35 or 40 years, have shown a doubling of the risk of developing PE (Bianco *et al.,* 1996; Lawoyin and Ani, 1996; Lee *et al.,* 2000; Chen *et al.,* 2000). Data from US shows that the risk for PE appears to increase more abruptly after the mid-30s (Saftlas *et al.,* 1990) and similarly reported in a study by Mittendorf *et al.,* (1996) which shows that the risk for PE increases by 30-40% for every additional year of age past 34 years. In a study which demonstrated the association of advanced maternal age with PE, the authors reported that the increase in risk of PE was significantly higher in mothers older than 40 years compared to younger mothers and this association remained statistically significant regardless of adjustment for parity (Ziadeh and Yahaya, 2001). The association of advanced maternal age with PE may be related to the underlying progressive vascular endothelial damage associated with aging (Naeye, 1983; Eisenberg and Schenker 1997).

These results were consistent with results of a systematic review in which the authors reported that maternal age above 40 years is associated with doubling in risk of PE (Duckitt and Harrington 2005). The association of PE with maternal age is also reported in a large study which examined the risk factors for PE in a multivariate approach, thus accounting for confounding effects and interactions, and reported that the risk for late onset PE increases by 4% every year over the age of 32 years (Poon *et al.,* 2010). In a large prospective observational cohort study of more than 75,000 singleton pregnancies examining the association of maternal age with adverse pregnancy outcomes, the authors reported that the risk of PE was significantly higher in women with advanced maternal age more than 40 years. Compared to younger women, the risk of developing early-PE was higher than late-PE. They reported that even after adjusting for other maternal characteristics, maternal age of 35-39 years and > 40 years remained significantly associated with risk of developing PE (Khalil *et al.,* 2013a).

Parity

The relationship between women in their first pregnancy and risk of PE is reported widely. A systematic review of controlled studies showed that nulliparity increases the

likelihood of PE by a factor of about three (Duckitt and Harrington, 2005). Similar results were reported by Luo *et al.,* (2007) in a systematic review of 26 studies reporting that the summary crude odds ratio was 2.42 for risk of PE among primiparous vs multiparous women. They further reported that this elevated risk for PE remained even after adjusting for other risk factors such as maternal age, race and body mass index and the summary adjusted odds ratio was 2.71 (Luo *et al.,* 2007).

Women with a previous pregnancy, albeit a miscarriage appears to provide some degree of reduction in risk for PE (Dekker *et al.,* 1998). An explanation for this association between nulliparity and risk of PE is provided by the immune maladaptation hypothesis which states that the fetal-placental unit contains paternal antigens are antigenic to the nulliparous mother who mounts an abnormal immune response resulting in manifestations of PE (Dekker, 1999). This hypothesis is supported by observations that suggest that multiparity reduces the risk of PE and that this protective effect is lost with change of partner (Robillard *et al.,* 1993). Another support for immune maladaptation hypothesis in explaining the association of nulliparity and PE is the finding that in multiparous women conceived by donor insemination, the risk of PE is increased (Need *et al.,* 1983). There are studies suggesting that regular sperm exposure over a prolonged period prior to conceiving may result in the mother's immune system being exposed to paternal antigens and therefore affording some protection against PE in nulliparous mothers (Marti and Herrmann 1977; Dekker 2002; Dekker and Robillard, 2005). Although the immune maladaptation theory is a proposed hypothesis to explain the increased risk but the evidence for this from biological studies is not strong (Luo *et al.,* 2007). There are however, recent studies which suggest that differences in angiogenic factor profile or reactivity to insulin resistance may explain the primiparity-associated PE risk (Wolf *et al.,* 2002 and 2005). There is evidence that women in their first pregnancies had a significantly increased level of anti-angiogenic factor sFlt-1 compared to women in their second pregnancies and this difference persisted despite adjusting for other confounding factors which are associated with PE (Wolf *et al.,* 2005). In summary,

there is an increased risk for PE associated with nulliparity but further research into the mechanisms for this need to be carried out.

Change of partner

There is evidence from some studies that the risk of PE in a second pregnancy is lower only if the mother's partner remains the same. The basis for this association is that the risk may be reduced with repeated maternal exposure or adaptation to specific foreign antigens of the partner, while a new partner presents with new antigens that results in a risk of PE that is similar to that of a first pregnancy (Robillard *et al.,* 1993; Trupin *et al.,* 1996). However, it is likely that the increased risk of PE associated with a change of partner might be attributable to a longer inter-pregnancy interval.

Inter-pregnancy interval

In a large study examining 550,000 women who had two or more singleton deliveries including 200,000 women who had three or more singleton deliveries, the authors demonstrated that an association between the risk of PE and interval is more significant than the association between the risk and a change of partner, with the risk in a second or third pregnancy directly related to the time elapsed since the previous delivery (Skjaerven *et al.,* 2002). In addition, when the inter-pregnancy interval is 10 years or more the risk of PE is about the same as that in nulliparous women. After adjustment for the presence or absence of a change of partner, maternal age and year of delivery, the probability of PE is increased by a factor of 1.12 for each year increase in the inter-pregnancy interval. Similar results were reported by another study where the authors stratified the analysis in mothers with a subsequent pregnancy according to whether they had previous PE or not. In women without previous PE, the risk of subsequent PE increased with increasing time interval between deliveries regardless of change in paternity for the second pregnancy. Whereas in women with a previous PE, there was no significant difference in the risk of PE according to time interval between deliveries in women with same or new paternity pregnancies (Trogstad *et al.,* 2001). Additional evidence for the importance of the inter-pregnancy interval derives from a cross-

sectional study which reported a significant increase in the risk of PE in women with inter-pregnancy intervals of more than 59 months compared with those with intervals of 18-23 months (Conde-Agudelo *et al.,* 2000).

Parous women with history of previous preeclampsia

There is evidence from observational studies that a previous history of PE increases the risk of developing PE in future pregnancies. Women who have PE in a first pregnancy are seven to ten times more likely to develop PE in a second pregnancy (Campbell *et al.,* 1985; Sibai *et al.,* 1986; Lie *et al.,* 1998; Lee *et al.,* 2000; Mostello *et al.,* 2002; Duckitt and Harrington, 2005), whilst women with PE in their second pregnancy are also seven times more likely to have had a history of PE in their first pregnancy than women in their second pregnancy who do not develop PE (Eskenazi *et al.,* 1991; Stone *et al.,* 1994).

There are varying reports in the literature with regard to quantifying such recurrence risks. In an individual patient data meta-analysis examining the recurrence of hypertensive disorders in pregnancy, the authors reported their results based on data from 94 studies including more than 99,000 pregnancies. The recurrence rate for all hypertensive disorders for pregnancy was 20.7% and in particular was 13.8% for PE and 8.6% for GH. The authors further reported that there was an inverse relationship between the gestational age at delivery in the first pregnancy and the risk of PE in a subsequent pregnancy (van Oostwaard *et al.,* 2015).

There are studies which provide results separately for early onset and late onset PE and it was demonstrated that previous history of PE increases twice as much the risk for early-PE (< 32 weeks) as opposed to late-PE (Odegard *et al.,* 2000). There are other studies reporting their results on the recurrence risk for a subsequent early-PE (< 34 weeks) given a previous history of early onset-PE in the index pregnancy but the recurrence risks range from 5 to 17% (van Rijn *et al.,* 2006; Langenveld *et al.,* 2010). In a systematic review examining the risks of early delivery at < 34 weeks following

early-onset PE in an index pregnancy, the authors reported results from 11 studies including 2377 women and found that the pooled recurrence risk for recurrence of early disease is in the region of 8%. (Langenveld *et al.,* 2011).

Assisted reproductive technologies

There are several studies which report that use of ART double the risk for PE (Maman *et al.,* 1998; Jackson *et al.,* 2004; Shevell *et al.,* 2005; Lambert-Messerlian *et al.,* 2006; Trogstad *et al.,* 2009). It is unclear whether either IVF and ovulation induction equally influence the risk of PE or they have different contributions. In a large observational study, the authors showed that it is IVF but not ovulation induction that increases the risk for PE (Shevell *et al.,* 2005) and one smaller case-control study reported that both techniques increase the likelihood of developing hypertension in pregnancy (Maman *et al.,* 1998). In a conflicting report form another study, the authors reported that it is ovulation induction rather than IVF that increases the risk of PE by 2-fold (Trogstad *et al.,* 2009).

In a large prospective observational cohort study, the authors examined more than 40,000 pregnancies including 634 that conceived after IVF and 682 that conceived following ovulation induction and reported that the risk of PE was significantly increased in those conceived following IVF but not ovulation induction. The unadjusted odds ratio for risk of PE was 1.76. They further reported that when the risk was examined separately for early and late PE, after adjusting for maternal characteristics, there was a significant increase in the risk for early-PE (OR 3.28) but not for late-PE. These results raise the possibility that IVF, independent of maternal characteristics, somehow contributes to the process of impaired trophoblastic proliferation that is a hallmark of early and severe disease (Chaveeva *et al.,* 2011).

In another cohort study of 47,088 pregnancies following assisted reproductive technology, the authors reported that the risk of PE was higher in IVF pregnancies compared to those conceived spontaneously and in addition, the risk was higher in

frozen-thawed cycles compare to fresh cycle pregnancies (Opdahl *et al.,* 2015). Similarly, when the risk of PE was compared between women having autologous ovum IVG with those having donor oocyte IVF, there was an increased risk in those that had the donor IVF (Simeone *et al.,* 2016). There is some evidence from IVF pregnancies with ovum donation that there is impaired autophagy of extravillous trophoblast and immunological changes in decidua basalis which may induce impairment in spiral artery remodelling and contribute to subsequent development of PE (Nakabayashi *et al.,* 2015). This was supported from a systematic review of 19 studies including more than 86,000 pregnancies which reported that the risk of PE is higher in oocyte donation IVF cycles compared to other methods of assisted conception (OR 2.54) and natural conception (OR 4.34) (Masoudian *et al.,* 2015).

Obesity

Obesity is an important risk factor for developing PE. The results from many of the previously published studies, which treated BMI as a binary variable showed an overall doubling to quadrupling of the risk of PE with an increase in pre-pregnancy or booking BMI (Eskenazi *et al.,* 1991; Stone *et al.,* 1994; Mittendorf *et al.,* 1996; Conde-Agudelo and Belizán, 2000; Mostello *et al.,* 2002; Duckitt and Harrington, 2005). In a Norwegian population-based study, the authors reported that a high pre-pregnancy BMI is not an independent risk factor for early-PE but it increases the risk of late-PE by two-fold (Odegard *et al.,* 2000).

In a large prospective observational cohort study of more than 45,000 singleton pregnancies examining the association of maternal BMI with pregnancy complications, the authors reported that the risk of PE increased with increasing maternal BMI in spite of adjustment for other maternal characteristics known to be associated with risk of PE. Although the risk for total PE was increased but univariate analysis demonstrated a higher risk for late disease compared to early disease (Syngelaki *et al.,* 2011a). In another cohort study examining the association between maternal BMI and risk of severe PE, the authors reported that there was no significant

difference between the 4 BMI categories with regard to risk of severe PE but in those that are overweight, obese or morbidly obese, the risk of severe late-onset PE is increased (Durst *et al.,* 2015).

The mechanisms linking obesity with increased risk of PE may be explained by effect of metabolic factors in obese mothers which impact on various stages of pathogenesis of PE such as cytotrophoblast migration and placental ischaemia, release of soluble factors in maternal circulation and impact on endothelial cell dysfunction (Spradley *et al.,* 2015). There is direct and indirect evidence from studies to suggest that obesity related metabolic factors may lead to impaired spiral artery remodelling.

There are studies reporting that the maternal serum level of PlGF examined in early second trimester is lower in obese pregnant women compared to their lean counterparts (Ghosh *et al.,* 2013). This is consistent with observations that obese women have reduced placental vascularity characterized by villi having large diameters and low numbers of capillaries (Dubova *et al.,* 2011). Similarly, there is evidence that maternal serum levels of sFlt-1 and leptin in an obese hypertensive pregnancy were greater than those in obese normotensive pregnancy in early and late pregnancy (Mise *et al.,* 1998; Hendler *et al.,* 2005; Masuyama *et al.,* 2012; Straughen *et al.,* 2013).

Another biomarker for obesity that is altered in pregnancies with PE is adiponectin. There is evidence that maternal serum levels of adiponectin in the first trimester are significantly higher in women who subsequently develop early-PE than in women who develop late-PE or those who remain normotensive. The authors report that adiponectin levels are however not related to biophysical and biomarker of impaired placentation such as uterine artery PI and serum PAPP-A and these altered levels may be secondary to a different mechanism such as endothelial dysfunction (Nanda *et al.,* 2011).

Racial origin

There is considerable evidence in the literature which suggests that ethnic or racial origin is an important predictor of PE. One small series (Eskenazi *et al.,* 1991) has demonstrated that African race is associated with a 12-fold increase in the risk of PE while larger studies have reported a 20-50% increase in the risk (Mittendorf *et al.,* 1996; Sibai *et al.,* 1997; Knuist *et al.,*1998; Mostello *et al.,*2002; Caughey *et al.,* 2005).

The risk for PE is higher not only in women of Afro-Caribbean Race but also women of South Asian racial origin. These differences in risks for PE are also supported by evidence from metabolic profile of non-pregnant women which suggests susceptibility to cardiovascular disease. In both Afro-Caribbean and South Asian women, the risk for chronic hypertension and cardiovascular disease is increased but in particular, Afro-Caribbean women are more prone to stroke and renal failure whereas South Asian women are more at high risk of coronary heart disease (Cappuccio 1997a; Cappuccio *et al.,* 1997b; Ramraj and Chellapa 2008). Another retrospective cohort study including 67,746 pregnancies examining the risk of developing PE reported that in East Asian women of Chinese descent the risk of PE is significantly lower compared to Caucasian women and the possible factors associated with this may be difference in BMI and lifestyle factors such as length of cohabitation with partner (Xiao *et al.,* 2014). Similar findings were also reported in Hispanic women where the risk for PE among Hispanic-black women was significantly increased compared to non-Hispanic White women (Ghosh *et al.,* 2014).

In a large prospective observational cohort study of more than 79,000 singleton pregnancies examining the association of maternal racial origin with pregnancy outcomes, the authors reported that the risk of PE was significantly higher in women of Afro-Caribbean and South Asian racial origin compared to Caucasian women. This increase in risk remained significant even after adjusting for other maternal characteristics known to be associated with a risk for PE. In fact, after chronic

hypertension, Afro-Caribbean race was the second highest risk factor associated with risk for developing PE with an OR of 2.60 (Khalil *et al.,* 2013b).

Family history of preeclampsia

The risk of PE is also shown to be increased by a factor of 3-4 times when there is a history of PE in the family i.e. mother or sister (Arngrimsson *et al.,* 1990; Cincotta and Brennecke, 1998). In a study investigating the risk of PE in 94 families spanning over at least 3 generations, the authors reported that the prevalence of PE is higher in in daughters-in-law (23% vs 10%) (Arngrimsson *et al.,* 1990). There are other reports which have also reported that a family history of PE in a mother, sister or both triples the risk of PE (Cincotta and Brennecke, 1998).

Cigarette smoking

There is evidence from case-control and observational studies that in women who smoke, the risk of PE is lower (Conde-Agudelo *et al.,* 1999). In a systematic review of 28 cohort and 7 case-control studies including more than 800,000 women, the authors reported that cigarette smoking in pregnancy is associated with an overall 30% reduction in the risk of PE (Conde-Agudelo *et al.,* 1999). There was an inverse dose-response relationship with the risk for PE decreasing as the number of cigarettes smoked increased. Similarly, a meta-analysis of 9 cohort studies reported that smokers of less than 10 cigarettes per day and 10 or more cigarettes per day had 20% and 30% reductions respectively in the risk of PE.

Pre-existing medical conditions

There are some medical conditions that predispose a mother to developing PE. These include a history of type I diabetes mellitus, chronic hypertension, renal disease, autoimmune diseases and anti-phospholipid syndrome. These reported risks for PE in cohort and case-control studies were analysed in a systematic review by Duckitt and

Harrington (2005) and they reported summary relative risks for PE given these pre-existing medical conditions (Table 1.5).

*Diabetes mellitus*

The likelihood of PE is more than triple if type I diabetes is mellitus present before pregnancy (Garner *et al.,* 1990; Ros *et al.,* 1998; Lee *et al.,* 2000).

*Chronic hypertension*

Pre-existing hypertension has been shown to double the risk for PE, after controlling for other maternal characteristics, (Conde-Agudelo *et al.,* 2000).

**Table 1.5.** Relative risks of pre-existing medical conditions for the development of preeclampsia (modified from Duckitt and Harrington, 2005).

| Pre-existing condition | Type of study | N | RR (95% CI) |
|---|---|---|---|
| Type 1 diabetes mellitus | Cohort | 56,968 | 3.56 (2.54-4.99) |
| Hypertension | | | |
| Systolic $\geq$ 130 mm Hg vs < 130 mm Hg at booking | Cohort | 906 | 2.37 (1.78-3.15) |
| Diastolic $\geq$ 80 mm Hg vs < 80 mm Hg at booking | Cohort | 907 | 1.38 (1.01-1.87) |
| Antiphospholipid antibodies vs none | Cohort | 1,802 | 9.72 (4.34-21.75) |
| Antiphospholipid antibodies | Case-control | 760 | 6.12 (0.35-108.35) |

RR = relative risk; CI = confidence interval

*Autoimmune disease*

A matched case-control study by Stamilio *et al.* (2000) has found that women who developed PE were six times more likely to have an autoimmune disease. The presence of antiphospholipid antibodies (anti-cardiolipin antibodies or lupus anticoagulant or both) has been observed to significantly increase the risk of developing PE in both

cohort and case-control studies (Branch *et al.*, 1989; Sletnes *et al.*, 1992; Pattison *et al.*, 1993; Yasuda *et al.*, 1995; Dreyfus *et al.*, 2001).

### 1.4.3 Biophysical markers

Uterine artery Doppler

The examination of impedance to flow in the uterine arteries has been shown to correlate with not only histological studies but also clinical severity of PE. There is evidence from studies that suggest that screening based on uterine artery Doppler in not only the second but also the first trimester of pregnancy is effective.

Doppler ultrasound provides a non-invasive method for the assessment of the uteroplacental circulation. Several studies using colour Doppler imaging of the distal branches of uterine arteries demonstrate a significant decrease in resistance in the spiral arteries with advancing gestation during the first-trimester which is in keeping with physiological changes (Carbillon *et al.*, 2001). There is an initial fall in the impedance to flow until 24-26 weeks of gestation due to the effects of trophoblastic invasion of the spiral arteries and their conversion from narrow muscular vessels to low resistance wide non-muscular channels (Campbell *et al.*, 1983). The continuing fall in impedance may be due to hormonal effect in pregnancy on the elasticity of arterial walls.

The observation of abnormal uteroplacental flow velocity waveforms, as a result of persistent high impedance to flow in the uterine arteries, constitutes indirect evidence of abnormal placentation. Previous histological findings from placental bed biopsies of pregnancies affected by PE have shown good correlation with high resistance in uterine artery Doppler waveforms (Olofsson *et al.*, 1993). Cross-sectional studies in pregnancies with PE have shown that impedance to flow in the uterine arteries is increased due to an inability to convert maternal placental arteries into low resistance vessels (Aardema *et al.*, 2001).

*Second-trimester uterine artery Doppler*

The assessment of uterine artery has evolved from a blind technique, using continuous wave Doppler (Hanretty *et al.,* 1989) to real-time ultrasound in order to positively identify the vessels (Bower *et al.,* 1993a,b). The correct vessel is identified using pulsed wave Doppler and distinguishes uterine artery blood flow from adjacent high resistance internal iliac vessels and lower resistance arcuate arteries. The use of colour flow along with pulsed wave Doppler makes it easier to identify the vessels of interest and obtain accurate measurements.

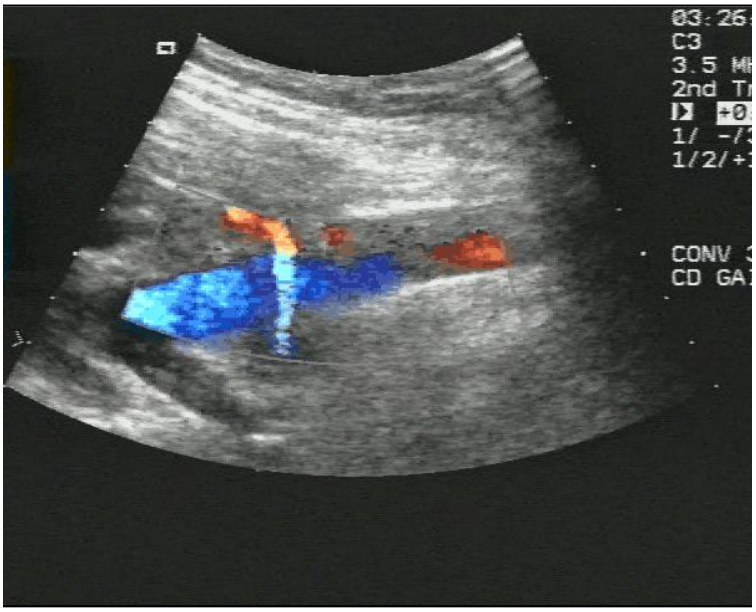



**Figure 1.3**. Transabdominal technique in obtaining uterine artery waveform at the crossover with iliac artery.

Transabdominally, the uterine artery can be identified by holding the transducer in the longitudinal axis and lateral to the uterus. In that position the scan shows the bifurcation of the common iliac artery into external and internal iliac arteries and there is apparent cross-over of the uterine artery and the external iliac artery (Figure 1.3).

Transvaginally, the ultrasound probe is placed into the lateral fornices and the uterine artery is identified at the level of the internal cervical os (Figure 1.4). The Doppler gate is placed over the uterine artery, at an angle of less than 60°.

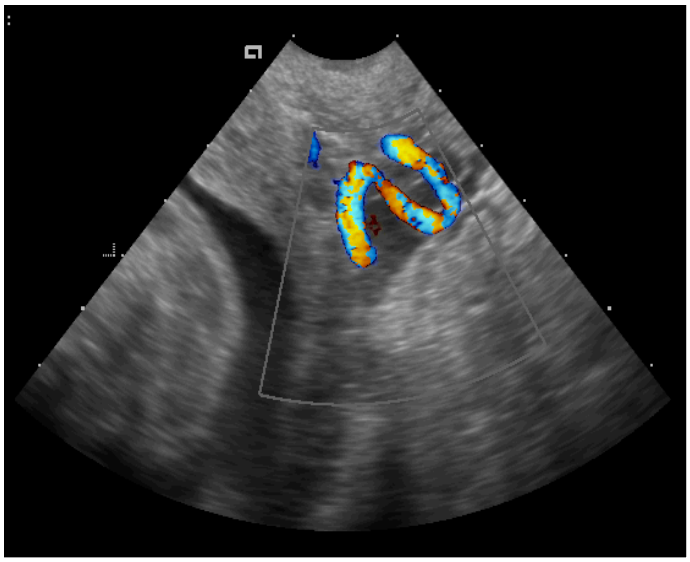


**Figure 1.4.** Transvaginal technique in obtaining uterine artery waveform lateral to uterine cervix.

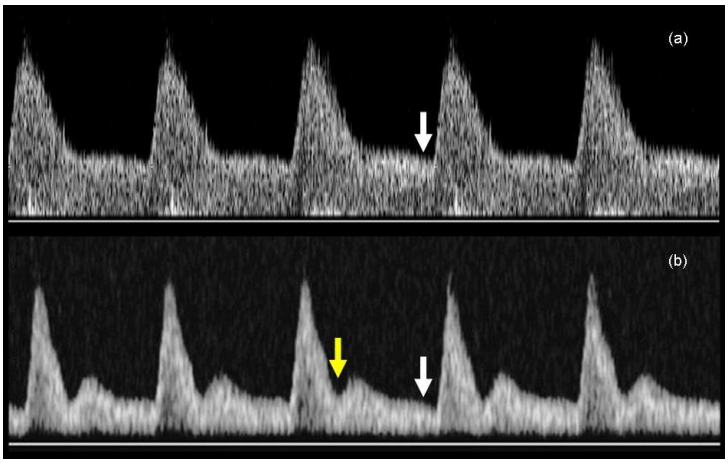



**Figure 1.5.** Pulsed wave signals of uterine artery blood flow (a) normal waveform: note the systolic unslope is less steep with an abundance of end-diastolic flow velocity (arrow); (b) abnormal waveform suggesting impaired placentation: note the steep systolic upslope with an early diastolic notch (yellow arrow) and reduced end-diastolic flow velocity (white arrow).

The pulsed Doppler is applied to obtain flow velocity waveform and when 3 consistent waveforms are obtained, the image is frozen and the pulsatility indices are obtained by automatically or manually tracing these waveforms.

The indices derived from the flow velocity waveforms are described below. The possible range of the resistance index is between 0 and 1 and that of the pulsatility index is greater than 0.

$$\text{Resistance index} = \frac{(\text{Peak systolic velocity - minimum diastolic velocity})}{\text{Peak systolic velocity}}$$

$$\text{Pulsatility index} = \frac{(\text{Peak systolic velocity - minimum diastolic velocity})}{\text{Mean velocity}}$$

The normal waveform of uterine artery at 22-23 weeks' gestation where the placentation is complete, the systolic upslope is less steep, with an abundance of diastolic flow velocity (Figure 1.5). In contrast, the high resistance waveform is characterised by steep systolic upslope with an early diastolic notch and reduced end diastolic flow velocity (Figure 1.5). Albaiges *et al*. (2003) compared velocity and impedance indices irrespective of notch status in a high-risk population defined by an increased uterine artery impedance > 95th centile or the presence of bilateral notches at a follow-up assessment from 24 weeks of gestation. The study concluded that uterine artery mean impedance indices perform better than velocity indices in the prediction of adverse pregnancy outcomes irrespective of notch status. Quantitative uterine artery indices provide a more objective method of calculating individual risk for adverse outcome and remove the subjectivity of an operator-dependent assessment of a notch.

Some studies further examined the association of abnormal Doppler findings with the severity of PE and they have shown that uterine artery Doppler is better in predicting more severe disease. Harrington *et al*. (1996) have found that bilateral notching at 24 weeks of gestation identified 55% of women who later developed PE and 81% with

PE requiring delivery before 35 weeks of gestation. Similarly, Kurdi *et al*. (1998) have found that bilateral notches and a mean resistance index of greater than 0.55 identified 62% of women who later developed PE and 88% with PE requiring delivery before 37 weeks while Albaiges *et al*. (2000) have shown that the detection rates of increased PI or bilateral notches in predicting all PE and early onset PE (delivery before 34 weeks) were 45% and 90%, respectively.

*First trimester uterine artery Doppler*

There is evidence from studies conducted in the 1990s that there is a good correlation between first and second trimester uterine artery Doppler measurements. The hypothesis for these studies was that since the trophoblastic proliferation starts in the first trimester then the development of uteroplacental circulation in the second trimester is not a random event but a consequence of events in first trimester. These studies initially demonstrated that the impedance to flow in the uterine arteries increases with gestational age from 10 to 22 weeks and that the impedance to flow in uteroplacental circulation in second trimester depends on values in first trimester (Jurkovic *et al.,* 1991; Kaminipetros *et al.,* 1991). The feasibility, methodology and reproducibility of first trimester uterine artery Doppler measurements has been reported more than a decade ago (Martin *et al.,* 2001).

As with other biomarkers, the values of uterine artery Doppler should be adjusted for characteristics that affect the measurements in unaffected pregnancies. These relationships were examined in a study of more than 6,500 pregnancies in which the authors reported that uterine artery PI decreased with gestational age and maternal BMI and was higher in women of Afro-caribbean racial origin, in nulliparous women and in those parous women with a previous history of PE (Plasencia *et al.,* 2007). The studies examining the value of screening for PE by combination of maternal factors and uterine artery Doppler demonstrated that effective screening can be provided for PE, in particular early-PE, at a DR of about 80% for a FPR of 10% (Plasencia *et al.,* 2007; Poon *et al.,* 2009b). The relationship between first and second trimester uterine artery Dopplers and the change in PI between two trimesters in prediction of PI was

examined in a study of 3,107 pregnancies and the authors reported that the decrease in impedance to flow in uterine arteries between 12 and 23 weeks is steeper in pregnancies with a normal outcome than in those developing PE and therefore the rate of change in uterine artery PI between the two trimesters improves the performance of screening and this information can be used to follow-up women with high PI at 12 weeks (Plasencia *et al.,* 2008).

Blood pressure

The mainstay of early detection, prediction and diagnosis of hypertension in pregnancy is accurate measurement of BP. There is considerable evidence from studies suggesting that raised BP in women destined to develop PE can be observed as early as in the first-trimester of pregnancy (Moutquin *et al.,* 1985; Higgins *et al.,* 1997; Poon *et al.,* 2008 and 2011a). The limitation of using booking BP for early prediction of PE is that it is difficult to distinguish undiagnosed pre-existing hypertension from those without as the booking measurement may the first health screen performed in young women. This limitation makes one-off BP assessment a difficult screening tool for PE in retrospective studies. Careful prospective evaluation of booking BP using standardised measurements may be of value.

A systematic review examined the value of using BP to predict PE using data from more than 60,000 women including 3,300 cases with PE and concluded that MAP is significantly better at predicting PE than systolic or diastolic BP but regardless, the measurement of BP is not useful in prediction of PE (Cnossen *et al.,* 2008). However, there was considerable heterogeneity between studies in this review with major differences in study design, sample size, differences in use of machines used to measure BP, mixture of high-risk and low-risk populations and major differences in the DR between studies.

In a large prospective screening study of more than 9,000 pregnancies at 11-13 weeks' gestation, the authors examined the performance of systolic BP, diastolic BP and MAP

in screening for hypertensive disorders of pregnancy using validated automated devices. They reported that although systolic BP, diastolic BP and MAP were all increased in women who subsequently developed PE. The best performance of screening was provided by MAP and the DR of early-PE at a FPR of 10% increased from 47% based on maternal factors alone to 76% based on a combination of maternal factors and MAP (Poon *et al.*, 2011a).

Accurate measurement of MAP requires the adherence to a strict protocol (Poon *et al.*, 2012). First, the women should be in the sitting position with their arms supported at the level of the heart, second, a small (22 cm), normal (22 to 32 cm) or large (33 to 42 cm) adult cuff should be used depending on the mid-arm circumference, third, validated automated devices should be used, fourth, after rest for five minutes, two recordings of blood pressure should be made in both arms simultaneously and the final MAP calculated as the average of all four measurements.

### 1.4.4 Biochemical markers

A large number of biochemical markers have been put forward for the prediction of PE. Many such markers represent measurable manifestations of impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries. The reduced placental perfusion is though to lead to placental ischaemia with consequent release of inflammatory factors, platelet activation, endothelial dysfunction, maternal renal dysfunction or abnormal oxidative stress. The majority of studies have examined these markers during established disease and in the second trimester.

In biochemical testing it is necessary to make adjustments in the measured maternal serum metabolite concentration to correct for certain maternal and pregnancy characteristics as well as the machine and reagents used for the assays and is then expressed in a multiple of the expected median (MoM) of the normal (Kagan *et al.*, 2008a).

Pregnancy associated plasma protein-A

Maternal serum pregnancy associated plasma protein-A (PAPP-A) is a well-established biochemical marker for the effective screening for trisomies 21, 18 and 13 in combination with maternal age, fetal nuchal translucency thickness (NT) and maternal serum free ß-human chorionic gonadotrohpin (ß-hCG) at 11-13 weeks of gestation. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum free ß-hCG is increased whereas in trisomies 18 and 13 this is decreased. In unaffected pregnancies the median free ß-hCG and PAPP-A is 1.0 MoM. In trisomy 21 pregnancies the median free ß-hCG is 2.0 MoM and the median PAPP-A is 0.5 MoM whereas in trisomy 18 the respective values are 0.2 MoM and 0.2 MoM and in trisomy 13 they are 0.5 MoM and 0.3 MoM (Kagan *et al.,* 2008b, Nicolaides, 2011).

However, measurement of PAPP-A is not an effective method of screening for PE because only 8-23% of affected cases have serum levels below the 5th centile, which is about 0.4 MoM. Nevertheless, in the management of individual patients found coincidentally during first-trimester screening for trisomies to have a low levels of PAPP-A, their obstetrician should be aware of the increased risk for PE. At the 5th centile of normal for PAPP-A the reported odds ratios for PE varied between 1.5 and 4.6. Although a low PAPP-A in itself is not a strong indicator of PE, it has shown a significant improvement in detection by combining first-trimester PAPP-A measurement with second-trimester uterine artery Doppler velocimetry (Spencer *et al.,* 2005, 2007). This had led to the recommendation that the observation of a low first-trimester PAPP-A MoM in euploid pregnancies should be followed up further with 22- 24 weeks Doppler measurement to assess the risk of PE.

In established disease, there are reports suggesting that the maternal serum PAPP-A level is increased (Bersinger *et al.,* 2003; Bersinger and Odegard, 2004; Deveci *et al.,* 2009). In chromosomally normal pregnancies there is evidence that low maternal

serum PAPP-A in the first- and second-trimesters is associated with increased risk for subsequent development of PE (Table 1.6).

**Table 1.6.** Summary of studies of pregnancy associated plasma protein-A in the prediction of preeclampsia.

| Author | Total | Screening for preeclampsia | | | |
|---|---|---|---|---|---|
| | | N (%) | PAPP-A Cut off Centile (MoM) | DR (%) | OR or RR |
| Ong *et al.,* 2000 | 5,297 | 135 (2.6) | 5$^{th}$ centile (-) | 11.1 | 2.1 |
| Yaron *et al.,* 2002 | 1,622 | 27 (1.7) | 15$^{th}$ centile (0.50) | 22.2 | 1.7 |
| Smith *et al.,* 2002 | 8,839 | 331 (3.7) | 5$^{th}$ centile (-) | 10.6 | 2.3 |
| Dugoff *et al.,* 2004 | 33,395 | 764 (2.3) | 5th centile (0.42) | 7.9 | 1.5 |
| Spencer *et al.,* 2005 | 4,390 | 64 (1.5) | 5th centile (0.42) | 14.1 | 2.8 |
| Pilalis *et al.,* 2007 | 878 | 13 (1.5) | 5th centile (0.41) | 23.1 | 4.6 |
| Spencer *et al.,* 2007 | 47,770 | 222 (0.5) | 5th centile (0.42) | 14.6 | 3.7 |

PAPP-A = pregnancy associated plasma protein-A; MoM = multiple of median; DR = detection rate; OR = odds ratio; RR = relative risk.

PAPP-A is a syncytiotrophoblast-derived metalloproteinase which enhances the mitogenic function of the insulin-like growth factors by cleaving the complex formed between such growth factors and their binding proteins (Bonno *et al.,* 1994; Lawrence *et al.,* 1999). The insulin-like growth factor system is believed to play an important role in placental growth and development, it is therefore not surprising that low serum PAPP-A is associated with a higher incidence of PE.

Placental growth factor

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) sub-family. It is a glycosylated dimeric glycoprotein and it binds to VEGF receptor-1 to facilitate its actions on angiogenesis. PlGF is synthesised in villous and extravillous cytotrophoblast 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.,* 2007a). PE is associated with reduced placental production of PlGF and several studies reported that during the clinical phase of PE the maternal serum PlGF concentration is reduced (Table 1.7).

**Table 1.7.** Studies comparing maternal serum concentration of placental growth factor (PlGF) in pregnancies with preeclampsia and normotensive controls.

| Author | Gestation (w) | Preeclampsia | | Controls | | *p* |
|---|---|---|---|---|---|---|
| | | n | PlGF (pg/ml) | n | PlGF (pg/ml) | |
| **Studies during established preeclampsia** | | | | | | |
| Torry *et al.,* 1998 | 36 | 30 | 40.7 | 30 | 132.6 | <0.0001 |
| Reuvekamp *et al.,* 1999 | 34 | 28 | 54.1 | 28 | 497.9 | < 0.0001 |
| Livingston *et al.,* 2001 | 37 | 22 | 125.2 | 22 | 449 | 0.003 |
| Taylor *et al.,* 2003 | 35 | 31 | 75.6 | 31 | 308 | 0.001 |
| Masuyama *et al.,* 2005 | 30* | 5 | 116.6 | 5 | 800 | < 0.01 |
| | 38† | 10 | 166.6 | 10 | 266.6 | <0.05 |
| Crispi *et al.,* 2006 | 29* | 41 | 58.1 | 18 | 335 | < 0.05 |
| | 37† | 35 | 150 | 22 | 223 | <0.05 |
| Ohkuchi *et al.,* 2007 | 28* | 14 | 85 | 65 | 800 | <0.001 |
| | 37† | 20 | 220 | 65 | 550 | <0.001 |
| Teixiera *et al.,* 2008 | 33-35 | 32 | 30 | 9 | 490 | =0.0001 |

* Early onset preeclampsia, † Late onset preeclampsia

There is also evidence that reduced levels of serum PLGF are apparent during the second and even first trimester of pregnancy and several weeks before the clinical onset of the disease (Table 1.8).

Previous studies demonstrated that prediction of PE can be improved by combining the second-trimester uterine artery Doppler findings with maternal serum concentration of PlGF (Espinoza *et al.,* 2007) and the anti-angiogenic protein soluble fms-like tyrosine kinase 1 (sFlt-1; Stepan *et al.,* 2007). Although in pregnancies

developing PE reduced levels of PlGF are evident from the first-trimester, significant increase in levels of sFlt-1 become apparent only about five weeks before the onset of PE (Levine *et al.,* 2004). The authors examined serum levels of PlGF at various time points in pregnancy beginning from as early as first trimester to the development of PE. The PlGF concentrations steadily increased with gestational age, with levels peaking at 29-32 weeks' gestation and decreasing thereafter. In women with PE, the levels follow a similar pattern but were significantly lower than in controls from as early as 12-13 weeks of gestation (Levine *et al.,* 2004).

**Table 1.8.** Studies comparing maternal serum concentration of placental growth factor (PlGF) in pregnancies that develop preeclampsia and normotensive controls.

| Author | Gestation (w) | Preeclampsia | | Controls | | *p* |
|---|---|---|---|---|---|---|
| | | n | PlGF (pg/ml) | n | PlGF (pg/ml) | |
| **Studies in second-trimester before onset of preeclampsia** | | | | | | |
| Tjoa *et al.,* 2001 | 14-17 | 9 | 46.6 | 26 | 68.8 | NS |
| | 17-21 | 10 | 83.3 | 25 | 124.1 | 0.004 |
| Livingston *et al.,* 2001 | 11-20 | 22 | 98.8 | 22 | 56.3 | NS |
| Su *et al.,* 2001 | 14-19 | 27 | 0.55 MoM | 227 | 1.00 MoM | <0.001 |
| Tidwell *et al.,* 2001 | 16-20 | 14 | 101.4 | 25 | 175.5 | 0.011 |
| | 26-30 | 14 | 389.8 | 25 | 753.0 | 0.008 |
| Pollioti *et al.,* 2003 | 16-19 | 20 | 61.3 | 60 | 122.4 | <0.001 |
| Krauss *et al.,* 2004 | 22-29 | 44 | 286 | 177 | 441 | 0.024 |
| Crispi *et al.,* 2008 | 20-24 | 19* | 92 | 76 | 426 | <0.01 |
| | | 19† | 260 | 76 | 426 | 0.01 |
| Erez *et al.,* 2008 | 21-24 | 17* | 126.3 | 201 | 344.8 | <0.0001 |
| | | 35† | 273.4 | 201 | 344.8 | =0.03 |
| **Studies in first-trimester before onset of preeclampsia** | | | | | | |
| Tidwell *et al.,* 2001 | 5-15 | 14 | 20.1 | 25 | 58.5 | <0.0001 |
| Ong *et al.,* 2001 | 11-14 | 131 | 1.09 MoM | 400 | 0.98 MoM | NS |
| Thadani *et al.,* 2004 | 11-14 | 40 | 23 | 80 | 63 | <0.01 |
| Smith *et al.,* 2007 | 11-14 | 309 | | 937 | | |
| Erez *et al.,* 2008 | 9-14 | 17* | 20.3 | 201 | 35.4 | =0.002 |
| | | 35c | 26.2 | 201 | 35.4 | =0.003 |

* Early onset preeclampsia, † Late onset preeclampsia

Placental protein 13

Placental protein 13 (PP13) is a 32-kDa dimer protein and a member of a group of proteins that are highly expressed in the placenta (Than *et al.,* 1999, 2004). It is believed that it has a role in placental implantation and maternal artery remodelling (Burger *et al.,* 2004). Early studies suggest that maternal serum level of PP13 during the first-trimester is lower in women with PE requiring early delivery, compared to the level of PP13 in normotensive pregnancies (Nicolaides *et al.,* 2006; Chafetz *et al.,* 2007; Spencer *et al.,* 2007; Romero *et al.,* 2008b; Huppertz *et al.,* 2008; Gonen *et al.,* 2008; Khalil *et al.,* 2009). There is evidence from studies in the first-trimester of pregnancy that the maternal serum concentration of PP13 is reduced in women who subsequently develop PE (Table 1.9). However, the number of PE cases in these studies varied from 4 to 88, and the reported levels of PP13 in PE cases ranged from 0.07 MoM to 0.69 MoM.

**Table 1.9** Studies reporting on the association between maternal serum placental protein 13 (PP13) concentration and preeclampsia.

| Author | GA (wk) | Preeclampsia | | Control | | P value |
|---|---|---|---|---|---|---|
| | | n | PP13 MoM | n | PP13 MoM | |
| Nicolaides *et al.,* 2006 | 11-14 | 10 | 0.07 | 423 | 1.00 | < 0.001 |
| Chafetz *et al.,* 2007 | 9-12 | 47 | 0.20 | 290 | 1.00 | < 0.01 |
| Spencer *et al.,* 2007 | 11-14 | 88 | 0.69 | 446 | 1.00 | < 0.001 |
| Romero *et al.,* 2008a | 8-13 | 50 | 0.59 | 250 | 1.00 | < 0.001 |
| Huppertz *et al.,* 2008 | 5-10 | 4 | 0.12 | 41 | 1.00 | <0.005 |
| Gonen *et al.,* 2008 | 6-10 | 20 | 0.30 | 1178 | 1.01 | <0.001 |
| Khalil *et al.,* 2009 | 11-14 | 42 | 0.40 | 210 | 1.00 | < 0.001 |

GA= gestational age; MoM=Multiple of the unaffected median

Inhibin A

Inhibin A is a dimeric glycoprotein hormone produced by many tissues but in normal pregnancy the main source of circulating inhibin A is the placenta (Florio *et al.,* 2001; Muttukrishna *et al.,* 1997a; Petraglia, 1997). Several studies have reported that in patients with established PE there is a 1.5- to 8.5-fold increase in the maternal plasma inhibin A concentration (Muttukrishna *et al.,* 1997b; Silver *et al.,* 1999; Gratacos *et al.,* 2000; Zeeman *et al.,* 2002; Florio *et al.,* 2002; Bersinger *et al.,* 2003; Hanisch *et al.,* 2004; Hamar *et al.,* 2006; Paiwattananupant and Phupong, 2008) (Table 1.9). There is also evidence that increased levels of inhibin A precede the clinical onset of PE and may be evident from the first trimester of pregnancy (Cuckle *et al.,* 1998; Raty *et al.,* 1999; Sebire *et al.,* 2000; Grobman and Wang, 2000; D'Anna *et al.,* 2002; Davidson *et al.,* 2003; Florio *et al.,* 2003; Ay *et al.,* 2005; Wald *et al.,* 2006; Spencer *et al.,* 2006; Kim *et al.,* 2006; Zwahlen *et al.,* 2007; Kang *et al.,* 2008; Spencer *et al.,* 2008a) (Table 1.10).

The exact function of inhibin A in pregnancy is uncertain but there is evidence that inhibin A has autocrine and paracrine roles in the placenta thereby affecting trophoblastic function (Muttukrishna *et al.,* 1997b; Petraglia, 1997). The mechanism of increased maternal plasma levels of inhibin A in pregnancies destined to develop PE is uncertain. Immunohistochemical studies have localized inhibin A to the syncytiotrophoblast layer (McCluggage *et al.,* 1998) and it was postulated that the increased plasma levels in PE may be the consequence of reactive hyperplasia of the cytotrophoblast cells leading to increased production or due to functional alterations in syncytiotrophoblast giving rise to increased leakage of placental proteins into the maternal circulation (Aquilina *et al.,* 1999). There is evidence that in established PE the increased plasma levels may be secondary to increased placental m-RNA production (Silver *et al.,* 2002). In contrast, in vitro studies demonstrated that in placental explants low oxygen tension and hypoxia down regulate the expression of the inhibin A gene (Manuelpillai *et al.,* 2003).

**Table 1.10** Studies reporting on the association between maternal serum inhibin A concentration and preeclampsia.

| Author | GA (w) | Preeclampsia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | n | Inhibin A | n | Inhibin A | |
| **During preeclampsia** | | | | | | |
| Muttukrishna *et al.,* 1997b | 25-33 | 20 | 3.05 ng/mL† | 20 | 0.36 ng/mL | <0.001 |
| Silver *et al.,* 1999 | 25-42 | 60 | 2.6 MoM | 60 | 1.00 MoM | <0.0001 |
| Gratacos *et al*., 2000 | 30-37 | 20 | 2.18 ng/mL† | 60 | 0.50 ng/mL | <0.001 |
| Keelan *et al*., 2002 | 30-38 | 22 | 15.1 ng/mL‡ | 22 | 5.3 ng/mL | <0.001 |
| Zeeman *et al.,* 2002 | 34-40 | 77 | 1806.3 pg/ml‡ | 83 | 936.0 pg/ml | <0.001 |
| Florio *et al.,* 2002 | 25-38 | 21 | 2.46 MoM | 42 | 1.00 MoM | <0.001 |
| Bersinger *et al.,* 2003 | 25-39 | 19 | 3080 pg/mL† | 19 | 1510 pg/mL | <0.01 |
| Hanisch *et al.,* 2004 | 29-35 | 21 | 1325.5 pg/ml† | 11 | 346.1 pg/ml | <0.05 |
| Hamar *et al.,* 2006 | 26-38 | 31 | 1863.7 pg/ml† | 16 | 572.6 pg/ml | <0.05 |
| Paiwattananupant *et al.,* 2008 | 33-39 | 30 | 1229.7 pg/ml† | 30 | 839.1 pg/ml | <0.01 |
| **Before preeclampsia** | | | | | | |
| Cuckle *et al.,* 1998 | 13 –18 | 28 | 2.01 MoM | 701 | 1.00 MoM | <0.001 |
| Raty *et al.,* 1999 | 12-20 | 22 | 1.09 U/ml† | 7 | 0.85 U/ml | NS |
| Sebire *et al.,* 2000 | 10-14 | 9 | 233 pg/ml‡ | 759 | 167 pg/ml | <0.05 |
| Grobman *et al.,* 2000 | 14 –28 | 12 | 700.0 pg/mL† | 24 | 613.0 pg/mL | NS |
| D'Anna *et al.,* 2002 | 15-18 | 20 | 1.43 MoM | 40 | 1.03 MoM | NS |
| Davidson *et al.,* 2003 | 15-20 | 39 | 206 pg/ml‡ | 155 | 188 pg/mL | NS |
| Florio *et al.,* 2003 | 24 | 18 | 131.2 pg/ml† | 40 | 91.9 pg/ml | <0.05 |
| Ay *et al.,* 2005 | 16-18 | 14 | 3.36 MoM | 164 | 0.99 MoM | <0.001 |
| Wald *et al.,* 2006 | 15-20 | 96 | 1.39 MoM | 480 | 1.00 MoM | <0.05 |
| Spencer *et al.,* 2006 | 22-24 | 24 | 2.03 MoM | 144 | 1.05 MoM | <0.001 |
| Kim *et al.,* 2006 | 14-23 | 40 | 414 pg/ml‡ | 80 | 280 pg/ml | <0.001 |
| Zwahlen *et al.,* 2007 | 11-13 | 52 | 0.46 mg/ml‡ | 104 | 0.28 mg/ml | <0.05 |
| Kang *et al.,* 2008 | 10-21 | 32 | 1.73 MoM | 3044 | 1.00 MoM | <0.001 |
| Spencer *et al.,* 2008 | 11-14 | 64 | 1.24 MoM | 240 | 1.00 MoM | <0.001 |

† = mean values, ‡ = median values, MoM = multiple of median

Activin A

Similar to inhibin A, activin A is a glycoprotein hormone produced by many tissues but in normal pregnancy the main source is the placenta (Florio et al., 2001; Muttukrishna *et al.,* 1997a; Petraglia, 1997). Several studies have reported that in patients with PE there is a 2- to 9-fold increase in the maternal serum activin A concentration (Petraglia *et al.,* 1995; Muttukrishna *et al.,* 1997b; Silver *et al.,* 1999; D'Antona *et al.,* 2000; Yair *et al.,* 2001; Manuelpillai *et al.,* 2003; Keelan *et al.,* 2002; Florio *et al.,* 2002; Bersinger *et al.,* 2003; Diesch *et al.,* 2006). There is also evidence that increased levels of activin A precede the clinical onset of PE and may be evident from the first-trimester of pregnancy (Grobman and Wang, 2000; Davidson *et al.,* 2003; Florio *et al.,* 2003; Ong *et al.,* 2004; Ay *et al.,* 2005; Madazli *et al.,* 2005; Spencer et al., 2006; Banzola *et al.,* 2007; Spencer *et al.,* 2008a).

The role of activin A in the pathogenesis of PE is uncertain. However, there is evidence that activin A promotes trophoblastic invasion in early pregnancy (Bearfield *et al.,* 2005), and the reported increased maternal serum activin A level in patients with PE may reflect a placental compensatory mechanism to promote trophoblastic invasion in cases where this process is impaired (Muttukrishna *et al.,* 2006). Secretion of activin A by cytotrophoblasts *in vitro* is stimulated by the pro-inflammatory cytokine TNF (Mohan *et al.,* 2001). There is also evidence that in addition to the placenta another source for the increased circulating activin A levels in PE are peripheral mononuclear cells and endothelium activated by pro-inflammatory cytokines such as TNF-α (Tannetta *et al.,* 2003).

Previous studies reporting the maternal serum levels of Activin A in women with established PE reported contradictory results (Table 1.11). Studies examining the potential performance of serum Activin A in screening for PE are limited to second trimester of pregnancy.

**Table 1.11.** Studies reporting on the association between maternal serum activin A concentration and preeclampsia.

| Author | Gestation (w) | Preeclampsia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | n | Activin A | n | Activin A | |
| **During preeclampsia** | | | | | | |
| Petraglia *et al.,* 1997 | 29.0 (25-34) | 16 | 57.4 | 10 | 9.2 | <0.01 |
| Muttukrishna *et al., 1*997b | 29.2 (25-33) | 20 | 38.1 | 20 | 4.0 | <0.001 |
| Silver *et al.,* 1999 | 35.5 (25-42) | 60 | 3.0* | 60 | 1.0* | <0.0001 |
| D'Antona *et al.,* 2000 | 32.0 (26-39) | 16 | 4.6 | 38 | 1.0* | <0.001 |
| Yair *et al.,* 2001 | 36.3 (32-40) | 20 | 445.7 | 20 | 133.1 | <0.0001 |
| Manuelpillai *et al.,* 2001 | 34.0 (29-40) | 23 | 3.5* | 62 | 1.0* | <0.0001 |
| Keelan *et al.,* 2002 | 34.9 (30-38) | 22 | 33.4 | 22 | 8.0 | <0.001 |
| Florio *et al.,* 2002 | 31.0 (25-38) | 21 | 2.1* | 42 | 1.0 * | <0.001 |
| Bersinger *et al.,* 2003 | 31.6 (25-39) | 19 | 32.4 | 19 | 3.8 | <0.001 |
| Diesch *et al.,* 2006 | 35.0 (29 -41) | 34 | 12.1 | 44 | 7.1 | <0.001 |
| **Before preeclampsia** | | | | | | |
| Grobman *et al.,* 2000 | 21.7 (14 –28) | 12 | 11.3 | 24 | 11.1 | NS |
| Davidson *et al.,* 2003 | 16.6 (15-20) | 39 | 1.6 | 155 | 1.4 | NS |
| Florio *et al.,* 2003 | 24 | 18 | 2.7 | 40 | 1.8 | <0.05 |
| Ong *et al.,* 2004 | 12.0 (11-13) | 131 | 1.5* | 494 | 1.0* | <0.001 |
| Ay *et al.,* 2005 | 17.0 (16-18) | 14 | 12.3* | 164 | 1.0* | <0.001 |
| Madazli *et al.,* 2005 | 23.6 (21-26) | 14 | 26.5 | 108 | 8.6 | <0.001 |
| Spencer *et al.,* 2006 | 23.0 (22-24) | 24 | 2.1* | 144 | 1.0* | <0.001 |
| Banzola *et al.,* 2007 | 13.2 (11-15) | 56 | 1.8* | 168 | 1.0* | <0.001 |
| Spencer *et al.,* 2008a | 12.0 (11-13) | 64 | 1.2* | 240 | 1.0* | <0.05 |

All values are in ng/mL unless indicated * = multiple of the median (MoM)

P-selectin

P-selectin is an adhesion molecule found in platelets. In inflammatory and thrombogenic conditions, the molecule is expressed on the cell membrane and initiates interactions between endothelial cells leukocytes and platelets (Stenberg *et al.,* 1985; Larsen *et al.,* 1989; Johnston *et al.,* 1990; Hamburger *et al.,* 1990; Geng *et al.,* 1990). Platelet activation occurs in normal pregnancy (Holmes *et al.,* 2002; Gerbasi *et al.,* 1990), but this is exaggerated in PE which is characterised by platelet aggregation, vasoconstriction and endothelial injury (Nisell *et al.,* 1998; McCarthy *et al.,* 1993; Whigham, 1978; Holthe *et al.,* 2004; Norris *et al.,* 1994). The maternal plasma concentration of P-selectin, a marker for platelet activation, is increased during established PE and there is also evidence that this elevation may be evident from the first-trimester of pregnancy (Halim *et al.,* 1996; Heyl *et al.,* 1999; Aksoy *et al.,* 2002; Chaiworapongsa *et al.,* 2002; Chavarria *et al.,* 2008; Banzola *et al.,* 2007; Bosio *et al.,* 2001) (Table 1.12).

**Table 1.12** Studies reporting on the association between maternal plasma or serum P-Selectin and preeclampsia.

| Author | GA (w) | Preeclampsia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | n | P-selectin | n | P-selectin | |
| **During preeclampsia** | | | | | | |
| Halim *et al.,* 1996 | 33-40 | 30 | 901.3 ng/ml | 10 | 174 ng/ml | <0.001 |
| Heyl *et al.,* 1999 | 26-40 | 38 | 3.26 ng/ml | 20 | 3.99 ng/ml | NS |
| Aksoy *et al.,* 2000 | 28-36 | 28 | 28.2 ng/mL | 65 | 18.9 ng/mL | <0.001 |
| Chaiworapongsa *et al,* 2002 | 23-40 | 55 | 119.5 ng/ml | 100 | 92.6 ng/ml | <0.001 |
| Chavarria *et al.,* 2008 | 32-40 | 75 | 101.0 ng/ml | 125 | 80.0 ng/ml | <0.05 |
| **Before preeclampsia** | | | | | | |
| Banzola *et al.,* 2007 | 11-15 | 56 | 2.49 MoM | 168 | 1.00 MoM | <0.001 |
| Bosio *et al.,* 2001 | 10-14 | 20 | 85.5 ng/ml | 26 | 31.6 ng/ml | <0.001 |
| Chavarria *et al.,* 2008 | 20 | 75 | 91.2 ng/ml | 125 | 82.5 ng/ml | <0.05 |

Pentraxin 3

Innate defence mechanisms against pathogens and damaged tissues consist of a cellular and a humoral arm. Pentraxins (PTX), a superfamily of proteins highly conserved during evolution, are an essential component of the humoral immune system (Garlanda *et al.,* 2005). There are two types of PTXs. The short ones, such as C-reactive proteins, which are produced by the liver and the long ones, such as PTX3, which is expressed by many cells including vascular endothelial cells, monocytes, macrophages and fibroblasts (Garlanda *et al.,* 2005). Plasma levels of PTX3 are increased in shock, sepsis and vascular disorders such as myocardial infarction and small vessel vasculitis (Muller *et al.,* 2001; Peri *et al.,* 2000; Fazzini *et al.,* 2001). A suggested role for PTX3 is binding to dying or apoptotic cells and their constituents, thereby limiting their immunogenicity and reducing the risk of autoimmunity (Rovere *et al.,* 2000; Baruah *et al.,* 2006; Manfredi *et al.,* 2008). Apoptosis is a normal event during pregnancy (Huppertz and Kingdom, 2004; Smith *et al.,* 1997b) and PTX3 may play a role in preventing maternal alloimmunisation against the fetus (Rovere-Querini *et al.,* 2006). Two studies have reported that in patients with PE, there is a six to nine-fold increase in maternal plasma levels of PTX3 (Rovere-Querini *et al.,* 2006; Cetin *et al.,* 2006).

Plasminogen activator inhibitor-2

Plasminogen activators (PA) and plasminogen activator inhibitors (PAIs) are proteins which are crucial for maintaining hemostatic balance by preventing the deposition of excess fibrin. Fibrinolysis is activated by conversion of plasminogen to plasmin which depends on an intricate balance between PA and PAIs. There are two major types of PAIs, the PAI-1 which is primarily synthesized by endothelial cells and PAI-2 which is mainly produced by the trophoblast and found only in the plasma of pregnant women (Asted *et al.,* 1986; Kruithof *et al.,* 1995; Norris 2003; Bremme 2003). Longitudinal studies in pregnant women reported that the maternal plasma concentration of PAI-2

increases with gestational age until term and then declines to undetectable levels six weeks after delivery (Kruithof *et al.,* 1987; Halligan *et al.,* 1994; Coolman *et al.,* 2006).

There is evidence that established PE is associated with activation of platelets and coagulation cascade with up-regulation of intravascular coagulation (O'Riordan and Higgins 2003). This increase in fibrin and thrombus deposition is accompanied by reduced levels of PAI-2 in maternal blood (Table 1.13). The possible explanation for these reduced levels in maternal circulation is due to decreased production by a hypoxic placenta. There is some suggestion that in addition to its role in the coagulation cascade, PAI-2 is involved in trophoblastic invasion and therefore may play a role in the pathogenesis of PE (Asted *et al.,* 1998; Clausen *et al.,* 2002).

**Table 1.13.** Studies reporting on the association between maternal plasma plasminogen activator inhibitor-2 (PAI-2) concentration and preeclampsia.

| Author | Gestation (wks) | Preeclampsia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | n | PAI-2 (ng/mL) | n | PAI-2 (ng/mL) | |
| **During preeclampsia** | | | | | | |
| Estelles *et al.,*., 1989 | 28-40 | 13 | 186.3 | 24 | 269.3 | <0.05 |
| Reith *et al.,* 1993 | 29-39 | 11 | 105.3 | 11 | 187.1 | <0.001 |
| Koh *et al.,* 1993 | 25-38 | 14 | 58.4 | 14 | 105.2 | <0.001 |
| Halligan *et al.,* 1994 | 33-40 | 12 | 48.5 | 32 | 183.5 | <0.001 |
| Lindoff *et al.,* 1994 | 34-40 | 36 | 172.1 | 40 | 212.0 | NS |
| He *et al.,* 1995 | 30-35 | 19 | 74.0* | 22 | 156.0* | <0.05 |
| Nakashima *et al.,* 1996 | 28-40 | 18 | 96.7 | 22 | 169.7 | <0.001 |
| Schjetlein *et al.,* 1999 | 24-42 | 200 | 120.0* | 97 | 158.0* | <0.0005 |
| Roes *et al.,* 2002 | 30-33 | 42 | 51.0* | 18 | 80.0* | <0.01 |
| Tanjung *et al.,* 2005 | 31-40 | 40 | 146.8 | 27 | 219.5 | <0.01 |
| Sartori *et al.,* 2008 | 31-36 | 35 | 36.9 | 68 | 62.8 | <0.01 |
| **Before preeclampsia** | | | | | | |
| Halligan *et al.,* 1994 | 9-16 | 4 | 13.3 | 32 | 20.5 | NS |
| Clausen *et al.,* 2002 | 17-19 | 71 | 78.8* | 71 | 67.6* | 0.002 |

The values of PAI-2 are mean except those indicated by * which are medians

Angiopoeitin-2

Angiopoietins are angiogenic proteins produced by the villous trophoblast and they are thought to play an important role in placental vascular development (Charnock-Jones *et al.,* 2004; Seval *et al.,* 2008). There are three stages in the vascular development of the placenta; firstly, vasculogenesis which refers to formation of a primitive vascular network from endothelial progenitor cells, secondly, branching angiogenesis and thirdly, non-branching angiogenesis both of which involve the formation of new blood vessels from already existing ones (Geva *et al.,* 2002; Yancopoulos *et al.,* 2000; Carmeliet *et al.,* 2000). The angiopoietins act on the second and third stages: angiopoietin-1 (Ang-1) causes endothelial maturation and vascular stabilization, whereas angiopoietin-2 (Ang-2) acts as an antagonist of Ang-1 thereby leading to further angiogenesis (Zhang *et al.,* 2001; Suri *et al.,* 1996; Maisonpierre *et al.,* 1997).

There is evidence from studies that in women with PE the serum levels of Ang-2 are significantly lower than in controls (Hirokoshi *et al.,* 2005; Nadar *et al.,* 2005) but it is uncertain whether the reduced levels of this protein in PE is a consequence of the disease or whether the decrease precedes the clinical onset of PE and is a reflection of the involvement of this growth factor in the pathogenesis of the disease.

Vascular endothelial growth factor and soluble fms-like tyrosine kinase-1

VEGF is a pro-angiogenic protein released by many cell types including the cytotrophoblast and it is involved in promoting angiogenesis and vasculogenesis. The VEGF protein is transcribed from the VEGF gene which is located on chromosome 6, which also encodes for various isoforms of VEGF including placental growth factor (PlGF) (Romero *et al.,* 2008b; Cheng *et al.,* 2013). These angiogenic factors exert their role in endothelial cells by mediating increased vascular permeability, which includes angiogenesis, vasculogenesis and growth of endothelial cells (Yamazaki *et al.,* 2006; Maharaj *et al.,* 2008). The soluble form of VEGF receptor-1 (VEGFR-1) (sFlt-1) is

primarily produced by the syncytiotrophoblast and is produced by alternative splicing of the Flt-1 gene which results in a truncated protein which cannot bind to PlGF or VEGF inside the cells but attaches to the transmembrane receptors thus acting as an antagonist to of PlGF and VEGF and preventing these angiogenic factors from interacting with their receptors (Maynard *et al* 2003; Levine *et al.*, 2004; Romero *et al.*, 2008b; Tache *et al.*, 2011). The sFlt-1 exerts anti-angiogenic effects by inhibiting biological activity of VEGF and PlGF (Kendall and Thomas, 1993). VEGF is important for maintaining endothelial function in fenestrated endothelium especially and in brain, liver and renal glomeruli (Esser *et al.*, 1998). The higher levels of sFlt-1 also counteract vasodilatory effects of nitric oxide induced by VEGF thereby leading to hypertension (Maynard *et al.*, 2003). In addition, sFlt-1 can induce proteinuria by blocking effects of VEGF (Eremina *et al.*, 2003). In pregnancies with PE the maternal plasma or serum concentration of free-VEGF and PlGF is decreased whereas the concentration of sFlt-1 is increased (Tables 1.14 and 1.15).

**Table 1.14.** Studies reporting the median maternal circulating free vascular endothelial growth factor (VEGF) concentration (pg/mL) in patients during or before preeclampsia compared to controls.

| Author | Gestation (wk) | Preeclampsia | | Control | | P value |
|---|---|---|---|---|---|---|
| | | n | VEGF | n | VEGF | |
| **During preeclampsia** | | | | | | |
| Lyall *et al.*, 1997 | 26-40 | 34 | 2.3 | 34 | 12.9 | <0.001 |
| Reuvekamp *et al.*, 1999 | 28-40 | 30 | 0.3* | 30 | 18.3* | <0.001 |
| Livingston *et al.*, 2000 | 27-40 | 21 | 6.4* | 21 | 18.7* | <0.001 |
| Maynard *et al.*, 2003 | 29-40 | 21 | 4.1 | 11 | 14.0 | <0.05 |
| Levine *et al.*, 2004 | 37-41 | 21 | 6.7 | 26 | 9.9 | <0.05 |
| Muy-Rivera *et al.*, 2005 | 35-39 | 131 | 10.9 | 175 | 13.6 | NS |
| Buhimschi *et al.*, 2006 | 23-40 | 42 | 0.1 | 13 | 1.6 | <0.001 |
| Lee *et al.*, 2007 | 29-40 | 20 | 21.3 | 20 | 134.0 | <0.001 |
| **Before preeclampsia** | | | | | | |
| Polliotti *et al.*, 2003 | 14-21 | 20 | 2.6 | 60 | 6.0 | <0.001 |
| Levine *et al.*, 2004 | 21-32 | 6 | 5.1 | 102 | 12.8 | <0.05 |

Values in * indicate mean values

**Table 1.15** Studies reporting the median maternal plasma soluble fms-like tyrosine kinase-1 (sFlt-1) concentration (pg/mL) in patients during or before preeclampsia compared to controls.

| Author | Gestation (wk) | Preeclampsia | | Control | | P value |
|---|---|---|---|---|---|---|
| | | n | sFlt-1 | n | sFlt-1 | |
| **During preeclampsia** | | | | | | |
| Tsatsaris *et al.*, 2003 | 30-38 | 19 | 2690 | 31 | 120 | <0.001 |
| Levine *et al.*, 2004 | 29-41 | 23 | 4382 | 23 | 1643 | <0.001 |
| Staff *et al.*, 2005 | 24-40 | 32 | 9932 | 38 | 3417 | <0.001 |
| Shibata *et al.*, 2005 | 28-40 | 22 | 5221 | 24 | 1857 | <0.001 |
| Buhimschi *et al.*, 2006 | 23-40 | 42 | 2026 | 13 | 434 | <0.001 |
| Masuyama *et al.*, 2007 | 33-40 | 30 | 5666* | 30 | 1204* | <0.01 |
| Stepan *et al.*, 2007a | 20-37 | 18 | 8388 | 15 | 2602 | <0.01 |
| Salahuddin *et al.*, 2007 | 28-40 | 19 | 74700* | 20 | 16600* | <0.01 |
| Lee *et al.*, 2007 | 29-40 | 20 | 1935* | 20 | 298* | <0.001 |
| De Vivo *et al.*, 2008 | 31-40 | 52 | 44870 | 52 | 12560 | <0.001 |
| Woolcock *et al.*, 2008 | 25-41 | 18 | 3130 | 18 | 470 | <0.001 |
| Kim *et al.*, 2009 | 23-40 | 62 | 2755* | 62 | 554* | <0.001 |
| Reddy *et al.*, 2009 | 37-41 | 10 | 10100 | 10 | 4900 | <0.05 |
| **Before preeclampsia** | | | | | | |
| Levine *et al.*, 2004 | 8-12 | 21 | | 20 | | NS |
| Thadani *et al.*, 2004 | 7-12 | 40 | 1048 | 80 | 973 | NS |
| Park *et al.*, 2005 | 16-23 | 32 | 730 | 128 | 441 | <0.05 |
| Kim *et al.*, 2007 | 14-23 | 46 | 3861 | 100 | 2353 | <0.001 |
| Rana *et al.*, 2007 | 11-13 | 39 | 3500* | 147 | 3000* | NS |
| Vatten *et al.*, 2007 | 4-12 | 154 | 135* | 392 | 166* | <0.01 |
| Erez *et al.*, 2008 | 6-15 | 56 | 1405 | 201 | 1788 | <0.05 |
| Baumann *et al.*, 2008 | 11-13 | 46 | 1764* | 92 | 1537* | <0.05 |
| Lim *et al.*, 2008 | 14-21 | 40 | 4945* | 100 | 2788* | <0.001 |

Values in * indicate mean values

There are studies reporting that maternal serum concentrations of sFlt-1 is altered in PE and that this precedes the clinical onset of the disease (Table 1.14). However, there is contradictory evidence concerning first-trimester maternal circulating levels of sFlt-1 in pregnancies that subsequently develop PE with some studies reporting an increase (Baumann *et al.,* 2008) and others a decrease (Erez *et al.,* 2008; Vatten *et al.,* 2007) or no difference (Levine *et al.,* 2004; Rana *et al.,* 2007; Thadani *et al.,* 2004) from normal. The maternal circulating levels of free-VEGF are also decreased prior to the clinical onset of PE (Levine *et al.,* 2004; Polliotti *et al.,* 2003) but there are no reports concerning the levels in the first-trimester of pregnancy.

Soluble endoglin

Another anti-angiogenic factor implicated in the pathogenesis of PE is soluble endoglin (sEng), a surface receptor of transforming growth factor-β1 (TGF- β1), which induces growth and proliferation of endothelial cells (Venkatesha *et al.,* 2006). The sEng protein has anti-angiogenic properties since it prevents binding of TGF- β1 to its receptors on endothelial cells this compromising its function and leading to impairment in production of nitric oxide (Levine *et al.,* 2006). There is evidence from animal studies that increased expression of sEng in mice leads to increased vascular permeability and hypertension. In combination with sFlt-1, the increased levels of sEng lead to severe vascular damage, nephrotic syndrome, proteinuria and growth restriction (Venkatesha *et al.,* 2006).

Several studies have reported that in women with established clinical disease, the concentration of sEng in maternal blood is increased (Table 1.16). However, there is contradictory evidence whether this increase precedes the clinical onset of disease or is merely a manifestation of disease rather than a cause and whether these alterations are detectable from first trimester of pregnancy (Rana *et al.,* 2007; Erez *et al.,* 2008; Bauman *et al.,* 2008; Lim *et al.,* 2009).

**Table 1.16**. Studies reporting on the association between maternal plasma soluble endoglin (sEng) concentration and preeclampsia.

| Author | Gestation (wk) | Preeclampsia | | Controls | | P value |
|---|---|---|---|---|---|---|
| | | n | sEng | n | sEng | |
| **During preeclampsia** | | | | | | |
| Stepan *et al.,* 2007 | 20-37 | 18 | 57.1 | 15 | 5.3 | <0.001 |
| Masuyama *et al.,* 2007[1] | 33-40 | 30 | 60.9 | 30 | 11.2 | <0.01 |
| Jeyabalan *et al.,* 2008 | 34-39 | 10 | 39.1 | 10 | 17.5 | 0.001 |
| De Vivo *et al.,* 2008 | 31-40 | 52 | 28.2* | 52 | 14.8* | <0.01 |
| Kim *et al.,* 2009 | 28-40 | 62 | 74.5 | 62 | 17.2 | <0.0001 |
| Reddy *et al.,* 2009 | 35-41 | 10 | 188.0* | 10 | 52.2* | <0.0001 |
| **Before preeclampsia** | | | | | | |
| Rana *et al.,* 2007 | 17-20 | 39 | 6.4 | 147 | 5.2 | <0.01 |
| Rana *et al.,* 2007 | 11-14 | 39 | 6.9 | 147 | 6.6 | NS |
| De Vivo *et al.,* 2008 | 24-28 | 52 | 14.6* | 52 | 9.1* | <0.001 |
| Erez *et al.,* 2008 | 21-24 | 56 | 7.1* | 201 | 5.9* | <0.05 |
| Erez *et al.,* 2008 | 9-14 | 56 | 7.7* | 201 | 7.2* | NS |
| Baumann *et al.,* 2008 | 11-14 | 46 | 5.5 | 92 | 4.8 | <0.01 |
| Lim *et al.,* 2009 | 14-19 | 60 | 10.5 | 124 | 3.9 | <0.001 |

All values are in mean unless indicated by * = median

## 1.5 PREVENTION OF PREECLAMPSIA

The term ‘primary prevention’ describes preventing the occurrence of a disease. The identification and manipulation of recognised risk factors might allow primary prevention. PE is a multi-system disorder of complex aetiology. There are several agents that have been used in an effort to prevent the disease and reduce its incidence such as dietary supplementation and pharmacological treatment aimed at interfering with the mechanisms that cause vasoconstriction, loss of vascular pressor refractoriness, platelet and endothelial activation and reduction of perfusion in PE.

### 1.5.1 Calcium

The hypothesis linking the use of calcium for prevention of PE comes from observations and studies reporting that women who had a high calcium intake in their diet had a lower incidence of PE (Hamlin 1962; Belizan *et al.*, 1988; Villar *et al.*, 1993). In the prevention of PE this is the most extensively researched dietary supplement. Low calcium intake may cause high BP by stimulating either parathyroid hormone or renin release, thereby increasing intracellular calcium in vascular smooth muscle and leading to vasoconstriction, hence high BP (Belizan *et al.*, 1988). Calcium supplementation may reduce parathyroid hormone release and intracellular calcium thus reducing smooth muscle contractility. There is some evidence suggesting that calcium supplementation affects utero-placental blood flow by lowering the resistance in uterine and umbilical vasculature (Caroli *et al.*, 2010).

A recent systematic review of randomised controlled trials comparing calcium administration vs placebo in preventing the occurrence of PE examined data from 14 studies including 15,730 women and reported that calcium supplementation of > 1g/day was associated with a reduction in risk of PE (RR 0.45, 95% CI 0.31 to 0.65), particularly for women with low-calcium diets (RR 0.36, 95% CI 0.20 to 0.65). However, the authors commented that these data should be interpreted with caution as there was a possibility of small-study effect or publication bias. This was also evident from the significant amount of heterogeneity between studies with $I^2$ values of between 70-80%. This limited evidence needs to be confirmed in larger, good quality trials before the treatment can be routinely advised (Hofmeyr *et al.*, 2014).

### 1.5.2 Folate

There is evidence from both animal and human studies which support a role for folic acid in prevention of PE (Wen *et al.*, 2008a and 2008b). The proposed mechanisms to explain the beneficial effect of folic acid in PE include a direct role in placental growth and development, through the effect of folic acid on lowering blood homocysteine

levels and lastly through the effect of folic acid in improving endothelial function. Hyperhomocysteinemia is a risk factor for PE and it is hypothesized that folic acid may have a role in prevention by reducing the levels of homocysteine (Lindblad *et al.*, 2005; Guven *et al.*, 2009). In addition, increased levels of homocysteine are suggested to lead to endothelial activation and folic acid may mitigate this endothelial dysfunction thereby contributing to preventing the development of PE (Powers *et al.*, 1998).

There is data from 4 retrospective cohort studies which examined the effect of folic acid in prevention of PE which suggest that regular use of folic acid supplementation reduces the risk of PE (Hernandez-Diaz *et al.*, 2002; Bodnar *et al.*, 2006; Wen *et al.*, 2008a; Catov *et al.*, 2009). There are two other studies which failed to find a protective effect of folic acid supplementation (Timmermans *et al.*, 2011; Li *et al.*, 2013). Based on these data, a systematic review reported that the OR for reduction in risk of PE as being 0.14 (95% CI = 0.06–0.31), showing a strong protective effect of folic acid supplementation (Wen *et al.*, 2013). An RCT is being carried out to examine the effectiveness of folic acid in a study entitled, The Effect of Folic Acid Supplementation in Pregnancy on Preeclampsia: the Folic Acid Clinical Trial (FACT) aims to recruit 3,656 high risk women to evaluate a new prevention strategy for PE: supplementation of folic acid throughout pregnancy. Pregnant women with increased risk of developing PE presenting to a trial participating center between 8 and 16 weeks of gestation will be randomized in a 1:1 ratio to folic acid 4.0 mg or placebo (Wen *et al.*, 2013).

### 1.5.3 Anti-oxidants

There is evidence linking oxidative stress in the pathophysiology of PE and therefore, there have been studies which have examined whether administration of anti-oxidants, which reduce the production of ROS and thereby oxidative stress, can be useful in prevention of PE (Vaughan and Walsh, 2002). Anti-oxidants are important in maintaining cellular integrity in normal pregnancy by inhibiting peroxidation reactions and thus protecting enzymes, proteins and cells from destruction by peroxides

(Rumbold *et al.,* 2006). The studies that have investigated the role of anti-oxidants in the prevention of PE have mostly involved the use of vitamins C and E. Vitamin C (ascorbic acid) scavenges free radicals in the aqueous phase, and the lipid soluble vitamin E (α-tocopherol) acts in vivo to prevent the formation of lipid peroxides and thus, protect cell membranes (Rumbold et al., 2006). The use of vitamin supplements may reduce oxidative stress by preventing endothelial damage and the subsequent development of PE. In a recent meta-analysis of seven studies including 5,969 women, the authors concluded that concomitant supplementation with vitamins C and E does not prevent PE in women at risk (pooled RR: 0.79; 95% CI 0.58-1.08), but does increase the rate of babies born with a low birth weight (pooled RR: 1.13; 95% CI of 1.00-1.27) (Rahmi *et al.,* 2009).

There are some reports suggesting that the use of magnesium supplements in pregnancy can potentially reduce the incidence of PE. (Conradt *et al.,* 1984). However, a Cochrane review of two randomised trials (474 patients) showed no apparent effect of magnesium treatment on the risk of PE or GH (Makrides and Crowther, 2001). Another anti-oxidant, which has been investigated in regard to prevention of PE is Zinc, which is an essential trace element required in many metabolic pathways. There appears to be a decrease in maternal and umbilical plasma zinc concentrations in PE when compared to normal pregnancy (Bassiouni *et al.,* 1979). It has been suggested that this is due to zinc and oestrogen competing for common binding sites on plasma proteins (Adeniyi, 1987). Bassiouni *et al.* (1979) suggested that low plasma zinc concentrations in PE may be a sign of zinc deficiency and recommended maintenance of adequate dietary zinc nutrition during pregnancy. However, two randomised controlled trials involving 1,288 patients failed to show any significant benefit (Mahomed *et al.,* 1989; Jonsson *et al.,* 1996).

### 1.5.4 Heparin

There has been interest in the use of low molecular weight heparin (LMWH) alone or in combination with an anti-platelet drug for prevention of hypertensive disorders in

pregnancy especially in women with known thrombophilic tendencies or a previous history of PE, but trials had been too small for reliable conclusions (Duley *et al.,* 2006a; 2006b; Walker *et al.,* 2006). There is however accumulating evidence, albeit based on smaller studies, for a role for LMWH as a potential pharmacological therapy for preventing PE. Two systematic reviews summarizing the published literature concluded that heparin significantly reduces the recurrence of PE and was associated with reductions in perinatal mortality, preterm birth, and infant birth weight <10th percentile in high-risk women (Dodd *et al.,* 2013; Rodger *et al.,* 2014). There is limited evidence from a meta-analysis suggesting that use of LMWH in addition to aspirin in women with a history of PE compared to aspirin alone was associated with a significant reduction in PE (RR 0.54, 95% CI 0.31 to 0.92) (Roberge *et al.,* 2015).

Although, the exact mechanism underlying the potential benefit of using LMWH in preventing PE is not known but it may be attributable to an anti-coagulant action of heparin within the placenta although none of the studies examining the role of LMWH included placental examination as part of the study methods or protocols. An alternative explanation is that LMWH exerts direct vascular actions in the maternal compartment to reverse the placenta-mediated systemic vascular dysfunction characteristic of PE (McLaughlin *et al.,* 2015). LMWH also significantly lowered systolic and diastolic blood pressure and reduced resistance indices in the uterine arteries, suggesting that LMWH can potentially 'normalize' maternal blood pressure and uteroplacental blood flow (Mello *et al.,* 2005). There is some evidence for a potential role of LMWH in prevent development of severe PE in high-risk women but large high quality RCTs are necessary to substantiate the results of smaller studies and to define the extent of any possible benefit in reducing the prevalence of PE.

### 1.5.5 Aspirin

The majority of interest and evidence in prevention of PE is based on use of Aspirin (acetylsalicylic acid). There have been studies reporting the potential for Aspririn in prevention of PE since the 80s. The basis for recommending low dose aspirin in the

prevention of PE is that Aspirin through its actions on prostaglandin pathways modifies the imbalance between thromboxane A2 and prostacyclin, which is altered in PE and contributes to the vasospasm and coagulation abnormalities. Wallenburg *et al.*, (1991) proposed that in normal pregnancy the levels of prostacyclin increase, therefore causing a reduced maternal response to angiotensin II and other vasopressors and by extension a reduction in systemic vascular resistance. In PE, eicosanoid synthesis is greatly altered with relatively low prostacyclin levels, high thromboxane A2 levels and a low prostacyclin to thromboxane A2 ratio in the maternal and fetal circulation. This imbalance causes the pressor effects of angiotensin II and catecholamines to be ineffectively opposed, resulting in the clinical syndrome of PE (Chavarria *et al.*, 2003). Low dose aspirin treatment in pregnancy inhibits biosynthesis of platelet thromboxane A2 with little effect on vascular prostacyclin production, thus altering the balance of the prostacyclin and preventing development of PE (Sibai *et al.*, 2005).

The enzyme cyclo-oxygenase plays a central role in the production of both prostacyclin and thromboxane A2. Aspirin inhibits endothelial cyclo-oxygenase (Dekker and Sibai, 2001) and this process is irreversible in platelets, where the enzyme is inhibited for their entire life-span. In contrast, the enzyme is re-synthesised in endothelial cells and prostacyclin production is re-established relatively rapidly. This selective inhibition of cyclo-oxygenase and the resulting alteration in the prostacyclin to thromboxane A2 ratio in the placenta forms the basis of using aspirin to prevent or delay the onset of PE.

There are early reports suggesting that low dose aspirin in high-risk women does reduce the prevalence of fetal growth restriction and PE (Beaufils *et al.*, 1985; Wallenburg *et al.*, 1986). However, a series of subsequent large randomised studies showed no effect in preventing these complications (Italian Study of Aspirin in Pregnancy, 1993; CLASP Collaborative Group, 1994). A meta-analysis by Duley *et al.*, (2001) assessed the effectiveness and safety of anti-platelet drugs (mainly aspirin) for the prevention of PE. This study, which examined more than 30,000 women from 39 trials, concluded that the use of an anti-platelet agent was associated with a 15%

reduction in the risk of PE (32 trials; 29,331 women; pooled RR 0.85) with an 8% reduction in the risk of preterm birth before 37 weeks and a 14% reduction in the risk of fetal or neonatal for women allocated to low dose aspirin. The PARIS (Perinatal Antiplatelet Review of International Studies) collaborative group (2005) meta-analysed individual patient data from 32,217 women from 31 randomised trials of anti-platelets for the prevention of PE. The relative risk of developing PE, of delivering before 34 weeks and of having a pregnancy with a serious adverse outcome was 0.90. There was no significant effect on the risk of death of the fetus or baby, having a small for gestational age infant or bleeding events for either the women or their babies. Thus the findings were similar to those of Duley et al. (2004) in that there was a moderate but consistent reduction in the relative risk of PE (Askie *et al.,* 2007).

A number of randomised studies have examined the value of prophylactic aspirin in women with increased impedance to flow in the uterine arteries. A meta-analysis examining nine randomised controlled trials with a total of 1,317 women, assessed the influence of gestational age at the time of administration of low dose aspirin on the incidence of PE in women at increased risk, on the basis of abnormal uterine artery Doppler and demonstrated that aspirin treatment commenced in early gestation was associated with a greater reduction in the incidence of PE than treatment started in late gestation: < 16 weeks of gestation resulted in a RR of 0.48 (95% CI 0.33-0.68), at 17-19 weeks RR 0.55 (95% CI 0.17-1.76), and at > 20 weeks RR 0.82 (95% CI 0.62-1.09) (Bujold *et al.,* 2009).

The authors further examined specifically the effect of early administration of aspirin before 16 weeks and reported that Aspirin was particularly effective in preventing early disease requiring delivery before term, rather than at term (RR 0.11, 95% CI 0.04–0.33 vs RR 0.98, 95% CI 0.42–2.33) and in severe, rather than mild PE (RR 0.22, 95% CI 0.08–0.57 vs RR 0.81, 95% CI 0.33–1.96) (Roberge *et al.,* 2012a and 2012b). They also suggested that not only PE but there was a 50% reduction in risk of FGR and 60% reduction in risk of perinatal death if treatment was commenced before 16 weeks rather than thereafter (Roberge *et al.,* 2013; Bujold *et al.,* 2014).

## 1.6 OBJECTIVES OF THE THESIS

- To examine the maternal serum or plasma levels of a series of biochemical markers thought to be involved in placentation or in the cascade of events leading from impaired placentation to the development of the clinical symptoms of the PE at 11-13 weeks' gestation and determine which markers are significantly altered in women who subsequently develop PE

- To examine the performance of screening for early, intermediate and late-PE by a combination of the *a priori risk*, derived from maternal factors, in combination with various biochemical markers and biophysical markers including uterine artery Doppler and MAP.

- To investigate the performance of screening for PE based on a survival time model which treats gestation at delivery as a continuous variable and using Bayes theorem to combine information from maternal characteristics and biomarker MoM values.

# CHAPTER 2

## PATIENTS AND METHODS

## 2.1 STUDY POPULATION

This was a prospective screening study for hypertensive disorders in women attending their routine first hospital visit in pregnancy at King's College Hospital, Medway Maritime Hospital and University College Hospital between March 2006 and September 2010. In this visit, which is held at $11^{+0}$-$13^{+6}$ weeks' gestation, all women have an ultrasound scan to confirm gestational age from the measurement of the fetal CRL, to diagnose any major fetal abnormalities and to measure fetal nuchal translucency (NT) thickness and maternal serum free ß-hCG and PAPP-A as part of screening for chromosomal abnormalities (Snijders *et al.*, 1998; Kagan *et al.*, 2008b).

All women attending for this visit were invited to participate in the research study entitled 'Early Prediction of Pregnancy Complications', which was approved by the Ethics Committee (REC reference number: 02-03-033, R&D reference number: 03WH06) (Section 2.2). We obtained a written informed consent from women agreeing to participate in the study and in these women we recorded maternal characteristics and medical history, measured the uterine artery pulsatility index (PI) by transabdominal colour Doppler and mean arterial pressure (MAP) by automated devices and stored plasma and serum at -80°C for subsequent biochemical analysis.

The inclusion criteria for this study were singleton pregnancies delivering a phenotypically normal livebirth or stillbirth at or after 24 weeks of gestation. We excluded pregnancies with major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks.

The studies presented in the following chapters are based on case-control studies investigating the biochemical markers for early prediction of PE (Chapter 3) and studies based on data collected as a part of the prospective screening study for hypertensive disorders (Chapter 4 and 5).

### 2.1.1 Case-control studies

The case-control studies involved measurement of maternal serum or plasma concentration of PLGF, Activin-A, Inhibin-A, PP13, P-selectin, Pentraxin-3, sEng, Plasminogen activator inhibitor-2, Angiopoietin-2 and soluble fms-like tyrosine kinase-1 at 11-13 weeks' gestation in pregnancies complicated by PE and unaffected controls. These were nested case-control studies drawn from the larger prospective screening study on the basis of availability of stored samples from pregnancies that developed early and late-PE. Each case was matched with controls that had blood collected and stored on the same day and delivered a phenotypically normal neonate appropriate for gestational age at term and did not develop any pregnancy complications. None of the samples were previously thawed and refrozen.

### 2.1.2 Screening studies

Prediction of preeclampsia from maternal factors, biophysical and biochemical markers

In the first screening study, described in Chapter 4, we prospectively screened 36,743 singleton pregnancies. We excluded 3,141 cases because they had missing outcome data (n=2,005) or the pregnancies resulted in miscarriage, termination or the birth of babies with major defects (n=1,136). In the remaining 33,602 cases there were 752 (2.2%) that developed PE and 32,850 that were unaffected by PE.

The measurements of maternal serum PAPP-A were available in all cases. Uterine artery PI was available in 21,673 of the 32,850 pregnancies, including 583 (2.7%) that developed PE and MAP was available in 13,946 of the 32,850 pregnancies, including 431 (3.0%) that developed PE. The maternal characteristics and history in each PE group and the unaffected pregnancies in the screening population and in the subgroups with measurements of uterine artery PI and MAP are compared in Table 2.1 to 2.3.

**Table 2.1.** Maternal and pregnancy characteristics in the total screening population.

| Characteristic | Unaffected pregnancies (n=32,850) | Early preeclampsia (n=112) | Intermediate preeclampsia (n=187) | Late preeclampsia (n=453) |
|---|---|---|---|---|
| Maternal age in years, median (IQR) | 32.3 (27.9-36.0) | 31.3 (25.7-36.2) | 32.1 (28.1-36.8) | 31.3 (27.2-36.3) |
| Maternal weight in kg, median (IQR) | 65.0 (59.0-75.0) | 72.0 (63.0-85.0)* | 70.0 (61.0-85.0)* | 72.0 (63.3-85.0)* |
| Fetal Crown-Rump Length in mm, median (IQR) | 64.0 (59.2-69.5) | 64.9 (58.1-72.1) | 62.5 (57.4-68.2) | 63.1 (58.9-69.4) |
| Racial origin | | | | |
| Caucasian, n (%) | 23,765 (72.3) | 47 (42.0) | 90 (48.1) | 247 (54.5) |
| African, n (%) | 6,051 (18.4) | 53 (47.3)* | 75 (40.1)* | 165 (36.4)* |
| South Asian, n (%) | 1,430 (4.4) | 8 (7.1) | 14 (7.5) | 20 (4.4) |
| East Asian, n (%) | 651 (2.0) | 0 | 2 (1.1) | 10 (2.2) |
| Mixed, n (%) | 953 (2.9) | 4 (3.6) | 6 (3.2) | 11 (2.4) |
| Parity | | | | |
| Nulliparous, n (%) | 15,698 (47.8) | 65 (58.0) | 104 (55.6) | 291 (64.2)* |
| Parous with no previous preeclampsia, n (%) | 16,196 (49.3) | 27 (24.1)* | 55 (29.4)* | 105 (23.2)* |
| Parous with previous preeclampsia, n (%) | 956 (2.9) | 20 (17.9)* | 28 (15.0)* | 57 (12.6)* |
| Cigarette smoker, n (%) | 2,695 (8.2) | 2 (1.8) | 12 (6.4) | 29 (6.4) |
| Family history of preeclampsia, (n, %) | 1,480 (4.5) | 13 (11.6)* | 17 (9.1)* | 34 (7.5)* |
| Conception | | | | |
| Spontaneous, n (%) | 31,618 (96.2) | 104 (92.9) | 174 (93.0) | 435 (96.0) |
| Assisted, n (%) | 1,232 (3.8) | 8 (7.1) | 13 (7.0) | 18 (4.0) |
| History of chronic hypertension, n (%) | 322 (1.0) | 15 (13.4)* | 18 (9.6)* | 29 (6.4)* |
| History of pre-existing diabetes, n (%) | 245 (0.7) | 3 (2.7) | 8 (4.3)* | 2 (0.4) |

Comparison between each outcome group and unaffected controls ($\chi^2$ - test and Fisher's exact test for categorical variables and Mann-Whitney test with *post hoc* Bonferroni correction for continuous variables): * Critical significance level $p < 0.0167$.

**Table 2.2.** Maternal and pregnancy characteristics in the case-control study for uterine artery pulsatility index (PI).

| Characteristic | Unaffected pregnancies (n=21,090) | Early preeclampsia (n=86) | Intermediate preeclampsia (n=143) | Late preeclampsia (n=354) |
|---|---|---|---|---|
| Maternal age in years, median (IQR) | 32.1 (27.7 (35.9) | 31.4 (25.5-36.4) | 32.0 (28.0-36.8) | 31.6 (27.3-36.4) |
| Maternal weight in kg, median (IQR) | 65.0 (59.0-75.0) | 73.5 (63.0-85.0)* | 70.0 (61.0-86.0)* | 73.0 (64.0-86.0)* |
| Fetal Crown-Rump Length in mm, median (IQR) | 63.9 (58.9-69.3) | 65.0 (57.9-71.4) | 62.7 (57.0-69.0) | 63.4 (58.9-69.9) |
| Racial origin | | | | |
| Caucasian, n (%) | 13,889 (65.9) | 31 (36.0) | 57 (39.9) | 166 (46.9) |
| African, n (%) | 5,164 (24.5) | 43 (50.0)* | 66 (46.2)* | 157 (44.4)* |
| South Asian, n (%) | 929 (4.4) | 8 (9.3) | 12 (8.4) | 14 (4.0) |
| East Asian, n (%) | 417 (2.0) | 0 | 2 (1.4) | 7 (2.0) |
| Mixed, n (%) | 691 (3.3) | 4 (4.7) | 6 (4.2) | 10 (2.8) |
| Parity | | | | |
| Nulliparous, n (%) | 10,221 (48.5) | 46 (53.5) | 76 (53.1) | 220 (62.1)* |
| Parous with no previous preeclampsia, n (%) | 10,276 (48.7) | 21 (24.4)* | 46 (32.2)* | 83 (23.4)* |
| Parous with previous preeclampsia, n (%) | 593 (2.8) | 19 (22.1)* | 21 (14.7)* | 51 (14.4)* |
| Cigarette smoker, n (%) | 1,696 (8.0) | 1 (1.2) | 11 (7.7) | 21 (5.9) |
| Family history of preeclampsia, (n, %) | 886 (4.2) | 12 (14.0)* | 14 (9.8)* | 25 (7.1)* |
| Conception | | | | |
| Spontaneous, n (%) | 20,235 (95.9) | 80 (93.0) | 132 (92.3) | 338 (95.5) |
| Assisted, n (%) | 855 (4.1) | 6 (7.0) | 11 (7.7) | 16 (4.5) |
| History of chronic hypertension, n (%) | 229 (1.1) | 14 (16.3)* | 13 (9.1)* | 26 (7.3)* |
| History of pre-existing diabetes, n (%) | 158 (0.7) | 3 (3.5) | 6 (4.2)* | 2 (0.6) |

Comparison between each outcome group and unaffected controls ($\chi^2$ - test and Fisher's exact test for categorical variables and Mann-Whitney test with *post hoc* Bonferroni correction for continuous variables): * Critical significance level $p < 0.0167$.

**Table 2.3.** Maternal and pregnancy characteristics in the case-control study for mean arterial pressure.

| Characteristic | Unaffected pregnancies (n=13,515) | Early preeclampsia (n=69) | Intermediate preeclampsia (n=111) | Late preeclampsia (n=251) |
|---|---|---|---|---|
| Maternal age in years, median (IQR) | 32.1 (27.7-35.7) | 30.1 (25.4-36.3) | 32.4 (28.8-37.0) | 31.7 (27.3-36.6) |
| Maternal weight in kg, median (IQR) | 65.0 (59.0-75.0) | 75.0 (63.0-86.0)* | 70.0 (61.0-85.0)* | 74.0 (64.0-87.0)* |
| Fetal Crown-Rump Length in mm, median (IQR) | 63.3 (58.5-68.6) | 63.5 (56.4-71.2) | 62.0 (56.9-67.9) | 62.1 (58.5-68.9) |
| Racial origin | | | | |
| Caucasian, n (%) | 8,925 (66.0) | 24 (34.8) | 48 (43.2) | 123 (49.0) |
| African, n (%) | 3,243 (24.0) | 34 (49.3)* | 46 (41.4)* | 107 (42.6)* |
| South Asian, n (%) | 618 (4.6) | 7 (10.1) | 10 (9.0) | 10 (4.0) |
| East Asian, n (%) | 253 (1.9) | 0 | 1 (0.9) | 4 (1.6) |
| Mixed, n (%) | 476 (3.5) | 4 (5.8) | 6 (5.4) | 7 (2.8) |
| Parity | | | | |
| Nulliparous, n (%) | 6,680 (49.0) | 37 (53.6) | 59 (53.2) | 155 (61.8)* |
| Parous with no previous preeclampsia, n (%) | 6,649 (47.9) | 18 (26.1)* | 36 (32.4)* | 60 (23.9)* |
| Parous with previous preeclampsia, n (%) | 366 (2.7) | 14 (20.3)* | 16 (14.4)* | 36 (14.3)* |
| Cigarette smoker, n (%) | 1,110 (8.2) | 1 (1.4) | 8 (7.2) | 17 (6.8) |
| Family history of preeclampsia, (n, %) | 551 (4.1) | 10 (14.5)* | 10 (9.0)* | 19 (7.6)* |
| Conception | | | | |
| Spontaneous, n (%) | 13,073 (96.7) | 64 (92.8) | 103 (92.8) | 237 (94.4) |
| Assisted, n (%) | 442 (3.3) | 5 (7.2) | 8 (7.2) | 14 (5.6) |
| History of chronic hypertension, n (%) | 143 (1.1) | 12 (17.4)* | 11 (9.9)* | 18 (7.2)* |
| History of pre-existing diabetes, n (%) | 107 (0.8) | 2 (2.9) | 4 (3.6)* | 2 (0.8) |

Comparison between each outcome group and unaffected controls ($\chi^2$ - test and Fisher's exact test for categorical variables and Mann-Whitney test with *post hoc* Bonferroni correction for continuous variables): * Critical significance level $p < 0.0167$.

Competing risks model in early screening for PE by biophysical and biochemical markers

In the second screening study, described in Chapter 5, we prospectively screened 65,771 singleton pregnancies (Table 2.4). We excluded 6,887 cases because they had missing outcome data (n=2,133), the pregnancies resulted in miscarriage, termination or the birth of babies with major defects (n=1,586) or the birth of SGA neonates in the absence of PE (n=3,168). In the remaining 58,884 cases there were 1,426 (2.4%) that developed PE and 57,458 that were unaffected by PE.

**Table 2.4.** Maternal and pregnancy characteristics in the outcome groups.

| Characteristic | Unaffected (n=61,606) | Preeclampsia (n=1,482) |
|---|---|---|
| Maternal age in years, median (IQR) | 31.3 (26.7-35.2) | 31.2 (26.4-36.2) |
| Maternal weight in kg, median (IQR) | 65.0 (58.8-75.0) | 72.0 (62.0-85.0)* |
| Fetal CRL in mm, median (IQR) | 63.4 (58.4-69.0) | 62.9 (58.3-68.6) |
| Racial origin | | |
| Caucasian, n (%) | 47,025 (76.3) | 865 (58.4) |
| Afro-Caribbean, n (%) | 8,860 (14.4) | 478 (32.3)* |
| South Asian, n (%) | 2,872 (4.7) | 81 (5.5) |
| East Asian, n (%) | 1,415 (2.3) | 26 (1.8) |
| Mixed, n (%) | 1,434 (2.3) | 32 (2.2) |
| Parity | | |
| Nulliparous, n (%) | 29,680 (48.2) | 910 (61.4) |
| Parous with no previous PE, n (%) | 30,343 (49.3) | 373 (25.2)* |
| Parous with previous PE, n (%) | 1,583 (2.6) | 199 (13.4)* |
| Cigarette smoker, n (%) | 6,419 (10.4) | 117 (7.9)* |
| Family history of PE, (n, %) | 2,512 (4.1) | 126 (8.5)* |
| Conception | | |
| Spontaneous, n (%) | 59,351 (96.3) | 1,400 (94.5) |
| Assisted, n (%) | 2,255 (3.7) | 82 (5.5)* |
| History of chronic hypertension, n (%) | 550 (0.9) | 142 (9.6)* |
| History of type 1 DM, n (%) | 253 (0.4) | 15 (1.0)* |
| History of type 2 DM, n (%) | 160 (0.3) | 13 (0.9)* |
| History of SLE or APS | 106 (0.2) | 7 (0.4)* |

* Significance value $p<0.05$

The measurements of maternal serum PAPP-A were available in all cases. Uterine artery PI was available in 45,885 of the 58,884 pregnancies, including 1,245 (2.7%) that developed PE, MAP was available in 35,215 of the 58,884 pregnancies, including

979 (2.8%) that developed PE and serum PLGF was available in 14,252 of the 58,884 pregnancies, including 385 (2.7%) that developed PE.

In the PE group, compared to unaffected pregnancies, there was a higher mean maternal weight and prevalence of Afro-Caribbean racial origin, family and personal history of PE, chronic hypertension, diabetes mellitus and systemic lupus erythematosus or anti-phospholipid syndrome and a lower prevalence of cigarette smokers.

## 2.2 ETHICAL COMMITTEE APPROVAL

The study entitled Early Prediction of Pregnancy Complications, was approved by the King's College Hospital Ethics Committee (Ref: 02-03-033, R & D reference number 03WH06). The patients received an information leaflet outlining the details of the research study (Table 2.5) and a written informed consent (Table 2.6) was obtained from the women agreeing to participate in the study.

## 2.3 BIOCHEMICAL MARKERS

### 2.3.1 Placental growth factor

Duplicate serum sample of 100 µl was used to measure PlGF concentration by a quantitative enzyme linked immunoassay (ELISA) technique using Quantikine® human PlGF immunoassay (R&D systems Europe Ltd., Abingdon, UK). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and PlGF concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 7 pg/mL and the between-batch imprecision was 8.3% at a PlGF concentration of 48 pg/mL, 5.6% at 342 pg/mL and 5.1% at 722 pg/mL. Samples whose coefficient of variation of the duplicates exceeded 15% were reanalysed.

**Table 2.5** Patient information sheet for the study.

**PATIENT INFORMATION SHEET**

**Research Study: Early prediction of pregnancy complications**

We would like to invite you to take part in a research study. Before you decide whether to do so it is important for you to understand why the research is being done and what it will involve. Please take time to read this leaflet. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

**What is the purpose of the study?**

We are looking for new ways through scientific research to improve the care of pregnant women and their unborn babies. As part of this work, we are inviting all women that attend for the 11-13 weeks scan to participate in a large study on preeclampsia (high blood pressure of pregnancy) and fetal growth restriction (poor fetal growth).

Preeclampsia and fetal growth restriction are two important complications of pregnancy which can have serious implications for mother and baby. These problems can affect any pregnant woman, irrespective of previous healthy pregnancies and irrespective of how healthy the mother is.

Our aim is to try and identify the women who are at high risk of developing these problems and to do so as early in pregnancy as possible.

**Why have I been chosen?**

All pregnant women attending for the 11-13 weeks scan are welcome to take part in this study.

**Do I have to take part?**

It is up to you to decide whether you would like to take part. If you decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. Once you have decided to take part you are still free to withdraw at any time without giving any reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

**What will happen to me if I take part?**

The study consists of three components which are done at the time of the 11-13 week scan:

*1. Maternal blood markers*
This involves us saving some of the blood that we take from you as part of the test to determine the risk for Down's syndrome. Since new tests may become available in the future we feel it would be prudent to store some of your blood and urine sample for future studies.

*2. Measurement of blood flow from the mother to the placenta*
During your visit we will use ultrasound to examine your baby. We will also use ultrasound to look at the vessels that supply blood to the uterus and the placenta. This extra scan takes a couple of minutes to do. It is not uncomfortable and does not carry any risks to you or your baby.

*3. Blood pressure measurement*
During your visit we will measure your blood pressure. Usually this measurement is taken from your left arm. We are trying to find out if it is better to use the reading from the left or the right arm or the average one from both arms. We would take blood pressure measurements from both of your arms simultaneously.

**What are the possible benefits of taking part?**
If we find that you have high blood pressure we will arrange for any follow up tests and monitoring that would be necessary. This will have a direct benefit for you. In addition, the information we get from the study may help us to help you and/or other women in the future.

**What are the possible disadvantages and risks of taking part?**
The blood pressure measurement may also be uncomfortable because of the inflation of the cuffs. If you find this examination intolerable please let us know, we will stop immediately.

**Will my taking part in this study be kept confidential?**
Yes. All the information about your participation in this study will be kept confidential.

**What if I want to complain?**

If you have a concern about any aspect of this study, you can ask to speak with one of the researchers who will do their best to answer your questions. By agreeing to take part in the study you do not lose any legal rights. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital (Contact: Mr Tim Hiles on 0207 346 3983).

**What will happen to the results of the research study?**
It is hoped that the results will be published in medical journals and perhaps also in the press. You may request a copy of any published documents in relation to the study. You will not be identified in any of these reports.

**Who is organising and funding the research?**
This research is carried out by the team of Professor Kypros Nicolaides and it is funded by the Fetal Medicine Foundation (Registered charity: 1037116).

**Table 2.6** Consent form for the study.

**CONSENT FORM**

**Research Study: Early prediction of pregnancy complications**

We are requesting your permission to participate in the research study that essentially involves the following:

1. Maternal blood analysis
2. Measurement of blood flow to the placenta
3. Measurement of blood pressure

*We hope that you find it worthwhile to take part in this study. If you should decide to participate, please sign the consent below. We would ask you to sign three copies of this form, one for your own records, one for our research, and one for your medical notes. Thank you.*

**1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.**

**2. I understand that my participation is voluntary, and that I am free to withdraw at any time, without affecting my medical care or legal rights.**

- I agree / disagree to have a sample of my blood taken for current testing and storage for future tests
- I agree / disagree to the measurement of flow in the uterine arteries
- I agree / disagree to the measurement of my blood pressure.

ID number: ……..Date: ……

Patient's name: ……Patient’s signature: ……

Doctor's name: ……Doctor’s signature: …….

### 2.3.2 Activin-A

Duplicate serum samples of 75µl were used to measure total Activin A concentration by a solid phase sandwich ELISA (Enzyme Linked Immunoadsorbent Assay) using Oxford Bio-Innovation total Activin A immunoassay kits (Oxford Bio-Innovation Limited, Oxfordshire, UK). The lower limit of detection of the assay was 0.078 ng/mL and the between-batch imprecision was 15.8% at Activin A concentration of 1.13 ng/mL, 8.3% at 1.79 ng/mL and 12.3% at 3.85 ng/mL. All samples were analysed in duplicate and those with a coefficient of variation exceeding 15% were reanalysed.

### 2.3.3 Inhibin-A

Duplicate plasma samples of 50 µl were used to measure inhibin A concentration by a quantitative enzyme linked immunoassay (ELISA) technique using DSL-10-28100 inhibin A immunoassay (Diagnostic systems laboratories, Inc. Webster, Texas, USA). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and inhibin-A concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 1.0 pg/mL and the between-batch imprecision was 15.4% at an inhibin-A concentration of 94.5 pg/mL and 8.2% at 361.0 pg/mL. All samples were analysed in duplicate and those with a coefficient of variation exceeding 10% were reanalysed.

### 2.3.4 Placental protein-13

DELFIA® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) research reagents (Perkin Elmer Life and Analytical Sciences, Turku, Finland) were used to measure PP13 in maternal serum samples (25 µl/well in duplicate). The concentration of PP13 measured was directly proportional to the fluorescence measured on time-resolved fluorometer at 615 nm. The coefficient of variation (CV) was 4.1% at a PP13

concentration of 16.6 pg/ml, 2.0% at 60.4 pg/ml and 2.7% at 136.2 pg/ml. Samples with duplicate CV's greater than 10% were reanalysed.

### 2.3.5 P-selectin

Duplicate plasma sample of 100 µl was used to measure P-selectin concentration by a quantitative enzyme linked immunoassay (ELISA) technique using human soluble P-selectin/CD62P immunoassay (R & D Systems Europe, Ltd., Abingdon, United kingdom). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and P-selectin concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 0.5 ng/mL and the between-batch coefficient of variation (CV) was 12.3% at a P-selectin concentration of 383.0 ng/mL, 11.9% at 212.0 pg/mL and 14.9% at 21.3 ng/ml. All samples were analysed in duplicate and those with a coefficient of variation exceeding 15% were reanalysed.

### 2.3.6 Pentraxin-3

Duplicate plasma samples were analysed using a PTX3 enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems Europe Ltd, Abingdon, United Kingdom). The assay was performed according to the manufacturer's instructions. Sample pre-treatment was performed manually. The ELISA assay was performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Substrate and Stop reagents were added manually. Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland). The concentrations of PTX3 were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 0.025 ng/mL and the between-batch imprecision was 8.4% at PTX3 concentration of 0.9 ng/mL, 8.3% at 3.4 ng/mL and 5.6% at 9.0 ng/mL. All samples were analysed

in duplicate and those with a large coefficient of variation (more than 20% for values of less than 1 ng/mL and more than 15% for values of 1 ng/mL or more) were reanalysed.

### 2.3.7 Soluble endoglin

Plasma sEng was measured by enzyme linked immunoassay (ELISA) technique using DuoSet® human sENG (R&D Systems Europe Ltd., Abington, UK) according to manufacturer's instructions. The lower limit of detection of the assays was 5 pg/mL. Samples whose coefficient of variation of the duplicates exceeded 15% were reanalysed.

### 2.3.8 Plasminogen Activator Inhibitor-2

Maternal plasma samples diluted 1:10 were analysed in duplicate using a DELFIA® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) PAI-2 sandwich immunoassay using a monoclonal antibody and a goat polyclonal antibody against human PAI-2 (Omnio AB, Sweden). Assays were performed manually using a 2-step protocol. Fluorescence readings were measured on a VICTOR2D plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland). The concentrations of PAI-2 were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The between-batch coefficient of variation was less than 5% in all the samples analysed. The investigators were blinded to the pregnancy outcome during the sample analysis.

### 2.3.9 Angiopoietin-2

Duplicate serum samples of 50 µL were analysed using a DELFIA® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) Ang-2 sandwich immunoassay (PerkinElmer Life and Analytical Sciences, Turku, Finland) using a two-step protocol. The analysis was done using two monoclonal anti-human angiopoietin antibodies (R

& D Systems, Minnesota, USA; catalogue number MAB098 and MAB0983). Firstly, standards and samples (50µL/well) in duplicates were added to the wells followed by assay buffer (150µL/well) and then incubated at room temperature for 2 hours. The plate was then washed four times and then DELFIA Enhancement solution (200µl/well) was dispensed. Fluorescence readings were measured on a VICTOR2D plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland). The concentrations of Ang-2 were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The between-batch coefficient of variation was less than 5 % in all the samples analysed.

### 2.3.10 Soluble fms-like tyrosine kinase-1

Plasma sFlt-1 was measured by enzyme linked immunoassay (ELISA) technique using DuoSet® human sFlt-1 (R&D Systems Europe Ltd., Abington,UK). The lower limit of detection of the assays was 15 pg/mL for sFlt-1. Samples whose duplicate values differed by more than 15% were analysed.

## 2.4 BIOPHYSICAL MARKERS

### 2.4.1 Blood pressure

The blood pressure (BP) was taken by validated automated devices (3BTO-A2, Microlife, Taipei, Taiwan; Reinders *et al*., 2005) that were calibrated before and at regular intervals during the study (every 1,000 inflations). The recordings were made by doctors who had received appropriate training on the use of these machines. Women were allowed to rest for 5 minutes and the BP measurements were taken in a quiet room with temperature of between 20°C and 24°C. The women were in the seating position, with their arms supported at the level of the heart and either a small (< 22 cm), normal (22-32 cm) or large (33-42 cm) adult cuff was used depending on the mid-arm circumference (Pickering *et al.*, 2005; Poon *et al*., 2012).

The BP was measured in both arms simultaneously (Figure 2.1) and two recordings were made in both arms (National Heart Foundation of Australia, 2004). The final MAP was calculated as the average of all 4 measurements from both arms along with its measurements of systolic BP and diastolic BP for the subsequent analysis of results (National Heart Foundation of Australia, 2004).

**Figure 2.1** Measurement of blood pressure.

### 2.4.2 Uterine artery Doppler

For the measurement of uterine artery pulsatility index (PI) by transabdominal ultrasound, a sagittal section of the uterus was obtained and the cervical canal and internal cervical os were identified. Subsequently, the transducer was gently tilted from side to side and colour flow mapping was used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os (Figure 2.2; Plasencia *et al.,* 2007).

Pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel and care was taken to ensure that the angle of insonation was less than 30º. When three similar consecutive waveforms were obtained, the uterine artery PI was measured from the left and right arteries (Figure 2.3). All operators who participated in the study had obtained the Fetal Medicine Foundation Certificate of competence in obstetric Doppler imaging (http://www.fetalmedicine.com). The results of the Doppler studies were not given to the women or their doctors and did not influence the subsequent management of the pregnancies.

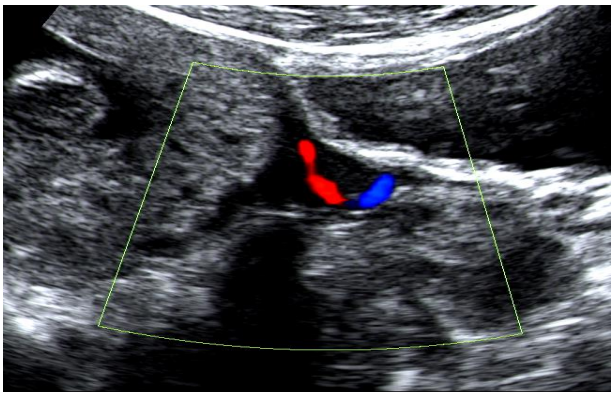


**Figure 2.2** The uterine artery as demonstrated by colour flow mapping along the side of the cervix and uterus at the level of the internal os.

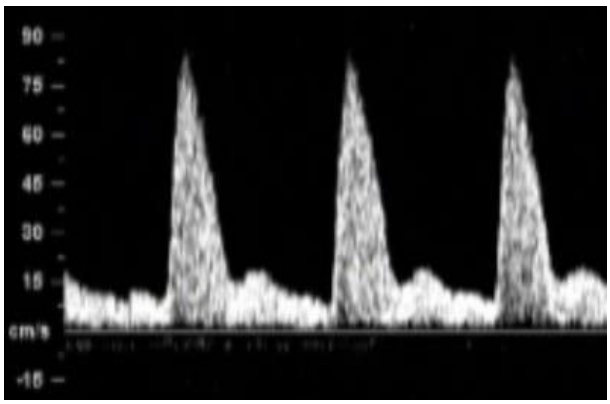



**Figure 2.3** Uterine artery Doppler waveform.

## 2.5 RECORDING OF INFORMATION

Women were asked to complete a questionnaire on age, racial origin (Caucasian, African, South Asian, East Asian and Mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous or assisted conception by either ovulation induction alone or in-vitro fertilisation), medical history (including chronic hypertension, diabetes mellitus, anti-phospholipid syndrome, thrombophilia, and sickle cell disease), medication (including anti-hypertensive, anti-depressant, anti-epileptic, aspirin, steroids, beta-mimetics, insulin, and thyroxine), parity (parous, nulliparous with no previous pregnancies, nulliparous with miscarriage or termination before 24 weeks), previous pregnancy with PE (yes or no) and family history of PE (mother, sister or both). The questionnaire was then reviewed by a doctor together with the patient.

The following measurements were made:

- Maternal weight and height were measured and the body mass index (BMI) was calculated in $Kg/m^2$,
- Stabilised measurement of systolic BP, diastolic BP and MAP,
- Uterine artery PI of the two arteries

## 2.6 OUTCOME MEASURES

The definitions of PE and GH were those of the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988). In GH the diastolic BP should be 90 mmHg or more on at least two occasions four hours apart developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria and in PE there should be GH with proteinuria of 300 mg or more in 24 hours or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available.

In PE superimposed on chronic hypertension significant proteinuria (as defined above) should develop after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

The obstetric records of all women with pre-existing or pregnancy associated hypertension were examined to determine if the condition was chronic hypertension, PE or GH. Similarly, for quality control we examined the records of 500 randomly selected cases without pregnancy associated hypertension.

## 2.7 STATISTICAL ANALYSIS

### 2.7.1 Case-control studies

Continuous data were presented as median and IQR and categorical data as n (%). Comparison between the outcome groups was by $\chi^2$-test or Fisher's exact test for categorical variables and Mann Whitney-U test for continuous variables. A p value of $p<0.05$ was considered significant. Where necessary *post hoc* correction for significance level was done with either Bonferroni correction or Dunn's test.

In case of each biochemical marker, the following steps were taken. First, the distribution of the biochemical marker was made Gaussian after logarithmic transformation (PLGF, Activin-A, Inhibin-A, PP-13, P-Selectin, PTX-3, sEng, PAI-2 and Ang-2). In case of sFlt-1, the distribution of was transformed using the equation: $Y= \log_{10}(\text{sFlt-1} - k)$ to approximate a Gaussian distribution. Distributions were confirmed to be Gaussian using Kolmogorov-Smirnov test, histograms or probability plots. Second, multivariate regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of the $\log_{10}$ transformed biochemical marker in the unaffected group. Then the distribution of $\log_{10}$ transformed biochemical marker expressed as MoM of the unaffected group, were determined in the PE group. The measured uterine artery PI

and PAPP-A were converted into MoM after adjustment for maternal characteristics (Poon *et al*., 2009b; Kagan *et al.*, 2008a). Third, non-parametric testing using either Mann-Whitney test with *post hoc* Bonferroni correction or Kruskall-Wallis test with *post hoc* Dunn's procedure was used to compare median MoM of biochemical marker between the outcome groups. Fourth, regression analysis was used to determine the significance of association between $\log_{10}$ transformed biochemical marker and $\log_{10}$ uterine artery MoM and $\log_{10}$ PAPP-A MoM in the outcome groups. Fifth, logistic regression analysis was used to determine if the $\log_{10}$ transformed maternal factor-derived *a priori* risks, $\log_{10}$ transformed biochemical marker, $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery MoM had a significant contribution in predicting PE. The detection and false positive rates were calculated as the respective proportions of PE (detection rate) and unaffected pregnancies (false positive rate) with MoM values above given cut-offs. The performance of screening was determined by receiver operating characteristic (ROC) curves analysis.

The statistical software package SPSS 16.0 (SPSS Inc.,Chicago, IL), Medcalc for windows, version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

### 2.7.2 Screening studies

Prediction of PE from maternal factors, biophysical and biochemical markers

Comparison between the early, intermediate and late PE groups with the unaffected pregnancies was by $\chi^2$-test or Fisher's exact test for categorical variables and Mann Whitney-U test for continuous variables, both with *post-hoc* Bonferroni correction (critical statistical significance $p<0.0167$).

The following steps were used to develop a model for predicting early, intermediate and late PE based on maternal characteristics. First, the association of continuous variables, such as maternal age, weight and height, with PE was assessed to determine

if this was linear or non-linear. Second, univariate analysis was performed to examine the individual variables contributing significantly to early, intermediate and late PE by assessing their odds ratios (ORs) and 95% confidence intervals. Third, logistic regression analysis with backward stepwise elimination of variables was used to develop the model. Fourth, to assess the predictive accuracy of our model we calculated the shrinkage factor using the equation $[\chi^2 - (df-1)]/ \chi^2$ where $\chi^2$ is the model chi-square derived from the log-likelihood statistic and df is the degree of freedom. This shrinkage factor was then applied to all the parameters in the model to adjust for over fitting. Fifth, the patient-specific risk for early, intermediate and late PE was calculated from the formula: odds / (1+odds), where odds= $e^Y$ and Y was derived from the logistic regression analysis. The distribution of risks was then used to calculate detection and false positive rates at different risk cut-offs and the performance of screening was determined by receiver operating characteristic (ROC) curves analysis.

The following steps were used to develop a model for predicting early, intermediate and late PE based on the combination of maternal characteristics and biophysical and biochemical markers. First, the measured serum PAPP-A and free ß-hCG were converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, weight, smoking, parity, racial origin and method of conception as previously described (Kagan *et al.*, 2008a). Second, the values of uterine artery PI, MAP, PLGF, PP13, inhibin-A, activin-A, sEng, PTX3 and P-selectin were log transformed to make their distribution Gaussian. Third, in the unaffected pregnancies multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and fetal CRL were significant predictors of each marker. In each case of PE and unaffected pregnancies the measurements were converted into a multiple of the normal median (MoM) and the MoM values in different groups were compared. Fourth, Guassian distributions of markers in early, intermediate, late-PE and unaffected pregnancies were fitted. These fitted distributions define the likelihood ratios for the screening tests that can be combined with the *a priori* maternal characteristics-derived risk to produce *a posteriori* risk. Fifth, the maternal factors-related *a priori* risks and $\log_{10}$MoM values of the biophysical and biochemical markers

were simulated for 500,000 pregnancies for each group of early, intermediate and late PE and 500,000 unaffected pregnancies. The maternal factors-related *a priori* risks for each of the PE groups were multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the *a posteriori* risks in the simulated samples of 500,000 PE and 500,000 unaffected pregnancies. Fifth, the *a priori* and *a posteriori* risks in each of the PE groups and unaffected pregnancies were used to calculate the detection rates at fixed false positive rates of 5% and 10%. The large size of the simulated population was chosen to ensure that the error resulting from simulation was negligble. The process of sampling with replacement from the screening data of PE and unaffected pregnancies ensured that the modelled screening performance reflects the screening population.

The statistical software package SPSS 16.0 (SPSS Inc.,Chicago, IL) was used for data analyses. Monte-Carlo simulations were programmed in R (The R Foundation for Statistical Computing, R version 2.11.0, ISBN 3-900051-070-0, http://www.r-project.org).

Competing risks model in early screening for PE by biophysical and biochemical markers

This model for early screening for PE was based on a survival time model for the time of delivery for PE. Bayes theorem was used to combine the prior information from maternal characteristics with biomarker multiple of the median (MoM) values. We used a competing risk model (Kalbfleisch and Prentice 2002). In this model it is assumed that if the pregnancy was to continue indefinitely all women would have developed PE and in this respect there is a competition between delivery before or after development of PE. We applied a model to represent the distribution of gestational age at delivery with PE. In pregnancies at low risk for PE the gestational age distribution is shifted to the right with the implication that in most pregnancies delivery will actually occur before the development of PE. In pregnancies at high-risk for PE the gestational age distribution is shifted to the left with the implication that in many pregnancies delivery will actually occur after the development of PE. Given

maternal characteristics and biomarker levels the risk of PE occurring at or before a specified gestational age was given by the area under the distribution curve.

The distribution of gestational age at delivery with PE was defined by two components: firstly, the prior distribution based on maternal characteristics and secondly, the distribution of MoM biomarker values with gestational age in pregnancies affected by PE. Although such model fitting was complicated because of the censoring in situations when delivery occurred before PE, fitting regression models to censored data was carried out routinely with standard statistical software.

The values of uterine artery PI, MAP, PAPP-A and PLGF were $\log_{10}$ transformed to make their distribution Gaussian. Each measured value in the unaffected and PE pregnancies was expressed as a multiple of the normal median (MoM) after adjustment for those characteristics found to provide a substantial contribution to the log transformed value (Wright *et al.,* 2010 and 2012; Pandya *et al.,* 2012). In each case of PE the measurements were converted into a MoM and regression analysis was used to determine the relationship between $\log_{10}$ MoM values with getational age at delivery.

In the estimation of performance of screening the values of uterine artery PI, MAP, PAPP-A and PLGF in the whole screened population were simulated based on the mean and SD of the $\log_{10}$ transformed marker values in the unaffected and PE pregnancies. In the PE group the mean and SD values used for simulation were specific for each gestational week at delivery, which were estimated from the regression analysis of the $\log_{10}$ MoM values of available data with getational age at delivery.

# CHAPTER 3

## BIOCHEMICAL MARKERS IN EARLY PREDICTION OF PREECLAMPSIA

## 3.1. INTRODUCTION

### 3.1.1 Background

A large number of maternal blood markers have been put forward for the prediction of PE (Chapter 1.4.4). Many such markers represent measurable manifestations of impaired placentation-related placental ischaemia and damage with subsequent release of inflammatory factors, platelet activation, endothelial dysfunction, maternal renal dysfunction or abnormal oxidative stress. The majority of studies have examined these markers either during established PE or in the second trimester before the clinical onset of the disease. However, there is some contradictory evidence that some markers may be altered from as early as the first trimester of pregnancy (Chapter 1.4.4).

### 3.1.2 Objectives

The objectives of the studies in this chapter are firstly, to investigate whether the maternal serum or plasma levels of a series of biochemical markers are altered in women who subsequently develop PE, secondly, to examine the association between these biochemical markers and established markers of placental dysfunction such as uterine artery PI and maternal serum PAPP-A and thirdly, to estimate the performance of screening for PE.

## 3.2 METHODS

The studies investigating the levels of maternal serum or plasma biochemical markers were nested case-control studies drawn from a larger prospective observational study at 11-13 weeks' gestation for early prediction of pregnancy complications. The description of study population (Section 2.1), data collection (Section 2.5) and outcome measures (Section 2.6) are outlined in Chapter 2.

We searched our database to identify cases that developed early and late PE with available stored samples. Each case was matched with controls that did not develop any pregnancy complication and had blood collected and stored on the same day. All sample analyses were in duplicate and in those where the CV exceeded 15%, the samples were reanalysed. Detailed description of biochemical (Section 2.3) and statistical analyses (Section 2.7) are presented in Chapter 2.

## 3.3 RESULTS

### 3.3.1 Placental growth factor

Serum PLGF was measured in 127 cases and 609 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between PLGF MoMs and serum PAPP-A MoM and uterine artery PI MoM in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ PLGF MoM with $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ PLGF significant independent contributions were provided by fetal CRL, maternal weight, cigarette smoking and ethnic origin: $\log_{10}$ expected PLGF = 1.150 + 0.008 x CRL in mm – 0.002 x weight in Kg + (0.199 if smoking, 0 if not) + (0.177 if Afro-Caribbean, 0.100 if South Asian, 0 if other racial origin); $R^2$=0.237, $p<0.0001$. In the control

group, the median PLGF MoM was 0.99 (IQR 0.80-1.29) (Table 3.1, Figure 3.1). The median PAPP-A MoM was 1.07 (IQR 0.74-1.46) and uterine artery PI MoM was 1.03 (IQR 0.84-1.24); (Table 3.1, Figure 3.1).

There was a significant association between $\log_{10}$ PlGF MoM and $\log_{10}$ PAPP-A MoM ($r=0.264$, $p<0.0001$; Figure 3.2), $\log_{10}$ uterine artery PI MoM ($r=0.102$, $p=0.012$; Figure 3.3), birth weight percentile ($r=0.114$, $p=0.005$) but not gestational age at delivery ($p=0.960$).

Preeclampsia

In both the early-PE and late-PE groups, PlGF and PAPP-A were lower and uterine artery PI was higher than in the controls (Figure 3.1, Table 3.1).

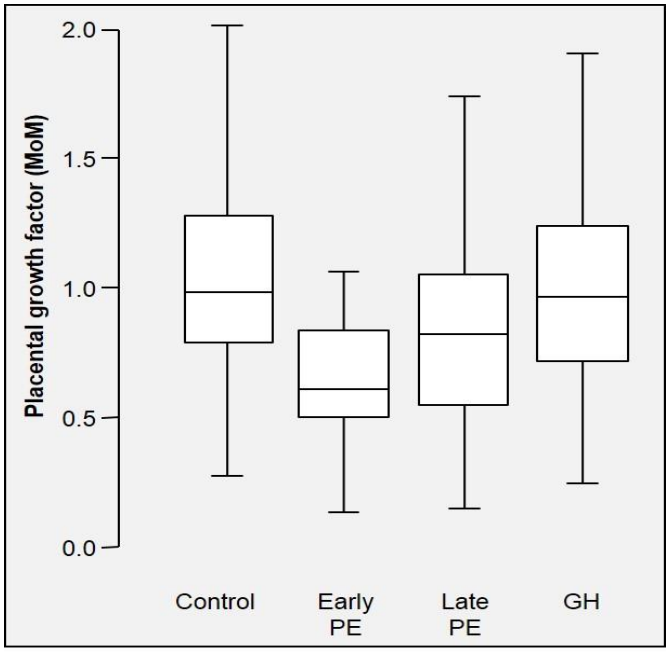



**Figure 3.1** Box-whisker plot of placental growth factor (PlGF) multiple of median (MoM) in the pregnancy outcome groups: controls, early preeclampsia (PE), late PE, gestational hypertension (GH).

**Table 3.1** Median (interquartile range) of maternal serum placental growth factor (PLGF) multiple of the median (MoM), pregnancy associated plasma protein-A (PAPP-A) MoM and uterine artery pulsatility index (PI) MoM in the control, early- and late-preeclampsia groups.

| Outcome group | PlGF MoM | PAPP-A MoM | Uterine artery PI MoM |
|---|---|---|---|
| Control | 0.99 (0.80-1.29) | 1.07 (0.74-1.46) | 1.03 (0.84-1.24) |
| Early-PE | 0.61 (0.48-0.84)‡ | 0.54 (0.39-0.96)‡ | 1.51 (1.20-1.65)‡ |
| Late-PE | 0.82 (0.55-1.06)‡ | 0.93 (0.57-1.31)* | 1.22 (0.93-1.45)‡ |

Mann-Whitney test: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.0001$

There was a significant association between $\log_{10}$ PlGF MoM and $\log_{10}$ PAPP-A MoM (r=0.325, p<0.0001; Figure 3.2), $\log_{10}$ uterine artery PI MoM (r=-0.279, p=0.001; Figure 3.3), gestational age at delivery (r=0.256, p=0.004) and birth weight percentile (r=0.338, p<0.0001).

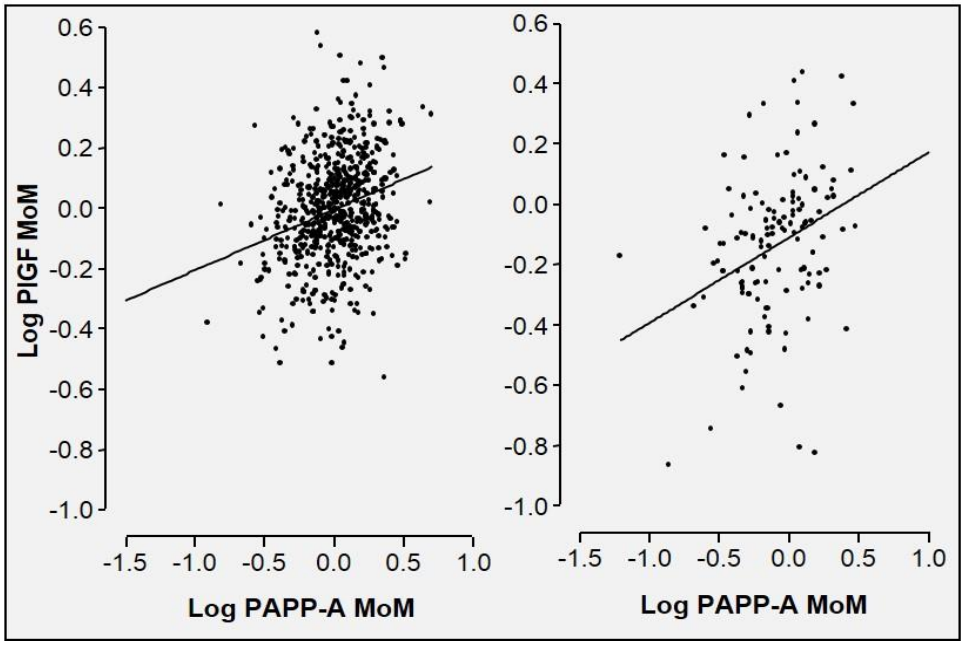



**Figure 3.2.** Relationship between placental growth factor (PLGF) multiple of the median (MoM) and pregnancy associated plasma protein-A (PAPP-A) MoM in the control group (left) and the preeclampsia group (right).

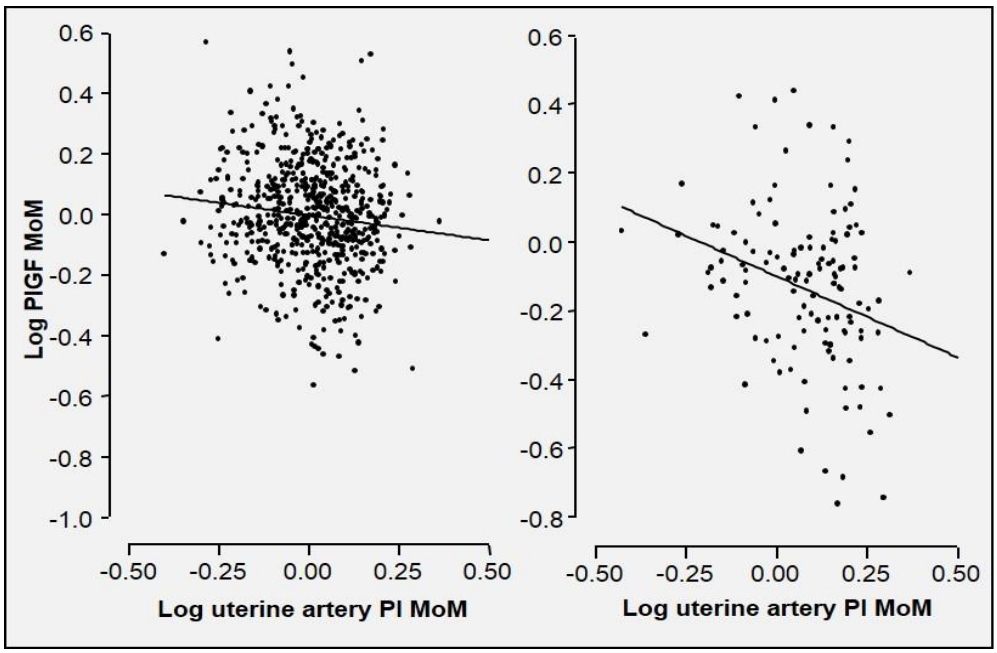



**Figure 3.3.** Relationship between placental growth factor (PLGF) ) multiple of the median (MoM) and uterine artery pulsatility index (PI) MoM in the control group (left) and the preeclampsia group (right).

Logistic regression analysis demonstrated that significant contributions for the detection of early-PE were provided from maternal factors, PLGF, PAPP-A and uterine artery PI ($R^2$=0.500, p<0.0001; Table 3.2).

**Table 3.2.** Logistic regression analysis for prediction of early-preeclampsia.

| **Independent variable** | **Early preeclampsia** | | | |
|---|---|---|---|---|
| | **OR** | **95% CI** | | ***p*** |
| $Log_{10}$ PLGF MoM | 0.01 | 0.00 | 0.17 | 0.002 |
| $Log_{10}$ uterine artery PI MoM | 2020561 | 5358.56 | $7.6E^{+08}$ | <0.0001 |
| $Log_{10}$ PAPP-A MoM | 0.16 | 0.03 | 0.97 | 0.046 |
| Chronic hypertension | 237.694 | 17.33 | 3260.52 | <0.0001 |
| Afro-Caribbean race | 3.17 | 1.17 | 8.56 | 0.023 |

Logistic regression analysis demonstrated that significant contributions for the detection of late-PE were provided from maternal factors, PLGF and uterine artery PI ($R^2$=0.290, p<0.0001; Table 3.3) but not PAPP-A (p=0.933).

**Table 3.3.** Logistic regression analysis for prediction of late-preeclampsia.

| Independent variable | Late preeclampsia | | | |
|---|---|---|---|---|
| | OR | 95% CI | | *p* |
| $Log_{10}$ PLGF MoM | 0.09 | 0.03 | 0.32 | <0.0001 |
| $Log_{10}$ uterine artery PI MoM | 14.03 | 1.89 | 103.91 | 0.010 |
| Body mass index in $Kg/m^2$ | 1.11 | 1.07 | 1.16 | <0.0001 |
| Afro-Caribbean race | 3.92 | 2.27 | 6.78 | <0.0001 |
| South Asian race | 2.95 | 1.16 | 7.55 | 0.024 |
| Mixed race | 4.71 | 1.74 | 12.75 | 0.002 |
| Parous – no previous PE | 0.28 | 0.16 | 0.48 | <0.0001 |
| Family history of PE | 4.22 | 1.71 | 10.41 | 0.002 |

The performance of screening of different methods by comparison of the AUROC curves along with the DR of early-PE and late-PE for different FPR in screening by maternal factors, serum PlGF, serum PAPP-A, uterine artery PI and by their combinations are given in Tables 3.4 and 3.5.

The AUROC for maternal factors in screening for early-PE was 0.762 with a DR of 39% and 49% for a FPR of 5 and 10%, respectively. The performance of screening was significantly improved by the combination of maternal factors with PLGF, PAPP-A and uterine artery PI with an AUROC of 0.936 and a corresponding DR of 76% and 86% for a FPR of 5% and 10% respectively. Similarly, the AUROC for maternal factors in screening for late-PE was 0.788 with a DR of 39% and 49% for a FPR of 5 and 10%, respectively. The performance of screening was significantly improved by the combination of maternal factors with PLGF and uterine artery PI with an AUROC of 0.817 and a corresponding DR of 30% and 49% for a FPR of 5 and 10% respectively (Table 3.4 and 3.5).

**Table 3.4.** Comparison of area under receiver operating characteristic curve (AUROCs) in screening for preeclampsia by maternal factors, placental growth factor (PLGF), pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations..

| Screening test | AUROC (95% CI) | |
|---|---|---|
| | Early PE | Late PE |
| History | 0.762 (0.654-0.870) | 0.788 (0.742-0.834) |
| PLGF | 0.797 (0.705-0.888) | 0.652 (0.589-0.714) |
| PAPP-A | 0.742 (0.639-0.846) | 0.576 (0.513-0.639) |
| Uterine artery PI | 0.826 (0.740-0.912) | 0.626 (0.560-0.692) |
| History with PLGF | 0.881(0.817-0.944) | 0.817 (0.775-0.859) |
| History with PAPP-A | 0.842 (0.747-0.937) | 0.788 (0.741-0.834) |
| History with uterine artery PI | 0.902 (0.833-0.971) | 0.801 (0.753-0.849) |
| History with PLGF and uterine artery PI | 0.941 (0.889-0.994) | 0.817 (0.773-0.861) |
| History with PLGF, PAPP-A, uterine artery PI | 0.936 (0.882-0.989) | - |

**Table 3.5.** Comparison of detection rate (DR) for fixed false positive rates (FPRs) of 5 and 10% in screening for preeclampsia by maternal factors, placental growth factor (PLGF), pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations.

| Screening test | DR (%) for fixed FPR | | | |
|---|---|---|---|---|
| | Early PE | | Late PE | |
| | 5% | 10% | 5% | 10% |
| History | 39.0 | 49.0 | 29.6 | 43.9 |
| PLGF | 27.6 | 51.7 | 19.4 | 32.7 |
| PAPP-A | 24.1 | 41.4 | 8.2 | 18.4 |
| Uterine artery PI | 37.9 | 65.5 | 16.3 | 27.6 |
| History with PLGF | 55.2 | 62.1 | 28.6 | 52.0 |
| History with PAPP-A | 51.7 | 69.0 | 29.6 | 46.9 |
| History with uterine artery PI | 69.0 | 75.9 | 29.6 | 51.0 |
| History with PLGF and uterine artery PI | 75.9 | 89.7 | 29.6 | 49.0 |
| History with PLGF, PAPP-A, uterine artery PI | 75.9 | 86.2 | - | - |

### 3.3.2 Activin A

Serum Activin A was measured in 126 cases and 214 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between Activin A MoMs and serum PAPP-A MoM and uterine artery PI MoM in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ Activin-A MoM with $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ Activin A significant independent contributions were provided by maternal weight and racial origin: $\log_{10}$ expected activin A = 0.455 - 0.003 x maternal weight in Kg + (0.082 if Afro-Caribbean, 0 if other racial origin); $R^2$=0.068, p=0.001.

In the control group, the median activin A MoM was 1.00 (IQR 0.76-1.28) (Table 3.7). The median PAPP-A MoM was 1.03 (IQR 0.70-1.45) and uterine artery PI MoM was 1.05 (IQR 0.84-1.30); (Table 3.6).

In the control group, there was a significant association between serum activin A and PAPP-A (r=0.330, p<0.0001) but there was no significant association between activin A and uterine artery PI (p=0.714; Figure 3.4).

**Table 3.6.** Median (interquartile range) of Activin A, pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM) and uterine artery pulsatility index (PI) MoM in the outcome groups.

| Outcome group | Activin A MoM | PAPP-A MoM | Uterine artery PI MoM |
|---|---|---|---|
| Control | 1.00 (0.76-1.28) | 1.03 (0.70-1.45) | 1.05 (0.84-1.30) |
| PE | 1.27 (0.96-1.68)* | 0.81 (0.53-1.25)* | 1.32 (0.99-1.56)* |

Significance value * $P < 0.05$

Preeclampsia

In PE, compared to controls, Activin A MoM and uterine artery PI MoM were higher and serum PAPP-A was lower (Table 3.7).

There was a significant association between serum Activin A and PAPP-A (r=0.277, p=0.002) but there was no significant association between Activin A and uterine artery PI (p=0.400; Figure 3.4).

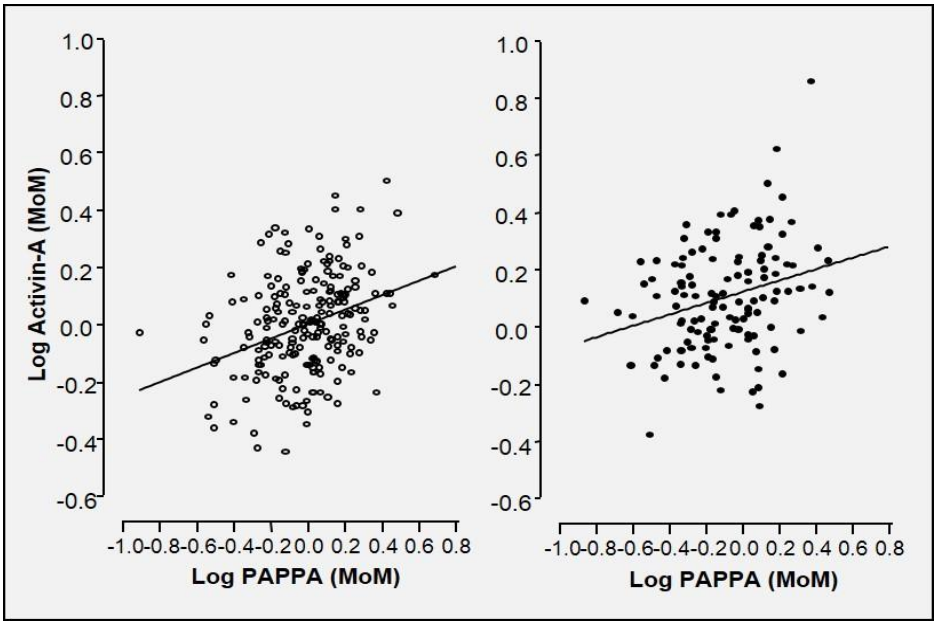



**Figure 3.4.** Relationship between maternal serum Activin A and pregnancy associated plasma protein-A (PAPP-A) in control group (left) and in those that developed preeclampsia (right).

Logistic regression analysis demonstrated that in the prediction of early-PE there were significant contributions from $\log_{10}$ uterine artery PI MoM (OR $1.7E^5$, 95% CI 247.8-1.2E8; p<0.0001), $\log_{10}$ PAPP-A MoM (OR 0.050, 95% CI 0.006-0.426; p=0.006), and $\log_{10}$ Activin A MoM (OR 164.2, 95% CI 4.6-5833.8; p=0.005), history of chronic hypertension (OR 108.0, 95%CI 6.1-1900.6; p=0.001), Afro-Caribbean racial origin (OR 3.6, 95% CI 1.1-12.3; p=0.041) and parous with no previous PE (OR 0.182, 95% CI 0.049-0.677; p=0.011) but not BMI (p=0.782) or family history of PE (p=0.305); $R^2$=0.569, p<0.0001.

The estimated DR of screening for early-PE by Activin A was 11.1% and 25.9% at respective FPR of 5% and 10%. The performance of screening for early-PE by a combination of history, PAPP-A and uterine artery PI (AUROC 0.921, 95%CI 0.879-0.952) was not significantly improved by the addition of Activin A (AUROC 0.927, 95%CI 0.886-0.956, p=0.787).

Logistic regression analysis demonstrated that in the detection of late-PE there were significant contributions from $\log_{10}$ uterine artery PI MoM (OR 36.0 95% CI 3.7-346.7; p=002), $\log_{10}$ Activin A MoM (OR 32.1% CI 6.0-171.7; p<0.001), BMI (OR 1.1, 95%CI 1.0-1.2; p=0.002), Afro-Caribbean ethnic origin (OR 3.4, 95% CI 1.8-6.4; p<0.001), parous with no previous PE (OR 0.171, 95% CI 0.09-0.324; p<0.001) and family history of PE (OR 4.5, 95%CI 1.4-14.7; p=0.014), but not $\log_{10}$ PAPP-A MoM (p=0.114) or history of chronic hypertension (p=0.215): $R^2$=0.382, p<0.0001.

The estimated DR of screening for late-PE by Activin A was 15.3% and 25.5% at respective FPR of 5% and 10%. The performance of screening for late-PE by a combination of history and uterine artery PI (AUROC 0.806, 95%CI 0.758-0.848) was not significantly improved by the addition of Activin A (AUROC 0.816, 95%CI 0.768-0.857; p=0.593).

### 3.3.3 Inhibin-A

Serum Inhibin-A was measured in 121 cases and 208 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between Inhibin-A MoMs and uterine artery PI MoMs in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ Inhibin-A MoM with $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ inhibin A, significant independent contributions were provided by maternal weight and racial origin: $\log_{10}$ expected inhibin A = 2.596 - 0.003 x maternal weight in Kg + (0.127 if Afro-Caribbean, 0 if other racial origins); $R^2$=0.078, p<0.0001 (Table 3.7).

In the control group, the median Inhibin-A MoM was 0.98 (0.72-1.43) (Table 3.8, Figure 3.5). The median PAPP-A MoM was 1.00 (0.69-1.45) and uterine artery PI MoM was 1.05 (0.83-1.30) (Table 3.7, Figure 3.5).

**Table 3.7.** Median (interquartile range) for Inhibin-A MoM, uterine artery pulsatility index (PI) multiple of the median (MoM) and pregnancy associated plasma protein-A (PAPP-A) MoM in the outcome groups.

| Outcome group | Inhibin A MoM | PAPP-A MoM | Uterine artery PI MoM |
|---|---|---|---|
| Control | 0.98 (0.72-1.43) | 1.00 (0.69-1.45) | 1.05 (0.83-1.30) |
| Early-PE | 1.55 (0.95-2.05)* | 0.62 (0.42-1.11)* | 1.56 (1.20-1.69)* |
| Late-PE | 1.24 (0.89-1.65)* | 0.95 (0.58-1.31) | 1.26 (0.92-1.44)* |

Significance value * P < 0.05; PE: preeclampsia

There was no significant association between $log_{10}$ Inhibin-A MoM and $log_{10}$ uterine artery PI MoM (p=0.120).

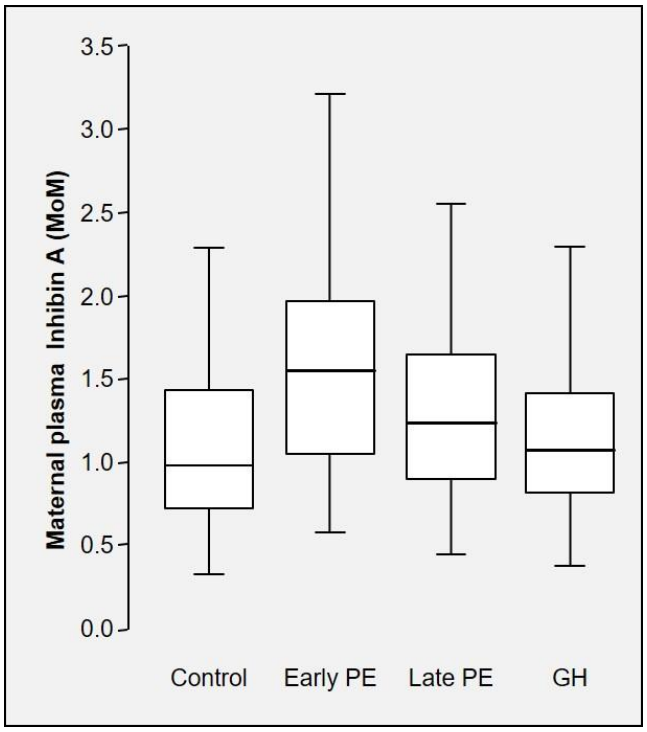



**Figure 3.5.** Box-and-whisker plot of inhibin-A multiple of the median (MoM) in the outcome groups.

Preeclampsia

Maternal plasma inhibin A and uterine artery PI were significantly higher in early-PE and late-PE than in controls (Table 3.7, Figure 3.5). Serum PAPP-A was significantly lower in early-PE than in controls but not in late-PE compared to controls (Table 3.7). There was no significant association between plasma Inhibin-A and uterine artery PI in the PE group (early-PE p=0.568 and late-PE p=0.492).

Logistic regression analysis demonstrated that in the prediction of early-PE there were significant contributions from $log_{10}$ uterine artery PI MoM (OR $4.442E^5$, 95% CI

413.0-4.8E$^8$; p<0.0001), $log_{10}$ PAPP-A MoM (OR 0.099, 95% CI 0.011-0.929; p=0.043), and $log_{10}$ Inhibin-A MoM (OR 249.6, 95% CI 9.3-6.688E$^3$; p=0.001), history of chronic hypertension (OR 143.3, 95%CI 8.3-2.463E$^3$; p=0.001), Afro-Caribbean racial origin (OR 5.5, 95% CI 1.4-21.8; p=0.016), mixed racial origin (OR 16.3, 95% CI 1.3-198.1; p=0.029) and parous with no previous PE (OR 0.159, 95% CI 0.039-0.651; p=0.011) but not BMI (p=0.190) or family history of PE (p=0.068); $R^2$=0.608, p<0.0001.

Logistic regression analysis demonstrated that in the detection of late-PE there were significant contributions from $log_{10}$ uterine artery PI MoM (OR 32.0, 95% CI 3.4-302.5; p=0.002), $log_{10}$ Inhibin-A MoM (OR 21.0, 95% CI 4.6-96.1; p<0.0001), BMI (OR 1.1, 95%CI 1.1-1.2; p<0.001), Afro-Caribbean racial origin (OR 3.8, 95% CI 2.0-7.3; p<0.0001) and parous with no previous PE (OR 0.123, 95% CI 0.062-0.243; p<0.0001) but not $log_{10}$ PAPP-A MoM (p=0.283), history of chronic hypertension (p=0.266) and family history of PE (p=0.063) $R^2$=0.377, p<0.0001.

The estimated DRs of early-PE at fixed FPRs of 5% and 10% in screening by maternal obstetric history and characteristics, Inhibin-A, PAPP-A, uterine artery PI and by their combinations are shown in Table 3.8 and the areas under the receiver operating characteristic curves are shown in Table 3.9. The estimated DR of screening for early-PE by Inhibin-A was 23.1% and 30.8% at respective FPR of 5% and 10% and the values increased to 84.6% and 88.5% in screening by a combination of maternal history and characteristics, Inhibin-A and uterine artery PI.

The estimated DR of late-PE at fixed FPR of 5% and 10% in screening by maternal obstetric history and characteristics, Inhibin-A, PAPP-A, uterine artery PI and by their combinations are shown in Table 3.8 and the AUROC curves are shown in Table 3.9. The estimated DR of screening for late-PE by Inhibin-A was 13.7% and 16.8% at respective FPR of 5% and 10% and the values increased to 36.8% and 55.8% in screening by a combination of maternal obstetric history and characteristics and plasma Inhibin-A.

**Table 3.8** Detection rate (DR) of early- and late-preeclampsia at fixed false positive rate (FPR) of 5% and 10% in screening by maternal obstetric history and characteristics, Inhibin-A, pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations.

| Screening test | Detection rate (%) | | | |
|---|---|---|---|---|
| | Early-PE | | Late-PE | |
| | FPR 5% | FPR 10% | FPR 5% | FPR 10% |
| History / characteristics | 47.5 | 57.7 | 31.6 | 43.2 |
| Inhibin A | 23.1 | 30.8 | 13.7 | 16.8 |
| PAPP-A | 23.1 | 42.3 | - | - |
| Uterine artery PI | 50.0 | 73.1 | 19.0 | 22.1 |
| History with | | | | |
| Inhibin A | 46.2 | 61.5 | 36.8 | 55.8 |
| PAPP-A | 69.2 | 69.2 | - | - |
| Inhibin A, PAPP-A | 57.7 | 73.1 | - | - |
| Uterine artery PI | 65.4 | 84.6 | 31.6 | 46.3 |
| Uterine artery PI, PAPP-A | 61.5 | 80.8 | - | - |
| Inhibin A, uterine artery PI | 84.6 | 88.5 | 33.7 | 42.1 |
| Inhibin A, PAPP-A, uterine artery PI | 80.8 | 88.5 | - | - |

**Table 3.9** Comparison of the performance of screening for preeclampsia by maternal obstetric history and characteristics, Inhibin-A, pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations by receiver-operating characteristics curve analysis.

| Screening test | AUROC, mean (95 %CI) | |
|---|---|---|
| | Early-PE | Late-PE |
| History / characteristics | 0.806 (0.749-0.854) | 0.783 (0.732-0.828) |
| Inhibin A | 0.679 (0.615-0.738) | 0.625 (0.568-0.680) |
| PAPP-A | 0.705 (0.643-0.763) | - |
| Uterine artery PI | 0.829 (0.774-0.875) | 0.623 (0.566-0.678) |
| History with | | |
| Inhibin A | 0.876 (0.827-0.916) | 0.815 (0.767-0.857) |
| PAPP-A | 0.866 (0.815-0.907) | - |
| Inhibin A and PAPP-A | 0.896 (0.849-0.932) | - |
| Uterine artery PI | 0.899 (0.853-0.934) | 0.799 (0.750-0.843) |
| Uterine artery PI and PAPP-A | 0.919 (0.877-0.951) | - |
| Inhibin A and uterine artery PI | 0.938 (0.899-0.965) | 0.823 (0.775-0.864) |
| Inhibin A, PAPP-A and uterine artery PI | 0.938 (0.899-0.965) | - |

### 3.3.4 Placental protein-13

Serum PP13 was measured in 208 cases and 416 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between PP13 MoMs and serum PAPP-A and uterine artery PI MoMs in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ PP13 MoM with $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ PP13 significant independent contributions were provided by maternal weight and smoking but not by racial origin (p=0.594), parity (p=0.870) or fetal CRL (p=0.707): log expected PP13 = 2.089 - 0.004 x maternal weight in Kg + (- 0.214 if smoker, 0 if non-smoker); $R^2$=0.154, p<0.0001.

In the control group, the median PP13 MoM was 1.02 (0.78-1.30) (Table 3.10, Figure 3.6). The median PAPP-A MoM was 1.08 (0.75-1.48) and uterine artery PI MoM was 0.97 (0.77-1.22). (Table 3.10, Figure 3.6). There was a significant association between $\log_{10}$ PP13 MoM and $\log_{10}$ PAPP-A MoM (r=0.269, p<0.0001) but not $\log_{10}$ uterine artery PI MoM (p=0.0.376).

Preeclampsia group

In those who subsequently developed early-PE, compared to controls, serum PP13 and PAPP-A were significantly decreased and uterine L-PI was increased (Table 3.10,

Figure 3.6). In those who subsequently developed late-PE, compared to controls, PAPP-A was decreased and uterine L-PI was increased but serum PP13 was not significantly different.

**Table 3.10** Median (interquartile range) of maternal serum placental protein 13 (PP13) multiple of the median (MoM), pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) MoM in the control, early- and late- preeclampsia groups.

| Outcome group | PP13 MoM | PAPP-A MoM | Uterine artery PI MoM |
|---|---|---|---|
| Control | 1.02 (0.78-1.30) | 1.08 (0.75-1.48) | 0.97 (0.77-1.22) |
| Early-PE | 0.83 (0.55-1.15)* | 0.55 (0.37-0.94)* | 1.61 (1.31-1.73)* |
| Late-PE | 0.96 (0.75-1.25) | 0.84 (0.55-1.18)* | 1.25 (0.88-1.52)* |

Mann-Whitney test: * $P < 0.0167$

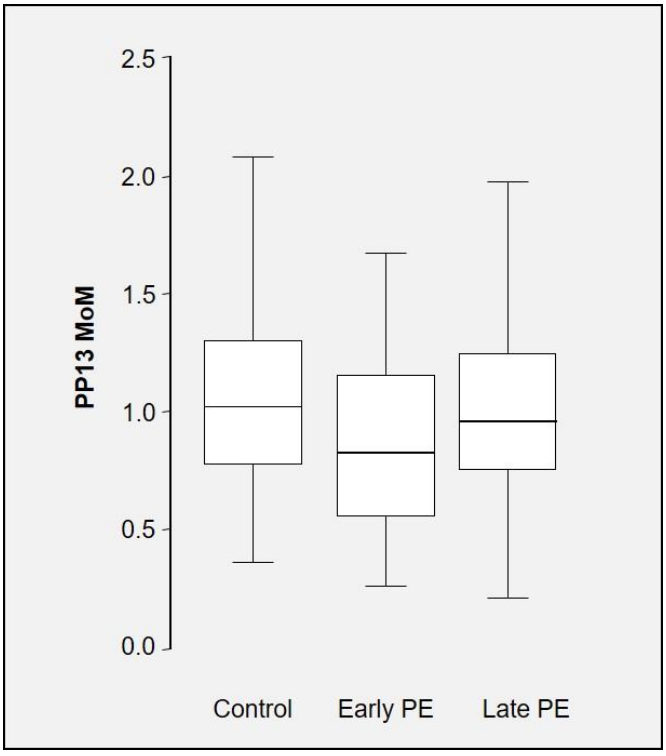



**Figure 3.6.** Box-and-whisker plot of placental protein-13 (PP13) multiple of the median (MoM) in outcome groups.

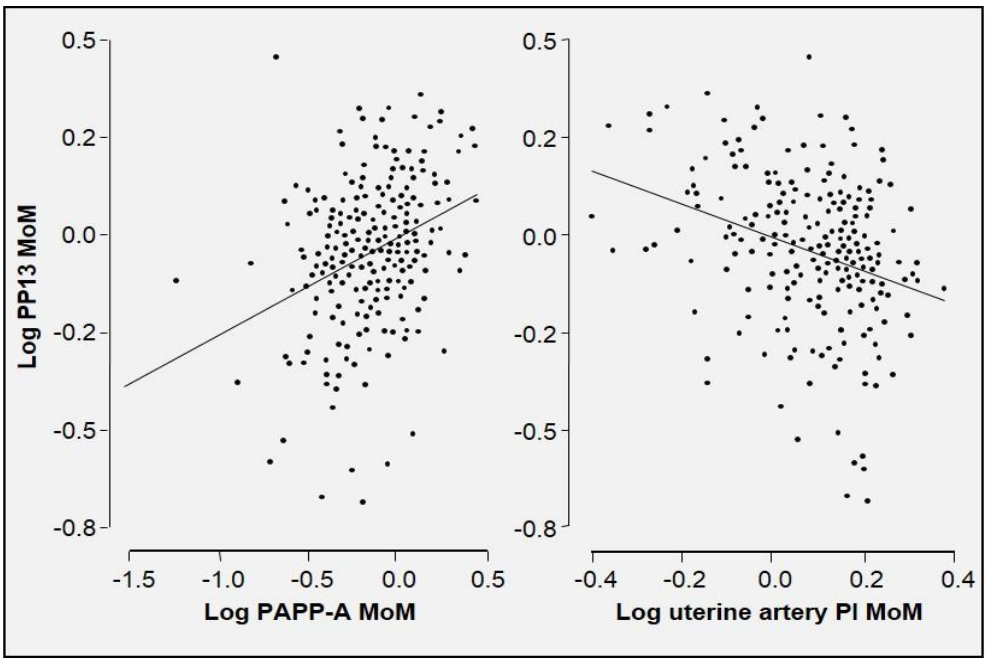



**Figure 3.7.** Relationship between maternal serum placental protein-13 (PP13) with pregnancy associated plasma protein-A (PAPP-A) and uterine artery PI in those that developed preeclampsia.

In the PE group there was a significant association between serum PP13 and both uterine artery L-PI (r=-0.342, p<0.0001) and serum PAPP-A (r=0.328, p<0.0001) (Figure 3.7).

Logistic regression analysis demonstrated that in the prediction of early-PE there were significant contributions from $\log_{10}$ maternal factor-derived *a priori risk* (OR 15.9, 95%CI 6.1-41.5), $\log_{10}$ uterine artery L-PI MoM (OR 5.1E4, 95% CI 1243.4-2.1E$^{6}$; p<0.0001) and $\log_{10}$ PAPP-A MoM (OR 0.07, 95% CI 0.02-0.27; p<0.0001).

Although $\log_{10}$ PP13 MoM did provide significant contribution to prediction of early-PE, it did not improve significantly the performance of screening for early-PE provided by the combination of the maternal factor-derived a priori risk, uterine artery L-PI and serum PAPP-A.

**Table 3.11** Detection rate (DR) of early-preeclampsia at fixed false positive rate (FPR) of 5% and 10% and comparison of screening performance by area under the receiver operating characteristic curves (AUROC) curve analysis in screening by maternal risk factor, placental protein 13 (PP13), pregnancy associated plasma protein-A (PAPP-A), uterine artery L-pulsatility index (PI) and by their combinations.

| Screening test | FPR 5% | FPR 10% | AUROC (95 %CI) |
|---|---|---|---|
| Maternal risk factor | 39.0 | 49.0 | 0.785 (0.745-0.822) |
| PP13 | 20.8 | 37.5 | 0.652 (0.606-0.695) |
| PAPP-A | 27.1 | 37.5 | 0.744 (0.701-0.783) |
| Uterine artery L-PI | 45.8 | 68.7 | 0.863 (0.829-0.893) |
| Maternal risk factor plus | | | |
| PP13 | 37.5 | 52.1 | 0.818 (0.779-0.852) |
| PAPP-A | 47.9 | 54.2 | 0.872 (0.838-0.901) |
| PP13 and PAPP-A | 52.1 | 60.4 | 0.878 (0.845-0.906) |
| Uterine artery L-PI | 58.3 | 75.0 | 0.920 (0.891-0.943) |
| Uterine artery L-PI and PP13 | 66.7 | 77.1 | 0.924 (0.896-0.946) |
| Uterine artery L-PI and PAPP-A | 64.6 | 81.2 | 0.936 (0.910-0.957) |

The estimated DR of early-PE at fixed FPR of 5% and 10% and their respective AUROC in screening by maternal factor-derived *a priori risk*, PP13, PAPP-A, uterine artery L-PI and by their combinations are shown in Table 3.11. The estimated DR of screening for early-PE by PP13 independently was 20.8% and 37.5% at respective FPR of 5% and 10%. The addition of PP13 did not improve the detection rate of early-PE that was achieved by a combination of maternal factor-derived *a priori risk*, uterine artery PI and serum PAPP-A. The maternal serum PP13 in late-PE was not significantly different from controls and therefore did not add value in screening for late-PE.

### 3.3.5 P-Selectin

Serum P-Selectin was measured in 121 cases and 208 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the

correlation between P-selectin MoMs and uterine artery PI MoMs in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ P-selectin MoM with $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ P-selectin significant independent contributions were provided by racial origin and method of conception but not CRL (p=0.180) or maternal weight (p=0.435) or smoking (p=0.224): log expected P-selectin = 1.482 + (- 0.050 if Afro-Caribbean, -0.251 if East Asian, 0 if other racial origins) + (-0.167 if conception with ovulation drugs, 0 if spontaneous or IVF conception); $R^2$=0.075, p<0.0001.

In the control group, the median P-selectin MoM was 1.01 (IQR 0.84-1.25) and the median uterine artery PI MoM was 1.04 (IQR 0.85-1.30). (Table 3.12). There were no significant associations between plasma P-selectin and uterine artery PI in the control group (p=0.379).

**Table 3.12.** Median (interquartile range) for P-selectin and uterine artery PI in the outcome groups.

| Outcome group | Plasma P-selectin MoM (median, IQR) | Uterine artery PI MoM (median, IQR) |
|---|---|---|
| Control | 1.01 (0.84-1.25) | 1.04 (0.85-1.30) |
| PE | 1.20 (0.94-1.43)* | 1.30 (0.98-1.56)* |

Significance level p<0.05

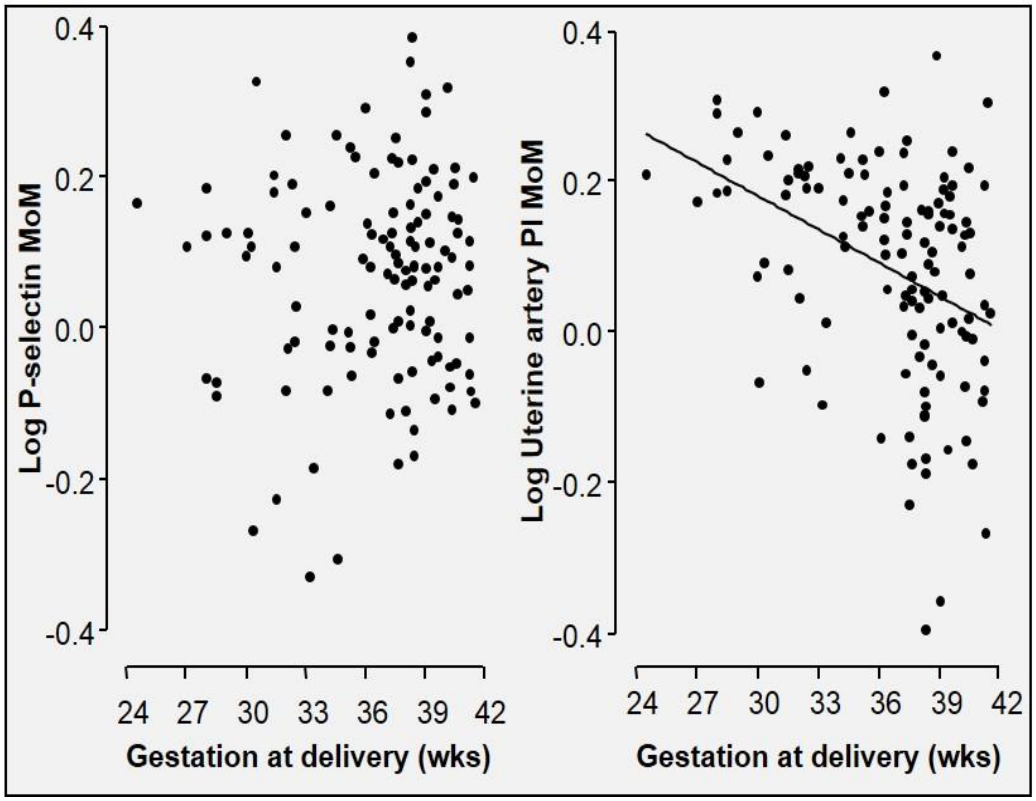



**Figure 3.8.** Relationship between maternal serum P-selectin (left) and uterine artery pulsatility index (PI) (right) with gestational age at delivery (weeks).

Preeclampsia group

Plasma P-selectin and uterine artery PI were higher in PE than in controls (Table 3.13). There were no significant associations between plasma P-selectin and uterine artery PI in the PE group (p=0.072). In the PE group, there was a significant association of uterine artery PI with gestation at delivery (r=-0.384, p<0.0001; Figure 3.8) but no significant association with P-selectin (p=0.579; Figure 3.8).

Multiple logistic regression analysis demonstrated that for prediction of early-PE, significant contributions were provided by maternal factors and uterine artery PI but not by maternal plasma P-selectin: Y= -3.470 +14.13 x $\log_{10}$ uterine artery PI MoM +

(1.083 if Afro-Caribbean, 0 if other racial origins) + (0 if nulliparous or parous with previous PE, -1.30 if parous without previous PE) + 4.875 if history of chronic hypertension; $R^2$=0.470, p<0.001.

In screening for early-PE by a combination of maternal factors and uterine artery PI the area under the ROC curve was 0.903 (95% CI 0.836-0.971) and at FPR of 5% and 10% the DR were 63.0% and 85.2%, respectively.

Multiple logistic regression analysis demonstrated that for prediction of late-PE significant contributions were provided by maternal factors, uterine artery PI and plasma P-selectin: Y = -3.678 + 4.926 x $\log_{10}$ P-selectin MoM + 3.345 x $\log_{10}$ uterine artery PI MoM + 0.105 x BMI in Kg/m2 + (1.326 if Afro-Caribbean, 0 if other racial origins) + (0 if nulliparous or parous with previous PE, - 1.979 if parous without previous PE) + 1.251 if family history of PE; $R^2$=0.397, P<0.0001.

In screening for late-PE by a combination of maternal factors and uterine artery PI the area under the ROC curve was 0.797 (95% CI 0.745-0.849) and at FPR of 5% and 10% the DR were 31.9% and 42.6%, respectively. In screening by a combination of maternal factors, uterine artery PI and plasma P-selectin the area under the ROC curve was 0.830 (95% CI 0.783-0.876) and at FPR of 5% and 10% the DR were 36.2% and 50.0%, respectively.

### 3.3.6 Pentraxin-3

Serum PTX3 was measured in 120 cases and 207 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between PTX3 MoMs and uterine artery PI MoMs in cases and controls were investigated. Linear regression analysis was used to examine the relationship between $\log_{10}$ PTX3 MoM with $\log_{10}$ uterine artery PI MoM.

Control group

Multiple regression analysis in the control group demonstrated that for $log_{10}$ PTX3 significant independent contributions were provided by racial origin and maternal weight but not fetal CRL (p=0.658), smoking (p=0.089), parity (p=0.923) or method of conception (p=0.170): $log_{10}$ expected PTX3 = -0.078 + (- 0.003 X maternal weight in kg) + (-0.122 if South Asian, 0 if other racial origins); $R^2$=0.042, p=0.004.

In the control group, the median PTX3 MoM was 0.99 (IQR 0.80-1.29) and uterine artery PI MoM was 1.03 (IQR 0.84-1.24). (Table 3.14). There was no significant association between plasma PTX3 and uterine artery PI in the control group (p=0.209).

Preeclampsia group

Plasma PTX3 MoM was significantly higher in early-PE compared to controls but not in late-PE (Table 3.14). Uterine artery PI MoM was significantly increased in both early-PE and late-PE than in controls (Table 3.13).

**Table 3.13.** Median (interquartile range) for plasma pentraxin 3 multiple of the median (MoM) and uterine artery pulsatility index (PI) MoM in the outcome groups.

| Outcome group | Plasma pentraxin MoM | Uterine artery PI MoM |
|---|---|---|
| Control | 0.97 (0.74-1.22) | 1.05 (0.84-1.31) |
| Early preeclampsia | 1.44 (0.83-2.00)* | 1.54 (1.20-1.68)* |
| Late preeclampsia | 1.11 (0.78-1.63) | 1.26 (0.94-1.45)* |

Adjusted significance level * P<0.01

There were no significant associations between plasma PTX3 and uterine artery PI in the PE group (p=0.693) (Figure 3.9).

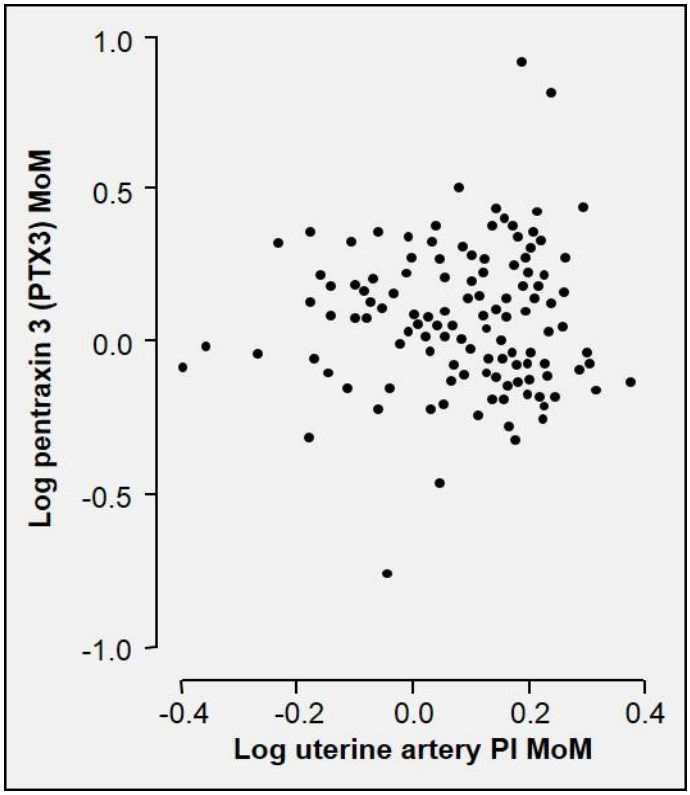



**Figure 3.9.** Association between pentraxin-3 (PTX3) uterine artery pulsatility index (PI) multiple of the median (MoM) in the preeclampsia group.

### 3.3.7 Soluble Endoglin

Serum sEng was measured in 90 cases and 180 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between sEng MoMs and serum PAPP-A, PLGF and uterine artery PI MoMs in cases and controls were investigated.

Linear regression analysis was used to examine the relationship between $\log_{10}$ sEng MoM with $\log_{10}$ PAPP-A MoM, $\log_{10}$ PLGF MoM and $\log_{10}$ uterine artery PI MoM. Multivariate logistic regression analysis was used to determine factors providing a significant contribution in prediction of early-PE and late-PE. Performance of screening was examined by AUROC curves analysis.

Control group

Multiple regression analysis in the control group demonstrated that for $log_{10}$ sEng significant independent contributions were provided by maternal weight but not by racial origin (p=0.602), parity (p=0.757), smoking (p=0.365) or fetal CRL (p=0.675): $Log_{10}$ expected sEng = 4.313 - 0.002 x maternal weight in kg; $R^2$=0.154, p=0.013.

In the control group, the median sEng MoM was 0.98 (0.78-1.30) (Table 3.15, Figure 3.10). The median PAPP-A MoM was 1.01 (0.77-1.32), PLGF MoM was 1.01 (0.83-1.33) and uterine artery L-PI MoM was 1.00 (0.80-1.23). (Table 3.14, Figure 3.10).

There was a significant association between plasma sEng and serum PAPP-A (r=0.176, p=0.019) but not serum PLGF (p=0.236) or uterine artery L-PI (p=0.726).

Preeclampsia

In early-PE, compared to controls, plasma sEng and uterine L-PI were significantly increased and serum PAPP-A and PlGF were decreased (Table 3.15, Figure 3.10). In late-PE, compared to controls, serum PlGF was decreased and uterine L-PI was increased but plasma sEng and serum PAPP-A were not significantly different.

In the PE group there was a significant association between plasma sEng and both uterine artery L-PI (r=0.212, p=0.045) and serum PlGF (r=-0.434, p<0.0001) but not serum PAPP-A (p=0.253).

Logistic regression analysis demonstrated that in the prediction of early-PE there were significant contributions from $log_{10}$ maternal factor-derived *a priori risk* [odds ratio (OR) 9.5, 95%CI 2.6-34.6; p=0.001], $log_{10}$ uterine artery L-PI MoM (OR 1.6E+05, 95% CI 353.0-8.1E+07; p<0.0001), $log_{10}$ PlGF MoM (OR 0.002, 95% CI 0.0-0.10; p=0.001) and $log_{10}$ sEng MoM (OR 465.2, 95%CI 5.2-4.1E+04; p=0.007), but not from $log_{10}$ PAPP-A MoM (p=0.240).

**Table 3.14** Median (interquartile range) of maternal serum soluble endoglin (sEng) multiple of the median (MoM), placental growth factor (PLGF) MoM, pregnancy associated plasma protein-A (PAPP-A) MoM and uterine artery L-pulsatility index (PI) MoM in the control, early- and late-preeclampsia groups.

| Outcome group | sEng MoM | PlGF MoM | PAPP-A MoM | Uterine artery PI MoM |
|---|---|---|---|---|
| Control | 0.98 (0.78-1.30) | 1.01 (0.83-1.33) | 1.01 (0.77-1.32) | 1.00 (0.80-1.23) |
| Early-PE | 1.38 (1.06-1.87)* | 0.61 (0.46-0.84)* | 0.56 (0.47-0.90)* | 1.65 (1.31-1.85)* |
| Late-PE | 0.90 (0.73-1.21) | 0.82 (0.53-1.03)* | 0.93 (0.57-1.18) | 1.31 (1.13-1.55)* |

Adjusted significance level * p<0.0167

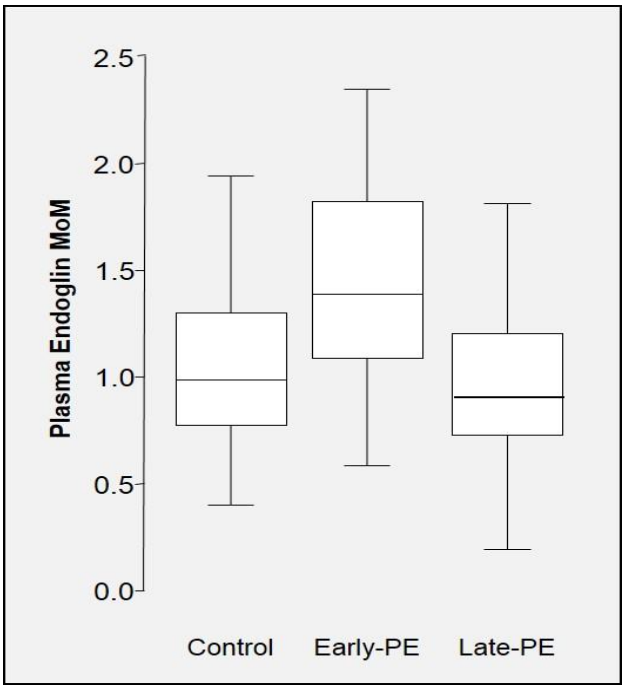



**Figure 3.10** Box-whisker plot of soluble endoglin (sEng) multiple of the median (MoM) in controls, early- and late- preeclampsia.

The patient-specific risk for early-PE was calculated from the formula: odds / (1+odds), where odds=eY and Y was derived from multivariate logistic regression analysis of the $\log_{10}$ transformed maternal factor-derived *a priori risk*, $\log_{10}$ uterine artery L-PI MoM, $\log_{10}$ PlGF MoM and $\log_{10}$ sEng MoM: Y= 0.991+ 2.249 X $\log_{10}$

maternal factor-derived *a priori risk* + 12.039 X $\log_{10}$ uterine artery L-PI MoM – 6.032 X $\log_{10}$ PlGF MoM + 6.142 X $\log_{10}$ sEng MoM; $R^2$=0.674, p<0.0001.

**Table 3.15.** Detection rate (DR) of early-preeclampsia at fixed false positive rate (FPR) of 5% and 10% and comparison of screening performance by ROC curve analysis in screening by maternal risk factor, soluble endoglin (sEng), placental growth factor (PLGF), uterine artery L- pulsatility index (PI) and by their combinations.

| | DR for Early preeclampsia | | |
|---|---|---|---|
| **Screening test** | **FPR 5%** | **FPR 10%** | **AUROC (95 %CI)** |
| Maternal factors | 29.6 | 40.0 | 0.751 (0.686-0.808) |
| sEng | 30.0 | 46.7 | 0.742 (0.677-0.800) |
| PlGF | 25.9 | 59.3 | 0.831 (0.772-0.880) |
| PAPP-A | 20.0 | 43.4 | 0.741 (0.676-0.799) |
| Uterine artery L-PI | 60.0 | 73.3 | 0.870 (0.817-0.913) |
| Maternal factors plus | | | |
| sEng | 33.3 | 56.7 | 0.865 (0.811-0.908) |
| PlGF | 48.1 | 66.7 | 0.902 (0.852-0.939) |
| PAPP-A | 46.7 | 53.3 | 0.839 (0.782-0.886) |
| sEng and PlGF | 55.6 | 74.1 | 0.923 (0.877-0.955) |
| sEng and PAPP-A | 63.3 | 73.3 | 0.905 (0.857-0.941) |
| Uterine artery L-PI | 63.3 | 76.7 | 0.908 (0.860-0.943) |
| Uterine artery L-PI and PAPP-A | 60.0 | 83.3 | 0.923 (0.878-0.955) |
| Uterine artery L-PI and sEng | 66.7 | 80.0 | 0.937 (0.895-0.966) |
| Uterine artery L-PI and PlGF | 77.8 | 85.2 | 0.945 (0.904-0.972) |
| Uterine artery L-PI, PAPP-A and sEng | 63.3 | 83.3 | 0.943 (0.903-0.971) |
| Uterine artery-L-PI, PlGF and sEng | 77.8 | 96.3 | 0.949 (0.909-0.975) |

The estimated DR of early-PE at fixed FPR of 5% and 10% and their respective AUROC curves in screening by maternal factor-derived *a priori risk*, sEng, PAPP-A, PLGF, uterine artery L-PI and by their combinations are shown in Table 3.15. The estimated DR of screening for early-PE by sEng independently was 30.0% and 46.7% at respective FPR of 5% and 10%.

The combination of maternal factor-derived *a priori risk*, uterine artery L-PI, PLGF and sEng achieved a DR of 77.8% and 96.3% for FPR of 5% and 10% respectively. The maternal plasma sEng in late-PE was not significantly different from controls and therefore did not add value in screening for late-PE.

### 3.3.8 Plasminogen activator inhibitor-2

Serum PAI-2 was measured in 119 cases and 204 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between PAI-2 MoMs and uterine artery PI MoMs in cases and controls were investigated. Linear regression analysis was used to examine the relationship between $\log_{10}$ PAI-2 MoM with $\log_{10}$ uterine artery PI MoM in PE and control groups.

Control group

Multiple regression analysis in the control group demonstrated that for $\log_{10}$ PAI-2 significant independent contribution was provided by maternal BMI but not by fetal CRL (p=0.365), smoking (p=0.800), parity (p=0.824) or racial origin (p=0.239): $\text{Log}_{10}$ expected PAI-2 = 1.895 + (- 0.006 X BMI in kg/m2); $R^2$=0.032, p=0.006.

In the control group, the median PAI-2 MoM was 0.96 (0.77-1.28). The median uterine artery PI MoM was 1.05 (0.83-1.29). (Table 3.16). There were no significant associations between plasma PAI-2 and uterine artery PI in the control group (p=0.14).

Preeclampsia group

There was no significant difference in maternal plasma PAI-2 MoM in patients with PE compared to controls whereas the uterine artery PI was significantly increased in PE compared to the controls (Table 3.16). There were no significant associations between plasma PAI-2 and uterine artery PI in the PE group (p=0.70).

**Table 3.16** Median (interquartile range) for maternal plasma plasminogen activator inhibitor-2 (PAI-2) and uterine artery pulsatility index (PI) in the outcome groups.

| Outcome group | PAI-2 MoM | Uterine artery PI MoM |
|---|---|---|
| Control | 0.96 (0.77-1.28) | 1.05 (0.83-1.29) |
| Preeclampsia | 1.07 (0.86-1.33) | 1.32 (0.98-1.56)* |

Significance level * P<0.01.

### 3.3.9 Angiopoietin-2

Serum Ang-2 was measured in 126 cases and 214 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared in the outcome groups.

Control group

Multiple regression analysis in the control group demonstrated that for $log_{10}$ Ang-2 significant independent contributions were provided by maternal weight but not fetal CRL (p=0.877), smoking (p=0.863), parity (p=0.797) or Afro-Caribbean racial origin (p=0.093). $Log_{10}$ expected Ang-2 = 0.995 + (- 0.003 x maternal weight in Kg); $R^2$=0.016, p=0.036.

Preeclampsia group

There was no significant difference in maternal serum Ang-2 levels in PE compared to controls. In contrast, uterine artery PI was significantly increased in PE compared to controls. (Table 3.17)

**Table 3.17** Median (interquartile range) for maternal serum angiopoetin-2 (Ang-2) and uterine artery pulsatility index (PI) in the outcome groups.

| Outcome group | Ang-2 MoM | Uterine artery PI MoM |
|---|---|---|
| Control | 1.07 (0.70-1.45) | 1.05 (0.83-1.29) |
| Preeclampsia | 0.96 (0.62-1.48) | 1.32 (0.99-1.56)* |

Significance level * P<0.01.

### 3.3.10 Soluble fms-like tyrosine kinase-1

Serum s-Flt-1 was measured in 90 cases and 180 controls. The measured values were converted to MoMs. The MoMs in cases and controls were compared and the correlations between sFlt-1 MoMs and serum PLGF and uterine artery PI MoMs in cases and controls were investigated. Linear regression analysis was used to examine the relationship between $\log_{10}$ sFlt-1 MoM with $\log_{10}$ PLGF MoM and $\log_{10}$ uterine artery PI MoM.

Control group

Multiple regression analysis in the control group demonstrated that $\log_{10}$ sFlt-1 did not change with fetal CRL (p=0.227), maternal age (p=0.874), racial origin (p=0.963), parity (p=0.524), maternal weight (p=0.987), method of conception (p=0.531) or smoking status (p=0.672).

In the control group, the median sFlt-1 was 6349 (IQR 3697-10153) (Table 3.18, Figure 3.11). The median PLGF MoM was 1.03 (IQR 0.83-1.33) and uterine artery PI MoM was 0.99 (IQR 0.80-1.23). (Table 3.18). There was no significant association between plasma sFlt-1 and serum PlGF (p=0.299), gestation at delivery (p=0.264) or birth weight centile (p=0.729) but there was a significant association between plasma sFlt-1 and uterine artery L-PI (r=-0.166, p=0.026).

Preeclampsia

In the pregnancies that subsequently developed early-PE and late-PE, the median plasma sFlt-1 concentration was not significantly different from that in the control group (Figure 3.11), whereas in both early-PE and late-PE the uterine artery L-PI was significantly increased and serum PlGF was significantly decreased (Table 3.18).

**Table 3.18** Median (interquartile range) of maternal serum placental growth factor (PLGF) multiple of the median (MoM), pregnancy associated plasma protein-A (PAPP-A) MoM and uterine artery pulsatility index (PI) MoM in the control, early- and late-preeclampsia groups.

| Outcome group | sFlt-1 pg/mL | PLGF MoM | Uterine artery PI MoM |
|---|---|---|---|
| Control | 6349 (3697-10153) | 1.03 (0.83-1.33) | 0.99 (0.80-1.23) |
| Early-PE | 7099 (4769-58270) | 0.61 (0.46-0.84)* | 1.65 (1.31-1.85)* |
| Late-PE | 6840 (4200-11381) | 0.82 (0.53-1.03)* | 1.31 (1.13-1.55)* |

Adjusted significance level * $P$ <0.0167

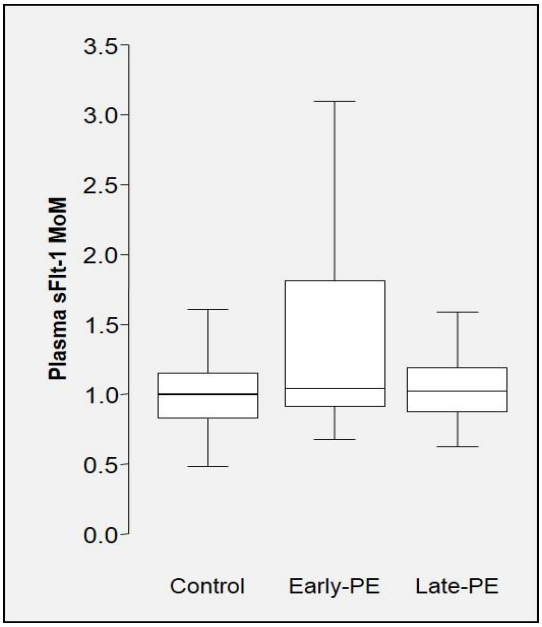



**Figure 3.11** Box-whisker plot of soluble fms-like tyrosine kinase-1 (sFlt-1) multiple of the median (MoM) in controls, early- and late- preeclampsia.

In the PE group there was no significant association between plasma sFlt-1 and uterine artery L-PI (p=0.736), serum PlGF (p=0.676), gestation at delivery (p=0.102) or birth weight centile (p=0.153).

## 3.4 DISCUSSION

The results of this study demonstrates that the maternal serum or plasma levels of PlGF, Activin A, Inhibin A, PP13, sEng, PTX-3 and P-selectin are significantly altered at 11-13 weeks' gestation in pregnancies that subsequently develop PE whereas there is no significant difference in the levels of PAI-2, Ang-2 and sFlt-1 in women who develop PE compared to controls. There is a significant reduction in levels of PlGF and PP-13 whereas the levels of Activin A, Inhibin A, sEng, PTX-3 and P-selection are increased in pregnancies destined to develop PE. The levels of PlGF, Inhibin A, PP-13 were significantly different in both early and late-PE but the levels of Activin A and P-Selectin were only altered in late-PE whereas PTX-3 was different from controls only in early-PE but not in late-PE.

There is a significant effect of maternal characteristics on the maternal serum or plasma concentration of these biochemical markers in unaffected pregnancies and consequently, the measured concentration of these markers must be adjusted for these maternal and pregnancy characteristics before comparison with pathological pregnancies (Kagan *et al.*, 2008a). The maternal characteristics affecting the serum or plasma levels include gestational age, maternal weight, racial origin, method of conception and smoking. There was an inverse relationship with maternal weight in case of almost all biochemical markers including PlGF, Activin A, Inhibin A, PP13, PTX-3, sEng, PAI-2 and Ang-2 except in case of P-selectin and sFlt-1. The association of maternal serum concentration with maternal weight reflects the effect of dilution of the levels of biomarker in plasma volume, which increases with maternal weight. The levels are also affected by racial origin and the concentration of PlGF, Activin A and inhibin A is higher in women of Afro-Caribbean origin compared to Caucasian women. The other factor affecting concentration is maternal smoking status and the

levels of PP-13 and P-selectin are lower in cigarette smokers compared to non-smokers whereas the levels of PlGF are higher in smokers.

The biochemical markers also demonstrate a relationship with established markers of placentation reflected in uterine artery Doppler and maternal serum PAPP-A. In the case of serum PlGF, Activin-A and PP-13, there was a significant linear relationship with maternal serum PAPP-A whereas there was a significant relationship between PlGF, PP-13 and sEng with uterine artery PI suggesting a common pathogenesis of impaired trophoblastic relationship. In the case of biochemical markers such as inhibin A, PTX-3 and P-selectin there was no association with either serum PAPP-A or uterine artery Doppler suggesting that the increased levels of this biomarker are secondary to a mechanism that is likely to be different than placental dysfunction. There was no association of Ang-2, PAI-2, sFlt-1 with neither serum PAPP-A nor uterine artery PI and the levels of these biomarkers are not altered in PE suggesting that they are not involved in the pathogenesis of PE at least in the first trimester of pregnancy. In the PE group, there was a significant association of PlGF and PP-13 with gestational age delivery which supports the hypothesis that these biochemical markers are associated with impaired trophoblastic proliferation which is reflected in their inverse relationship with gestational age at delivery and therefore severity of disease.

The biochemical markers providing a significant contribution to early screening for PE either alone or in combination with maternal factor derived *a priori* risk, uterine artery PI and serum PAPP-A were PlGF, Activin A, Inhibin-A, sEng, PP-13 and P-selectin. The DR of early-PE from maternal serum PlGF alone at a FPR of 10% was about 50% which improved to 90% when PlGF was added to the combination of maternal factors and uterine artery PI. Similarly, the estimated DR of screening for early-PE by Inhibin-A was 30.8% at FPR of 10% and this increased to 88.5% in screening by a combination of maternal factors, Inhibin-A and uterine artery PI. Although, Activin A was higher in women with PE but when added to the combination of maternal factors and uterine artery PI, the contribution was not significant. The estimated DR of screening for early-PE by sEng independently was 46.7% at FPR of

10%. The combination of maternal factor-derived *a priori risk*, uterine artery L-PI, PLGF and sEng achieved a DR of 96.3% for FPR of 10%. The estimated DR of screening for early-PE by PP13 independently was 37.5% at respective FPR of 10%. The addition of PP13 did not improve the detection rate of early-PE that was achieved by a combination of maternal factor-derived *a priori risk*, uterine artery PI and serum PAPP-A.

Similarly, in screening for late-PE, the DR of PlGF and inhibin-A for a FPR of 10% was 32 and 17%, respectively, which improved to 49 and 42 % when PlGF was added to the combination of maternal factors and uterine artery PI. Although the levels were increased in late-PE compared to controls but the addition of Activin-A to the combination of maternal factors and uterine artery PI did not provide a significant contribution. In case of P-selectin, addition to P-selectin to the combination of maternal factors and uterine artery PI increased the DR of late-PE from 53 to 50%.

These findings demonstrate that there are a series of biochemical markers which are placental products involved either in placentation or in the cascade of events leading from impaired placentation to placental ischemia and damage with release of inflammatory factors which cause platelet activation and endothelial dysfunction and consequent development of the clinical symptoms of the disease. These biochemical markers are altered in pregnancies from as early as 11-13 weeks' of gestation. It is also evident that there is a relation of these biochemical markers with established markers of placentation and the combination of these biochemical markers with maternal factor derived *a priori* risk and markers of placentation such as uterine artery Doppler significantly improves the performance of screening for PE. Such early screening would allow identification of women at high-risk and this could potentially improve pregnancy outcome because not only that these women would benefit from intensive maternal and fetal monitoring but they could be administered treatments that could potentially lead to prevention and reducing the prevalence of the disease. The findings from these case-control studies need to be validated in further larger prospective studies.

# CHAPTER 4

## PREDICTION OF PREECLAMPSIA FROM MATERNAL FACTORS, BIOPHYSICAL AND BIOCHEMICAL MARKERS AT 11-13 WEEKS

## 4.1 INTRODUCTION

### 4.1.1 Background

The traditional approach to screening for PE is based on risk factors identified from maternal characteristics and medical history (NICE 2008). An alternative approach is to combine maternal factors into an algorithm derived by multivariate analysis to estimate the individual patient-specific risk for PE. Such *prior* risk can then be combined with a series of biophysical and biochemical markers. Useful biophysical markers are uterine artery PI and MAP (Placensia *et al.,* 2007 and Poon *et al.,* 2008) and potentially useful biochemical markers are PLGF, PAPP-A, PP13, sEng, inhibin-A, activin-A, PTX3 and P-selectin (Chapter 3).

There is evolving evidence that both the degree of impaired placentation and the incidence of adverse fetal and maternal short-term and long-term consequences of PE are inversely related to the gestational age at onset of the disease (Chapter 1.3.3). Consequently, the end-point in screening for PE by first-trimester biophysical and biochemical markers should not be total PE but the condition should be subdivided according to gestational age at delivery.

This subdivision has so far been limited to early-PE, requiring delivery before 34 weeks and late PE. In our ongoing studies there are now sufficient data to allow further subdivision of the cases delivering at or after 34 weeks into intermediate PE and late PE groups, delivering at 34-37 weeks and after 37 weeks, respectively.

### 4.1.2 Objectives

The objectives of this chapter are to develop algorithms based a combination of maternal factors, uterine artery PI, MAP and serum biomarkers to estimate patient-specific risks for early-, intermediate- and late-PE and to evaluate the screening performance of such algorithms.

## 4.2 METHODS

### 4.2.1 Study population

The study comprised of two components. The first population included 36,743 pregnancies undergoing routine screening for pregnancy complications at 11-13 weeks. In all cases we recorded maternal factors and serum PAPP-A. Additionally, in 21,693 cases we measured uterine artery PI and in 13,946 we measured MAP. The measurements of uterine artery PI and MAP were not based on any pre-selection of the patients but were introduced into routine practice sequentially in time.

The second component of the study comprised of case-control studies, arising from the screened population, in which measurements of biochemical markers were carried out.

### 4.2.2 Statistical analysis

In this study we developed a model for predicting PE based on maternal characteristics in the whole screened population. We then expanded this model for prediction of PE to include the addition of firstly, uterine artery PI, MAP and serum PAPP-A derived from the screened population and secondly, serum or plasma PLGF, PP13, sEng, inhibin-A, activin-A, PTX3 and P-selectin derived from case-control studies. A detailed description of study population (Section 2.1) and statistical analyses (Section 2.7) are presented in Chapter 2.

## 4.3 RESULTS

The maternal characteristics and history in each PE group and the controls in the screening population and in the subgroups with measurements of uterine artery PI, MAP and serum PLGF are shown in Chapter 2 (Tables 2.1 to 2.4).

### 4.3.1 Biophysical and biochemical markers in unaffected pregnancies

Multiple regression analyses in the unaffected pregnancies demonstrated that for each marker significant independent contributions were provided by certain maternal characteristics. Expected $Log_{10}$ values for sEng, inhibin-A, activin-A, PTX3 and P-selectin are given in Chapter 3. Values for uterine artery PI, MAP, PLGF and PP13 are given below.

$Log_{10}$ uterine artery PI expected = 0.438 (SE 0.017) - 0.001 (SE 0.0001) x maternal age in years - 0.002 (SE 0.0004) x maternal weight in kg + $5.96e^{-06}$ (SE $2.55e^{-06}$) x (maternal weight in kg) $^2$ + [0.009 (SE 0.003) if cigarette smoker, 0 if not] - 0.002 (SE 0.0001) x fetal CRL in mm + [0.028 (SE 0.002) if Afro-Caribbean, 0.016 (SE 0.005) if Mixed, 0 if any other racial origin]; $R^2$=0.034, p<0.0001.

$Log_{10}$ MAP expected = 1.797 (SE 0.007) + 0.001 (SE 6.06 $e^{-05}$) x maternal age in years + 0.003 (SE $1.57e^{-04}$) x maternal weight in kg – $1.0e^{-04}$ (SE $1.01e^{-06}$) x (maternal weight in kg)$^2$ + [0.006 (SE $6.8e^{-04}$) if nulliparous, 0 if parous] – [0.008 (SE 0.001) if cigarette smoker, 0 in non-smoker] + [(0.066 (SE 0.003) if chronic hypertension, 0 if not] - $1.68e^{-04}$ (SE $4.25e^{-05}$) x fetal CRL in mm + [0.011 (SE 0.005) if diabetes mellitus type 1, 0 if not] – [0.002 (SE 0.001) if Afro-Caribbean, -0.004 (SE 0.002) if Mixed, 0 if any other racial origin]; $R^2$=0.161, p<0.0001.

$Log_{10}$ PlGF expected = 0.932 (SE 0.042) + 0.002 (SE 0.001) x maternal age in years - 0.002 (SE $3.0e^{-04}$) x maternal weight in kg + [0.174 (SE 0.015) if cigarette smoker, 0 if non-smoker] + 0.009 (SE $5.1e^{-04}$) x fetal CRL in mm + [0.177 (SE 0.010) if Afro-Caribbean, 0.0871 (SE 0.020) if South Asian, 0.045 (SE 0.022) if Mixed, 0 if any other racial origin]; $R^2$=0.289, p<0.0001.

$Log_{10}$ PP13 expected = 1.982 (SE 0.040) + 0.003 (SE 0.001) x maternal age in years – 0.004 (SE $3.9e^{-04}$) x maternal weight in kg – [0.214 (SE 0.019) if cigarette smoker, 0

if non-smoker] + [0.030 (SE 0.014) if Afro-Caribbean, 0 if any other racial origin]; $R^2$=0.174, p<0.0001.

### 4.3.2 Patient-specific risks for early, intermediate and late PE

The patient-specific *a priori* risk for early, intermediate and late PE based on maternal characteristics was calculated from the formula: odds / (1+odds), where odds= $e^Y$. The Y for each type of PE was derived from backward stepwise multivariate regression analysis.

The results of this analysis are summarized in Tables 4.1 to 4.3. The shrinkage coefficient for early, intermediate and late PE models were 0.95, 0.96 and 0.99 respectively, and all parameters in the model were adjusted accordingly.

The biophysical and biochemical results of each PE group and the unaffected pregnancies are compared in Table 4.4. The differences between the PE groups and unaffected pregnancies were sequentially greater in the early than intermediate or late disease, except for activin-A where the differences were greater for late than early disease.

The inter-correlations between biophysical and biochemical markers in PE and unaffected pregnancies are shown in Tables 4.5 to 4.8.

### 4.3.3 Performance of screening for preeclampsia

The estimated detection rates of early, intermediate and late PE at fixed FPRs of 5% and 10% in screening by maternal factors only and by combinations of maternal factors with biophysical and biochemical markers are given in Table 4.9. Receiver operating characteristic curves are shown in Figure 4.1.

**Table 4.1.** Logistic regression analysis to determine factors defining the *a priori* risk for the prediction of early preeclampsia by maternal history and characteristics.

| Independent variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | **p** | **OR** | **95% CI** | **P** |
| Age (per year) | 0.761 | 0.608-0.953 | 0.017 | - | - | - |
| $(Age)^2$ | 1.004 | 1.001-1.008 | 0.024 | - | - | - |
| Weight (per kg) | 1.026 | 1.016-1.037 | <0.0001 | 1.021 | 1.009-1.033 | 0.001 |
| Height (per cm) | 0.958 | 0.932-0.985 | 0.002 | 0.949 | 0.921-0.978 | 0.001 |
| Race origin | | | | | | |
| Caucasian (reference) | 1.000 | | | - | - | - |
| Afro-Caribbean | 4.429 | 2.987-6.566 | <0.0001 | 3.644 | 2.431-5.463 | <0.0001 |
| South Asian | 2.829 | 1.334-5.997 | 0.007 | 2.575 | 1.192-5.560 | 0.016 |
| East Asian | 0.000 | 0.000- | 0.992 | - | - | - |
| Mixed | 2.122 | 0.763-5.903 | 0.149 | - | - | - |
| Smoking | 0.203 | 0.050-0.824 | 0.026 | - | - | - |
| Assisted conception | 1.974 | 0.960-4.061 | 0.065 | 2.225 | 1.061-4.670 | 0.034 |
| History of diabetes | 3.663 | 1.155-11.613 | 0.017 | - | - | - |
| History of chronic hypertension | 15.621 | 8.970-27.205 | <0.0001 | 5.622 | 2.988-10.578 | <0.0001 |
| Family history of preeclampsia | 2.783 | 1.558-4.974 | 0.001 | 1.910 | 1.031-3.538 | 0.040 |
| Parity | | | | | | |
| Nulliparous (reference) | 1.000 | | | 1.000 | | |
| Parous with previous preeclampsia | 5.052 | 3.048-8.375 | <0.0001 | 2.235 | 1.259-3.966 | 0.006 |
| Parous without previous preeclampsia | 0.403 | 0.257-0.631 | <0.0001 | 0.333 | 0.211-0.525 | <0.0001 |

OR=odds ratio; CI=confidence interval; p=significance value

**Table 4.2.** Logistic regression analysis to determine factors defining the *a priori* risk for the prediction of intermediate preeclampsia by maternal history and characteristics.

| Independent variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | **p** | **OR** | **95% CI** | **p** |
| Age (per year) | 1.010 | 0.985-1.035 | 0.431 | - | - | - |
| Weight (per kg) | 1.026 | 1.018-1.034 | <0.0001 | 1.022 | 1.012-1.031 | <0.0001 |
| Height (per cm) | 0.967 | 0.947-0.988 | 0.002 | 0.957 | 0.936-0.980 | <0.0001 |
| Race origin | | | | | | |
| Caucasian (reference) | 1.000 | | | - | - | - |
| Afro-Caribbean | 3.273 | 2.406-4.452 | <0.0001 | 2.662 | 1.938-3.657 | <0.0001 |
| South Asian | 2.585 | 1.468-4.551 | 0.001 | 2.411 | 1.349-4.309 | 0.003 |
| East Asian | 0.811 | 0.199-3.301 | 0.770 | - | - | - |
| Mixed | 1.662 | 0.726-3.808 | 0.229 | - | - | - |
| Smoking | 0.767 | 0.427-1.379 | 0.376 | - | - | - |
| Assisted conception | 1.917 | 1.088-3.378 | 0.024 | 2.131 | 1.198-3.790 | 0.010 |
| History of diabetes | 5.948 | 2.897-12.211 | <0.0001 | 3.376 | 1.568-7.270 | 0.002 |
| History of chronic hypertension | 10.759 | 6.538-17.707 | <0.0001 | 4.198 | 2.408-7.319 | <0.0001 |
| Family history of preeclampsia | 2.120 | 1.284-3.499 | 0.003 | - | - | - |
| Parity | | | | | | |
| Nulliparous (reference) | 1.000 | | | 1.000 | | |
| Parous with previous preeclampsia | 4.421 | 2.898-6.744 | <0.0001 | 2.411 | 1.521-3.823 | <0.0001 |
| Parous without previous preeclampsia | 0.513 | 0.369-0.711 | <0.0001 | 0.432 | 0.309-0.603 | <0.0001 |

OR=odds ratio; CI=confidence interval; p=significance value

**Table 4.3.** Logistic regression analysis to determine factors defining the *a priori* risk for the prediction of late preeclampsia by maternal history and characteristics.

| Independent variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | **p** | **OR** | **95% CI** | **p** |
| Age (per year) | 0.840 | 0.745-0.948 | 0.005 | - | - | - |
| $(Age)^2$ | 1.003 | 1.001-1.005 | 0.007 | - | - | - |
| Weight (per kg) | 1.029 | 1.024-1.034 | <0.0001 | 1.028 | 1.022-1.034 | <0.0001 |
| Height (per cm) | 0.983 | 0.970-0.997 | 0.015 | 0.964 | 0.950-0.978 | <0.0001 |
| Race origin | | | | | | |
| Caucasian (reference) | 1.000 | | | - | - | - |
| Afro-Caribbean | 2.624 | 2.150-3.202 | <0.0001 | 2.123 | 1.735-2.598 | <0.0001 |
| South Asian | 1.346 | 0.851-2.129 | 0.205 | - | - | - |
| East Asian | 1.478 | 0.782-2.794 | 0.229 | - | - | - |
| Mixed | 1.111 | 0.605-2.309 | 0.735 | - | - | - |
| Smoking | 0.765 | 0.524-1.117 | 0.166 | - | - | - |
| Assisted conception | 1.062 | 0.661-1.707 | 0.804 | - | - | - |
| History of diabetes | 0.590 | 0.146-2.380 | 0.459 | - | - | - |
| History of chronic hypertension | 6.909 | 4.699-10.224 | <0.0001 | 3.019 | 1.967-4.632 | <0.0001 |
| Family history of preeclampsia | 1.720 | 1.208-2.449 | 0.003 | - | - | - |
| Parity | | | | | | |
| Nulliparous (reference) | 1.000 | | | 1.000 | | |
| Parous with previous preeclampsia | 3.216 | 2.404-4.304 | <0.0001 | 1.815 | 1.325-2.485 | <0.0001 |
| Parous without previous preeclampsia | 0.350 | 0.279-0.438 | <0.0001 | 0.289 | 0.230-0.362 | <0.0001 |

OR=odds ratio; CI=confidence interval; p=significance value

**Table 4.4.** Median and interquartile range (IQR) of pregnancy associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (hCG), uterine artery pulsatility index (PI), mean arterial pressure (MAP), placenal growth factor (PlGF), placental protein 13 (PP13), soluble endoglin (sEng), inhibin-A, activin-A, pentraxin-3 (PTX-3) and P-selectin in the control group and in those subsequently developing early, intermediate and late- preeclampsia.

| Variables | Control | | Early PE | | Intermediate PE | | Late PE | |
|---|---|---|---|---|---|---|---|---|
| | n | MoM | n | MoM | n | MoM | n | MoM |
| PAPP-A, median (IQR) | 32,850 | 1.02 (0.70-1.45) | 112 | 0.63 (0.40-1.14)* | 187 | 0.79 (0.53-1.11)* | 453 | 0.90 (0.62-1.29)* |
| Uterine artery PI, median (IQR) | 21,090 | 1.02 (0.84-1.23) | 86 | 1.47 (1.11-1.72)* | 143 | 1.28 (1.06-1.51)* | 354 | 1.11 (0.88-1.36)* |
| MAP, median (IQR) | 13,515 | 1.00 (0.95-1.06) | 69 | 1.10 (1.04-1.17)* | 111 | 1.08 (1.03-1.13)* | 251 | 1.06 (1.00-1.13)* |
| PlGF, median (IQR) | 2,143 | 0.99 (0.77-1.27) | 56 | 0.64 (0.46-0.82)* | 104 | 0.72 (0.55-0.91)* | 186 | 0.85 (0.68-1.12)* |
| PP13, median (IQR) | 1,210 | 1.00 (0.76-1.33) | 48 | 0.88 (0.57-1.23) | 70 | 0.93 (0.70-1.30) | 103 | 1.11 (0.89-1.49) |
| sEndoglin, median (IQR) | 181 | 0.99 (0.78-1.31) | 29 | 1.44 (1.03-1.92)* | 28 | 0.89 (0.70-1.21) | 32 | 0.99 (0.74-1.23) |
| Inhibin-A, median (IQR) | 403 | 0.98 (0.75-1.33) | 25 | 1.61 (0.90-1.94)† | 37 | 1.13 (0.83-1.70) | 62 | 1.32 (0.91-1.70)* |
| Activin-A, median (IQR) | 398 | 1.00 (0.76-1.30) | 26 | 1.25 (1.00-1.72)† | 41 | 1.22 (0.97-1.74)† | 61 | 1.36 (1.01-1.70)* |
| Pentraxin 3, median (IQR) | 291 | 0.99 (0.76-1.31) | 26 | 1.40 (0.82-2.04) | 37 | 1.40 (0.93-1.89)† | 60 | 1.02 (0.78-1.40) |
| P-Selectin, median (IQR) | 294 | 1.01 (0.84-1.24) | 26 | 1.21 (0.83-1.35) | 36 | 1.18 (0.90-1.43) | 62 | 1.16 (0.96-1.37)† |

MoM=multiple of the unaffected median. Comparisons between the outcome groups by Mann Whitney-U test with *post hoc* Bonferroni correction. Critical significance level p=0.0167; * p<0.0001; †p<0.01.

**Table 4.5.** Intercorrelations between pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM), uterine artery pulsatility index (PI) MoM, mean arterial pressure (MAP) MoM, placental growth factor (PlGF) MoM, placental protein 13 (PP13) MoM, soluble endoglin (sEng) MoM, inhibin-A MoM, activin-A MoM, pentraxin-3 (PTX-3) MoM and P-selectin MoM in unaffected pregnancies.

| Variable | PAPP-A | Uterine artery PI | MAP | PlGF | PP13 | sEndoglin | Inhibin-A | Activin-A | Pentraxin 3 | P-Selectin |
|---|---|---|---|---|---|---|---|---|---|---|
| **PAPP-A** | | | | | | | | | | |
| Pearson correlation (r) | 1.000 | -0.154 | -0.016 | 0.350 | 0.334 | 0.240 | 0.208 | 0.310 | 0.107 | -0.033 |
| Significance value (p) | - | <0.0001 | 0.064 | <0.0001 | <0.0001 | 0.001 | <0.0001 | <0.0001 | 0.068 | 0.572 |
| **Uterine artery PI** | | | | | | | | | | |
| Pearson correlation (r) | -0.154 | 1.000 | -0.047 | -0.149 | -0.113 | -0.078 | -0.043 | -0.010 | -0.073 | -0.044 |
| Significance value (p) | <0.0001 | - | <0.0001 | <0.0001 | <0.0001 | 0.295 | 0.387 | 0.850 | 0.214 | 0.452 |
| **MAP** | | | | | | | | | | |
| Pearson correlation (r) | -0.016 | -0.047 | 1.000 | 0.002 | 0.026 | 0.033 | 0.113 | 0.037 | 0.068 | 0.187 |
| Significance value (p) | 0.064 | <0.0001 | - | 0.943 | 0.375 | 0.674 | 0.030 | 0.483 | 0.263 | 0.002 |
| **PlGF** | | | | | | | | | | |
| Pearson correlation (r) | 0.350 | -0.149 | 0.002 | 1.000 | 0.066 | -0.090 | 0.092 | 0.132 | 0.001 | -0.016 |
| Significance value (p) | <0.0001 | <0.0001 | 0.943 | - | 0.036 | 0.231 | 0.106 | 0.020 | 0.988 | 0.781 |
| **PP13** | | | | | | | | | | |
| Pearson correlation (r) | 0.334 | -0.113 | 0.026 | 0.066 | 1.000 | 0.029 | 0.400 | 0.393 | 0.113 | 0.059 |
| Significance value (p) | <0.0001 | <0.0001 | 0.375 | 0.036 | - | 0.701 | <0.0001 | <0.0001 | 0.055 | 0.310 |
| **sEndoglin** | | | | | | | | | | |
| Pearson correlation (r) | 0.240 | -0.078 | 0.033 | -0.090 | 0.029 | 1.000 | 0.158 | 0.098 | 0.194 | 0.045 |
| Significance value (p) | 0.001 | 0.295 | 0.674 | 0.231 | 0.701 | - | 0.117 | 0.340 | 0.059 | 0.666 |
| **Inhibin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.208 | -0.043 | 0.113 | 0.092 | 0.400 | 0.158 | 1.000 | 0.224 | 0.113 | 0.157 |
| Significance value (p) | <0.0001 | 0.387 | 0.030 | 0.106 | <0.0001 | 0.117 | - | <0.0001 | 0.055 | 0.007 |
| **Activin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.310 | -0.010 | 0.037 | 0.132 | 0.393 | 0.098 | 0.224 | 1.000 | 0.052 | 0.060 |
| Significance value (p) | <0.0001 | 0.850 | 0.483 | 0.020 | <0.0001 | 0.340 | <0.0001 | - | 0.383 | 0.308 |
| **Pentraxin 3** | | | | | | | | | | |
| Pearson correlation (r) | 0.107 | -0.073 | 0.068 | 0.001 | 0.113 | 0.194 | 0.113 | 0.052 | 1.000 | 0.087 |
| Significance value (p) | 0.068 | 0.214 | 0.263 | 0.988 | 0.055 | 0.059 | 0.055 | 0.383 | - | 0146 |
| **P-Selectin** | | | | | | | | | | |
| Pearson correlation (r) | -0.033 | -0.044 | 0.187 | -0.016 | 0.059 | 0.045 | 0.157 | 0.060 | 0.087 | 1.000 |
| Significance value (p) | 0.572 | 0.452 | 0.002 | 0.781 | 0.310 | 0.666 | 0.007 | 0.308 | 0.146 | - |

**Table 4.6.** Intercorrelations between pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM), uterine artery pulsatility index (PI) MoM, mean arterial pressure (MAP) MoM, placental growth factor (PlGF) MoM, placental protein 13 (PP13) MoM, soluble endoglin (sEng) MoM, inhibin-A MoM, activin-A MoM, pentraxin-3 (PTX-3) MoM and P-selectin MoM in pregnancies destined to develop early-preeclampsia.

| Variable | PAPP-A | Uterine artery PI | MAP | PlGF | PP13 | sEndoglin | Inhibin-A | Activin-A | Pentraxin 3 | P-Selectin |
|---|---|---|---|---|---|---|---|---|---|---|
| **PAPP-A** | | | | | | | | | | |
| Pearson correlation (r) | 1.000 | -0.094 | 0.210 | 0.399 | 0.213 | -0.068 | -0.348 | -0.001 | 0.169 | -0.164 |
| Significance value (p) | - | 0.389 | 0.083 | 0.002 | 0.154 | 0.726 | 0.088 | 0.995 | 0.409 | 0.424 |
| **Uterine artery PI** | | | | | | | | | | |
| Pearson correlation (r) | -0.094 | 1.000 | -0.153 | 0.074 | -0.053 | 0.096 | -0.014 | 0.267 | -0.182 | 0.292 |
| Significance value (p) | 0.389 | - | 0.218 | 0.594 | 0.727 | 0.622 | 0.947 | 0.188 | 0.373 | 0.148 |
| **MAP** | | | | | | | | | | |
| Pearson correlation (r) | 0.210 | -0.153 | 1.000 | -0.067 | -0.050 | 0.086 | 0.338 | 0.202 | 0.320 | 0.065 |
| Significance value (p) | 0.083 | 0.218 | - | 0.660 | 0.759 | 0.677 | 0.124 | 0.367 | 0.137 | 0.769 |
| **PlGF** | | | | | | | | | | |
| Pearson correlation (r) | 0.399 | 0.074 | -0.067 | 1.000 | 0.159 | -0.269 | -0.412 | -0.276 | -0.335 | -0.115 |
| Significance value (p) | 0.002 | 0.594 | 0.660 | - | 0.394 | 0.183 | 0.040 | 0.172 | 0.094 | 0.577 |
| **PP13** | | | | | | | | | | |
| Pearson correlation (r) | 0.213 | -0.053 | -0.050 | 0.159 | 1.000 | -0.312 | -0.117 | -0.155 | -0.111 | -0.228 |
| Significance value (p) | 0.154 | 0.727 | 0.759 | 0.394 | - | 0.099 | 0.578 | 0.449 | 0.591 | 0.263 |
| **sEndoglin** | | | | | | | | | | |
| Pearson correlation (r) | -0.068 | 0.096 | 0.086 | -0.269 | -0.312 | 1.000 | -0.148 | 0.313 | -0.001 | 0.183 |
| Significance value (p) | 0.726 | 0.622 | 0.677 | 0.183 | 0.099 | - | 0.481 | 0.137 | 0.994 | 0.370 |
| **Inhibin-A** | | | | | | | | | | |
| Pearson correlation (r) | -0.348 | -0.014 | 0.338 | -0.412 | -0.117 | -0.148 | 1.000 | 0.169 | 0.362 | -0.040 |
| Significance value (p) | 0.088 | 0.947 | 0.124 | 0.040 | 0.578 | 0.481 | - | 0.430 | 0.075 | 0.851 |
| **Activin-A** | | | | | | | | | | |
| Pearson correlation (r) | -0.001 | 0.267 | 0.202 | -0.276 | -0.155 | 0.313 | 0.169 | 1.000 | 0.361 | 0.085 |
| Significance value (p) | 0.995 | 0.188 | 0.367 | 0.172 | 0.449 | 0.137 | 0.430 | - | 0.083 | 0.692 |
| **Pentraxin 3** | | | | | | | | | | |
| Pearson correlation (r) | 0.169 | -0.182 | 0.320 | -0.335 | -0.111 | -0.001 | 0.362 | 0.361 | 1.000 | -0.052 |
| Significance value (p) | 0.409 | 0.373 | 0.137 | 0.094 | 0.591 | 0.994 | 0.075 | 0.083 | - | 0.800 |
| **P-Selectin** | | | | | | | | | | |
| Pearson correlation (r) | -0.164 | 0.292 | 0.065 | -0.115 | -0.228 | 0.183 | -0.040 | 0.085 | -0.052 | 1.000 |
| Significance value (p) | 0.424 | 0.148 | 0.769 | 0.577 | 0.263 | 0.370 | 0.851 | 0.692 | 0.800 | - |

**Table 4.7.** Intercorrelations between pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM), uterine artery pulsatility index (PI) MoM, mean arterial pressure (MAP) MoM, placental growth factor (PlGF) MoM, placental protein 13 (PP13) MoM, soluble endoglin (sEng) MoM, inhibin-A MoM, activin-A MoM, pentraxin-3 (PTX-3) MoM and P-selectin MoM in pregnancies destined to develop intermediate-preeclampsia.

| Variable | PAPP-A | Uterine artery PI | MAP | PlGF | PP13 | sEndoglin | Inhibin-A | Activin-A | Pentraxin 3 | P-Selectin |
|---|---|---|---|---|---|---|---|---|---|---|
| **PAPP-A** | | | | | | | | | | |
| Pearson correlation (r) | 1.000 | -0.047 | -0.020 | 0.071 | 0.198 | 0.228 | 0.140 | 0.352 | 0.264 | 0.294 |
| Significance value (p) | - | 0.581 | 0.837 | 0.476 | 0.095 | 0.243 | 0.407 | 0.024 | 0.115 | 0.081 |
| **Uterine artery PI** | | | | | | | | | | |
| Pearson correlation (r) | -0.047 | 1.000 | -0.029 | -0.193 | -0.225 | 0.370 | 0.100 | 0.086 | -0.286 | -0.241 |
| Significance value (p) | 0.581 | - | 0.770 | 0.049 | 0.060 | 0.053 | 0.558 | 0.594 | 0.086 | 0.157 |
| **MAP** | | | | | | | | | | |
| Pearson correlation (r) | -0.020 | -0.029 | 1.000 | 0.048 | 0.085 | -0.013 | 0.048 | -0.013 | -0.121 | 0.229 |
| Significance value (p) | 0.837 | 0.770 | - | 0.660 | 0.490 | 0.950 | 0.782 | 0.936 | 0.480 | 0.187 |
| **PlGF** | | | | | | | | | | |
| Pearson correlation (r) | 0.071 | -0.193 | 0.048 | 1.000 | 0.146 | -0.488 | -0.173 | -0.186 | -0.262 | 0.225 |
| Significance value (p) | 0.476 | 0.049 | 0.660 | - | 0.254 | 0.008 | 0.307 | 0.244 | 0.118 | 0.187 |
| **PP13** | | | | | | | | | | |
| Pearson correlation (r) | 0.198 | -0.225 | 0.085 | 0.146 | 1.000 | 0.030 | 0.212 | 0.234 | 0.143 | 0.235 |
| Significance value (p) | 0.095 | 0.060 | 0.490 | 0.254 | - | 0.878 | 0.209 | 0.141 | 0.397 | 0.168 |
| **sEndoglin** | | | | | | | | | | |
| Pearson correlation (r) | 0.228 | 0.370 | -0.013 | -0.488 | 0.030 | 1.000 | 0.064 | 0.134 | 0.235 | 0.204 |
| Significance value (p) | 0.243 | 0.053 | 0.950 | 0.008 | 0.878 | - | 0.747 | 0.497 | 0.228 | 0.307 |
| **Inhibin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.140 | 0.100 | 0.048 | -0.173 | 0.212 | 0.064 | 1.000 | 0.517 | 0.190 | -0.157 |
| Significance value (p) | 0.407 | 0.558 | 0.782 | 0.307 | 0.209 | 0.747 | - | 0.001 | 0.260 | 0.361 |
| **Activin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.352 | 0.086 | -0.013 | -0.186 | 0.234 | 0.134 | 0.517 | 1.000 | 0.280 | -0.046 |
| Significance value (p) | 0.024 | 0.594 | 0.936 | 0.244 | 0.141 | 0.497 | 0.001 | - | 0.093 | 0.790 |
| **Pentraxin 3** | | | | | | | | | | |
| Pearson correlation (r) | 0.264 | -0.286 | -0.121 | -0.262 | 0.143 | 0.235 | 0.190 | 0.280 | 1.000 | 0.076 |
| Significance value (p) | 0.115 | 0.086 | 0.480 | 0.118 | 0.397 | 0.228 | 0.260 | 0.093 | - | 0.658 |
| **P-Selectin** | | | | | | | | | | |
| Pearson correlation (r) | 0.294 | -0.241 | 0.229 | 0.225 | 0.235 | 0.204 | -0.157 | -0.046 | 0.076 | 1.000 |
| Significance value (p) | 0.081 | 0.157 | 0.187 | 0.187 | 0.168 | 0.307 | 0.361 | 0.790 | 0.658 | - |

**Table 4.8.** Intercorrelations between pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM), uterine artery pulsatility index (PI) MoM, mean arterial pressure (MAP) MoM, placental growth factor (PlGF) MoM, placental protein 13 (PP13) MoM, soluble endoglin (sEng) MoM, inhibin-A MoM, activin-A MoM, pentraxin-3 (PTX-3) MoM and P-selectin MoM in pregnancies destined to develop late- preeclampsia.

| Variable | PAPP-A | Uterine artery PI | MAP | PlGF | PP13 | sEndoglin | Inhibin-A | Activin-A | Pentraxin 3 | P-Selectin |
|---|---|---|---|---|---|---|---|---|---|---|
| **PAPP-A** | | | | | | | | | | |
| Pearson correlation (r) | 1.000 | -0.123 | 0.125 | 0.254 | 0.346 | -0.047 | 0.435 | 0.352 | 0.106 | -0.006 |
| Significance value (p) | - | 0.020 | 0.048 | <0.0001 | <0.0001 | 0.799 | <0.0001 | 0.005 | 0.420 | 0.962 |
| **Uterine artery PI** | | | | | | | | | | |
| Pearson correlation (r) | -0.123 | 1.000 | -0.114 | -0.032 | -0.215 | -0.042 | -0.168 | -0.159 | 0.084 | -0.316 |
| Significance value (p) | 0.020 | - | 0.074 | 0.661 | 0.029 | 0.820 | 0.191 | 0.221 | 0.526 | 0.012 |
| **MAP** | | | | | | | | | | |
| Pearson correlation (r) | 0.125 | -0.114 | 1.000 | 0.060 | -0.058 | -0.149 | 0.006 | 0.054 | -0.199 | 0.047 |
| Significance value (p) | 0.048 | 0.074 | - | 0.477 | 0.572 | 0.416 | 0.962 | 0.682 | 0.134 | 0.719 |
| **PlGF** | | | | | | | | | | |
| Pearson correlation (r) | 0.254 | -0.032 | 0.060 | 1.000 | 0.161 | -0.360 | -0.019 | 0.009 | -0.144 | 0.006 |
| Significance value (p) | <0.0001 | 0.661 | 0.477 | - | 0.130 | 0.043 | 0.885 | 0.947 | 0.276 | 0.963 |
| **PP13** | | | | | | | | | | |
| Pearson correlation (r) | 0.346 | -0.215 | -0.058 | 0.161 | 1.000 | 0.109 | 0.410 | 0.364 | 0.299 | 0.118 |
| Significance value (p) | <0.0001 | 0.029 | 0.572 | 0.130 | - | 0.551 | 0.001 | 0.004 | 0.020 | 0.359 |
| **sEndoglin** | | | | | | | | | | |
| Pearson correlation (r) | -0.047 | -0.042 | -0.149 | -0.360 | 0.109 | 1.000 | 0.128 | 0.171 | 0.010 | 0.089 |
| Significance value (p) | 0.799 | 0.820 | 0.416 | 0.043 | 0.551 | - | 0.484 | 0.349 | 0.955 | 0.628 |
| **Inhibin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.435 | -0.168 | 0.006 | -0.019 | 0.410 | 0.128 | 1.000 | 0.286 | 0.212 | 0.206 |
| Significance value (p) | <0.0001 | 0.191 | 0.962 | 0.885 | 0.001 | 0.484 | - | 0.028 | 0.105 | 0.109 |
| **Activin-A** | | | | | | | | | | |
| Pearson correlation (r) | 0.352 | -0.159 | 0.054 | 0.009 | 0.364 | 0.171 | 0.286 | 1.000 | 0.325 | 0.037 |
| Significance value (p) | 0.005 | 0.221 | 0.682 | 0.947 | 0.004 | 0.349 | 0.028 | - | 0.013 | 0.780 |
| **Pentraxin 3** | | | | | | | | | | |
| Pearson correlation (r) | 0.106 | 0.084 | -0.199 | -0.144 | 0.299 | 0.010 | 0.212 | 0.325 | 1.000 | -0.082 |
| Significance value (p) | 0.420 | 0.526 | 0.134 | 0.276 | 0.020 | 0.955 | 0.105 | 0.013 | - | 0.536 |
| **P-Selectin** | | | | | | | | | | |
| Pearson correlation (r) | -0.006 | -0.316 | 0.047 | 0.006 | 0.118 | 0.089 | 0.206 | 0.037 | -0.082 | 1.000 |
| Significance value (p) | 0.962 | 0.012 | 0.719 | 0.963 | 0.359 | 0.628 | 0.109 | 0.780 | 0.536 | - |

**Table 4.9.** Performance of screening for early, intermediate and late-PE by maternal factors only and maternal factors with pregnancy associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI), mean arterial pressure (MAP), placental growth factor (PlGF), placental protein 13 (PP13), soluble endoglin (sEng), inhibin-A, activin-A, pentraxin-3 (PTX-3), P-selectin and their combinations.

| Screening test | Detection rate (95% confidence interval) for fixed false positive rate | | | | | |
|---|---|---|---|---|---|---|
| | Early PE | | Intermediate PE | | Late PE | |
| | 5% | 10% | 5% | 10% | 5% | 10% |
| Maternal factors | 33.0 (24.6-42.7) | 46.4 (36.9-56.1) | 27.8 (20.0-37.2) | 37.4 (28.6-47.2) | 24.5 (17.1-33.8) | 34.7 (26.1-44.4) |
| Maternal factors plus | | | | | | |
| Pregnancy associated plasma protein-A | 47.0 (37.5-56.7) | 58.3 (49.5-67.5) | 31.4 (23.1-41.0) | 43.2 (33.2-53.0) | 25.8 (17.7-34.5) | 37.2 (28.4-47.0) |
| Uterine artery pulsatility index | 54.1 (44.4-63.5) | 66.1 (56.4-74.6) | 36.9 (28.1-46.7) | 48.9 (39.3-58.6) | 27.1 (19.4-36.5) | 38.6 (29.7-48.4) |
| Mean arterial pressure | 49.7 (40.1-59.3) | 62.6 (52.8-71.5) | 41.1 (32.0-50.9) | 53.7 (44.0-63.2) | 33.1 (24.7-42.8) | 44.6 (35.2-54.4) |
| Placental growth factor | 53.5 (43.8-63.0) | 65.0 (55.3-73.6) | 40.3 (31.2-40.1) | 52.7 (43.0-62.2) | 27.0 (19.3-36.4) | 38.7 (29.7-48.5) |
| Placental protein 13 | 39.8 (30.8-49.6) | 51.9 (42.2-61.4) | 30.2 (22.1-39.8) | 41.4 (32.2-51.2) | 26.2 (18.6-35.6) | 37.8 (28.9-47.6) |
| sEndoglin | 46.2 (36.8-55.9) | 58.8 (49.0-68.0) | 31.0 (22.8-40.6) | 42.2 (33.0-52.0) | 25.7 (18.2-35.1) | 37.1 (28.3-46.9) |
| Inhibin-A | 44.4 (35.1-54.2) | 56.7 (46.9-66.0) | 32.6 (24.2-42.3) | 44.2 (34.9-54.0) | 30.8 (22.6-40.4) | 42.5 (33.3-52.3) |
| Activin-A | 40.4 (31.3-50.2) | 53.1 (43.4-62.6) | 33.7 (25.2-43.4) | 46.0 (36.6-55.7) | 34.1 (25.6-43.8) | 47.0 (37.5-56.7) |
| Pentraxin 3 | 37.8 (28.9-47.6) | 50.1 (40.5-59.7) | 31.2 (23.0-40.8) | 43.2 (33.9-53.0) | 25.6 (18.1-35.0) | 36.8 (28.0-46.6) |
| P-Selectin | 38.5 (29.6-48.3) | 50.5 (40.9-60.1) | 31.1 (22.9-40.7) | 42.6 (36.8-55.9) | 28.5 (20.6-38.0) | 40.5 (31.4-50.3) |
| Maternal factors plus biophysical markers | 66.5 (56.8-75.0) | 77.8 (68.7-84.8) | 48.7 (39.1-58.4) | 61.2 (51.4-70.2) | 34.3 (25.7-44.0) | 46.6 (37.1-56.3) |
| plus PAPP-A and PLGF | 77.8 (68.7-84.8) | 86.7 (78.7-92.0) | 56.6 (46.8-65.9) | 68.9 (59.3-77.1) | 35.2 (26.6-45.0) | 48.5 (38.9-58.2) |
| plus PAPP-A , PLGF, Inhibin-A and Activin-A | 83.4 (74.9-89.4) | 90.0 (82.6-94.5) | 63.6 (53.8-72.4) | 74.8 (65.5-82.3) | 47.9 (38.4-57.6) | 61.4 (51.6-70.4) |
| plus PAPP-A , PLGF, Inhibin-A , Activin-A, sEndoglin | 86.7 (78.7-92.1) | 92.1 (85.1-96.0) | 68.6 (59.0-76.9) | 79.5 (70.6-86.3) | 50.5 (40.9-60.1) | 64.2 (54.4-72.9) |
| Maternal factors plus all markers | 91.0 (83.8-95.2) | 95.2 (89.1-98.0) | 79.4 (70.5-86.2) | 88.3 (80.5-93.2) | 60.9 (51.1-69.9) | 71.1 (61.6-79.1) |

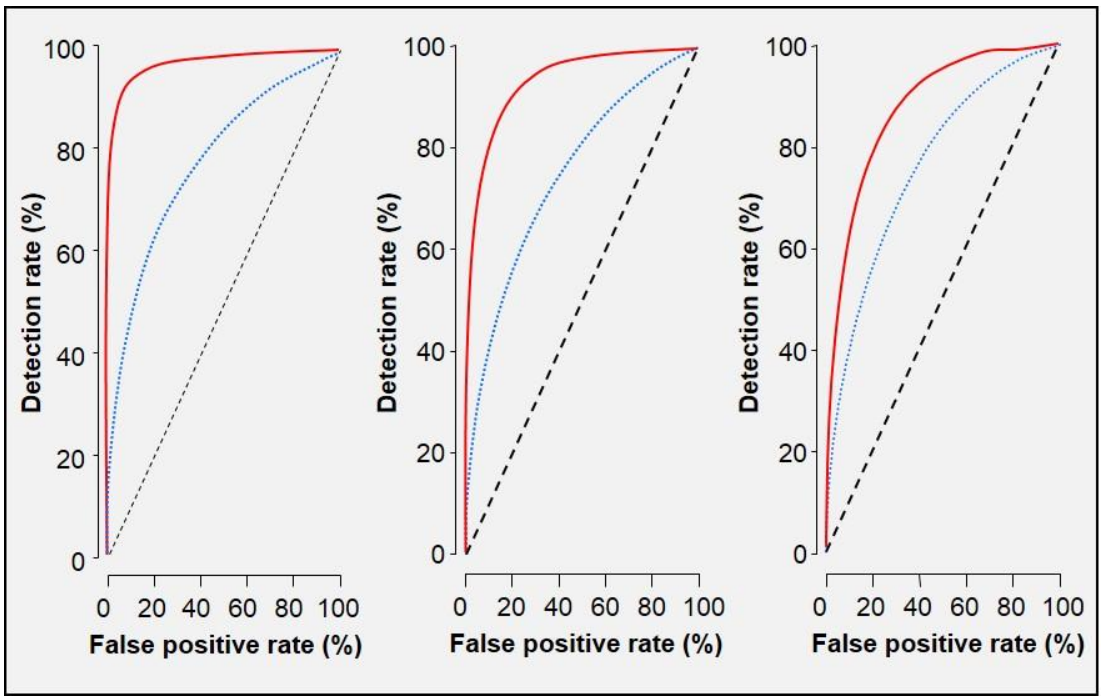



**Figure 4.1.** Receiver operating characteristics curves in the prediction of early (left), intermediate (middle) and late preeclampsia (right) by maternal factors only (—) and by a combination of maternal factors, biochemical and biophysical markers (—).

## 4.4 DISCUSSION

The results of this study demonstrate that the risk for PE is associated with maternal characteristics; it increases with maternal weight and decreases with height, it is higher in women of Afro-Caribbean and South Asian racial origin than in Caucasians, and it is increased in women conceiving after the use of ovulation induction drugs, in those with a personal or family history of PE and in those with pre-existing chronic hypertension or diabetes mellitus. In parous women with no previous PE the risk of developing PE in the current pregnancy was reduced by 60-70%. In general, the odds ratios for the factors in maternal history which defined the *a priori* risk for PE were inversely proportional to the gestation at delivery, with higher ratios for early disease compared to intermediate and late PE.

Algorithms that combine the various maternal characteristics at 11-13 weeks could potentially identify 33%, 28% and 25% of pregnancies that subsequently develop early, intermediate and late PE, at the FPR of 5%.

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. Algorithms which combine maternal characteristics and biophysical and biochemical tests at 11-13 weeks could potentially identify about 90%, 80% and 60% of pregnancies that subsequently develop early, intermediate and late PE, at the FPR of 5%.

An integrated first hospital visit at 11-13 weeks combining data from maternal characteristics and history with findings of biophysical and biochemical tests can define the patient-specific risk for a wide spectrum of pregnancy complications, including aneuploidies, fetal defects, miscarriage and fetal death, preterm delivery, fetal growth restriction and macrosomia, gestational diabetes, hypothyroidism and PE (Akolekar *et al.,* 2011a; Ashoor *et al.,* 2010; Beta *et al.,* 2011; Greco *et al.,* 2011; Karagiannis *et al.,* 2011; Nanda *et al.,* 2011; Nicolaides, 2011; Syngelaki *et al.,* 2011; Poon *et al,* 2011a and 2011b). Early estimation of patient-specific risks for these pregnancy complications would improve pregnancy outcome by shifting antenatal care from a series of routine visits to a more individualized patient and disease-specific approach both in terms of the schedule and content of such visits. In the case of PE effective early identification of the high-risk group could potentially improve outcome by directing such patients to specialist clinics for close surveillance and would be the basis for future studies investigating the potential role of pharmacological interventions, such as aspirin, starting from the first-trimester to improve placentation and reduce the prevalence of the disease.

# CHAPTER 5

## COMPETING RISKS MODEL IN EARLY SCREENING FOR PREECLAMPSIA BY BIOPHYSICAL AND BIOCHEMICAL MARKERS

## 5.1 INTRODUCTION

### 5.1.1 Background

PE has been defined as early or late on the basis of whether or not delivery occurs before 34 weeks' gestation. In general, the deviations from normal for the various proposed biomarkers have been greater in early-PE than in late-PE (Poon *et al.,* 2009a; Akolekar *et al.,* 2011b). In our previous study (Chapter 4), we demonstrated this approach to screening by expressing the effects of variables from maternal characteristics and history as odds ratios and the effect of biomarkers MoMs as likelihood ratios for early, intermediate or late PE. This has led to the view that early and late-PE may be different diseases with different biomarker profiles. An alternative view is that PE is a spectrum disorder the degree of which is reflected in both gestation at the time of delivery and the biomarker levels.

### 5.1.2 Objectives

The objectives of this chapter are to investigate the performance of screening for PE based on a survival time model which treats gestation at delivery as a continuous variable and using Bayes theorem to combine information from maternal characteristics and biomarker MoM values.

## 5.2 METHODS

The data for this study were derived from our prospective study for pregnancy complications in women attending for their routine first hospital visit in pregnancy at 11-13 weeks' gestation. We developed a model for predicting PE based on maternal characteristics in the whole screened population. We then expanded this model for prediction of PE to include the addition of uterine artery PI, MAP, PAPP-A and PLGF.

The approach for early screening for PE proposed in this chapter was based on a survival time model (Chapter 2).

## 5.3 RESULTS

### 5.3.1 Gestational age at delivery with preeclampsia given maternal characteristics

A standard Gaussian regression model for the gestation at delivery was fitted by treating deliveries for which PE did not occur as censored observations (Table 5.1). This specified the mean gestational age at delivery with PE for given variables from maternal demographic characteristics, medical and obstetric history; the smaller the mean gestational age the higher the risk for PE (Figure 5.1).

**Table 5.1.** Fitted regression model for posited gestational age in weeks at delivery with preeclampsia (PE). In this model the mean gestational age for delivery with PE is 55 weeks with a residual standard deviation of 7.11 weeks.

| | Coefficient | SE | 95% CI | p |
|---|---|---|---|---|
| Intercept | 55.0081 | 0.3465 | 54.3289 to 55.6873 | 0.00000 |
| Age above 30 years | -0.10367 | 0.02319 | -0.14912 to -0.05822 | 0.00001 |
| Weight in kg - 69 | -0.07259 | 0.00551 | -0.08339 to -0.06180 | 0.00000 |
| Height in m - 1.64 | 12.4007 | 1.3263 | 9.801129 to 15.0003 | 0.00000 |
| Ethnicity | | | | |
| Afro-Caribbean | -3.0357 | 0.2040 | -3.4355 to -2.6360 | 0.00000 |
| South Asian | -1.7709 | 0.3737 | -2.5034 to -1.0384 | 0.00000 |
| Previous History | | | | |
| Parous with PE | -3.0333 | 0.3174 | -3.6554 to-2.4112 | 0.00000 |
| Parous with no PE | 3.1440 | 0.2033 | 2.7455 to 3.5424 | 0.00000 |
| Mother had PE | -1.8841 | 0.3262 | -2.5234 to -1.2448 | 0.00000 |
| Conception by IVF | -1.7895 | 0.4782 | -2.7268 to -0.8523 | 0.00018 |
| SLE or APS | -4.0613 | 1.3186 | -6.6459 to -1.4768 | 0.00207 |
| Chronic hypertension | -6.2415 | 0.4179 | -7.0606 to -5.4223 | 0.00000 |
| Type 1 DM | -3.8525 | 0.9732 | -5.7600 to -1.9450 | 0.00008 |

In this model the mean gestational age for delivery with PE was 55 weeks with estimated standard deviation (SD) of 7.11 weeks (Table 5.1). Certain variables, including advancing maternal age over 30 years, increasing weight, Afro-Caribbean and South Asian racial origin, previous pregnancy with PE, conception by in vitro fertilization and a medical history of chronic hypertension, type 2 diabetes mellitus and systemic lupus erythematosus or antiphospholipid syndrome increase the risk for development of PE. The consequence of this increased risk is a shift to the left of the Gaussian distribution of the gestational age at delivery with PE (Figure 5.2).

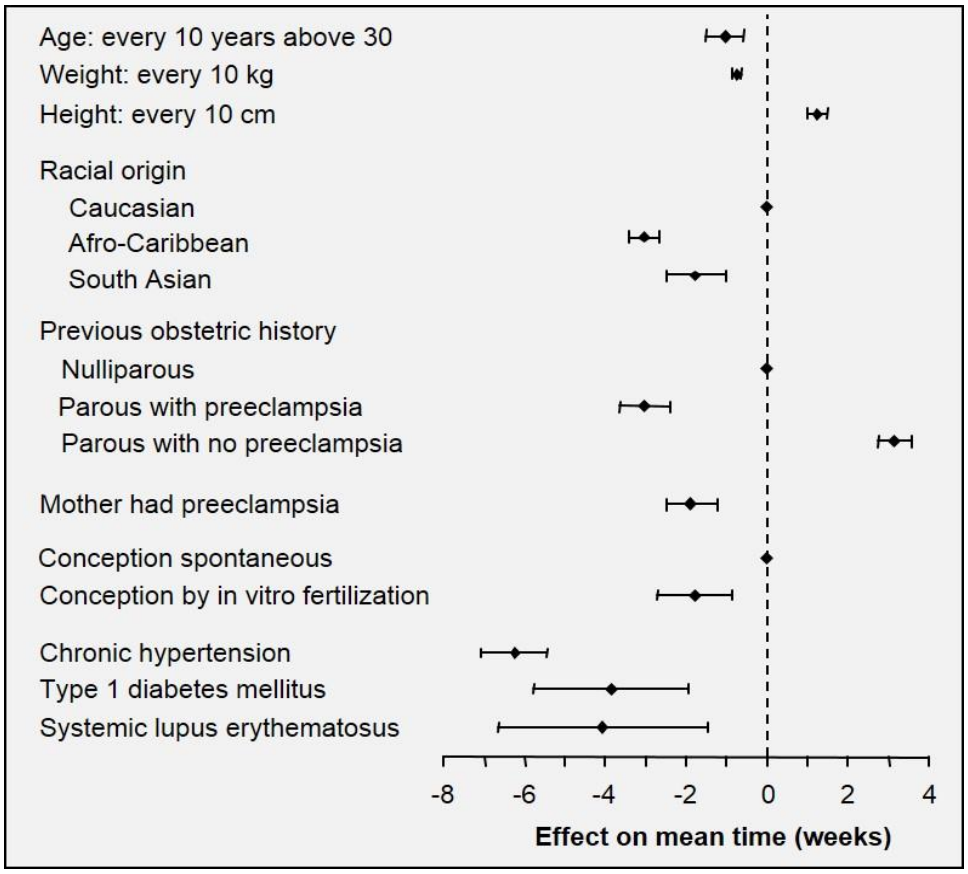



**Figure 5.1.** Effects of maternal characteristics (with 95% confidence intervals) on the gestational age at delivery for preeclampsia. This effect is expressed as gestational weeks by which the expected gestational age at delivery for preeclampsia is altered.

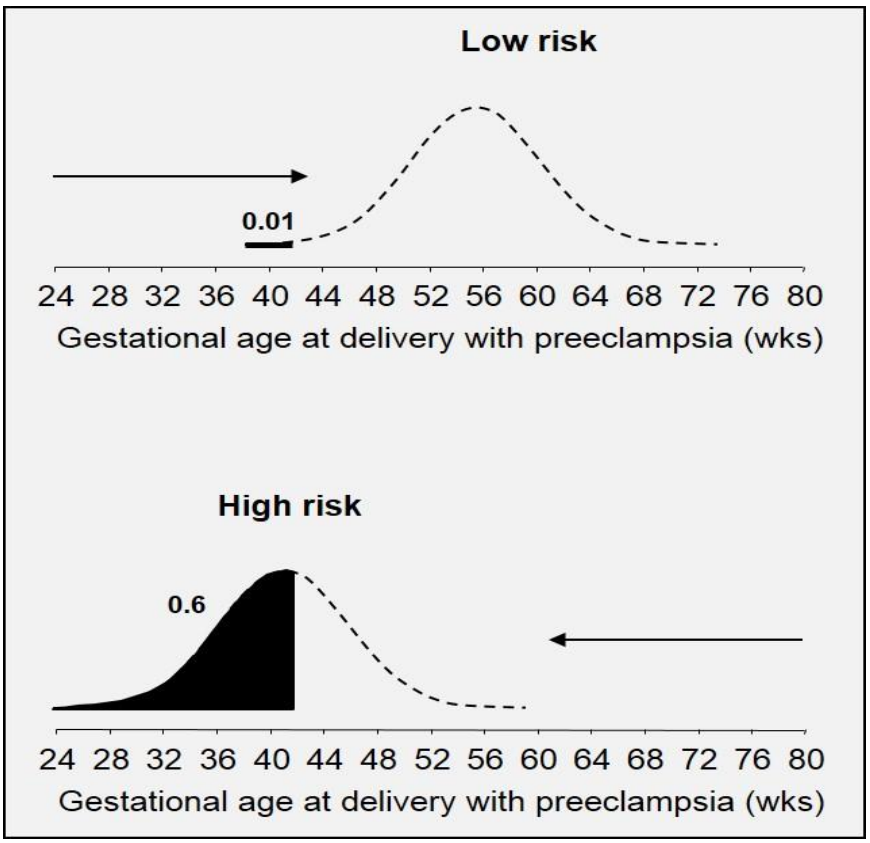



**Figure 5.2.** Distribution of gestational age at delivery for preeclampsia (PE). In pregnancies at low-risk for PE the gestational age distribution is shifted to the right and in most pregnancies delivery will occur before the development of PE. In pregnancies at high-risk for PE the distribution is shifted to the left. The risk of PE occurring at or before a specified gestational age is given by the area under the distribution curve (black). In the low-risk group the risk of PE at or before 34 weeks' gestation is 0.01 or 1% and in the high-risk group the risk is 0.6 or 60%.

### 5.3.2 Distribution of biomarkers in pregnancies with preeclampsia

Multiple regression analyses in the unaffected pregnancies demonstrated the significant independent contributions for the prediction of $\log_{10}$ uterine artery PI (Table 5.2; $R^2$=0.034, p<0.0001) and $\log_{10}$ MAP (Table 5.3; $R^2$=0.127, p<0.0001).

$\text{Log}_{10}$ uterine artery PI expected = 0.2638615843586 – [0.0008928915003 x (maternal weight – 69 kg)] + [0.0000089122525 x (maternal weight – 69 kg)$^2$] – [0.0046798061340 x (gestational age – 77 days)] + (0.0232056722206 if Afro-Caribbean racial origin, 0 if any other race); $R^2$=0.034, p<0.0001.

$Log_{10}$ MAP expected = 1.9299538440779 + [0.0011415204801 x (maternal weight – 69 kg)] – [0.0000080469298 x (maternal weight – 69 kg)$^2$]; $R^2$=0.127, p<0.0001.

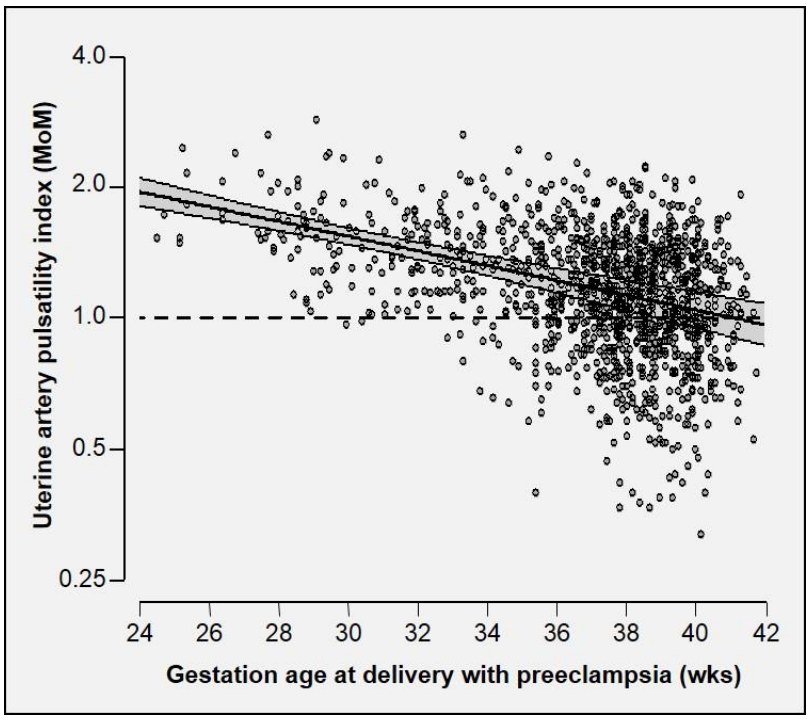
**Table 5.2.** Fitted regression model for uterine artery pulsatility index in unaffected pregnancies.

| Variable | Coefficient | 95% CI | p |
|---|---|---|---|
| Intercept | 0.2638615843586 | 0.2601668285800 to 0.2675563401372 | <0.0001 |
| Gestational age -77 days | -0.0046798061340 | -0.0049618816257 to -0.0043977306424 | <0.0001 |
| Weight-69 kg | -0.0008928915003 | -0.0009930145317 to -0.0007927684689 | <0.0001 |
| (Weight-69 kg)$^2$ | 0.0000089122525 | 0.0000056152695 to 0.0000122092354 | <0.0001 |
| Afro-Caribbean racial origin | 0.0232056722206 | 0.0200372989286 to 0.0263740455127 | <0.0001 |

**Table 5.3.** Fitted regression model for mean arterial pressure in unaffected pregnancies.

| Variable | Coefficient | 95% CI | p |
|---|---|---|---|
| Intercept | 1.9299538440779 | 1.9294356282676 to 1.9304720598883 | <0.0001 |
| Weight-69 kg | 0.0011415204801 | 0.0011033940268 to 0.0011796469335 | <0.0001 |
| (Weight-69 kg)$^2$ | -0.0000080469298 | -0.0000093217366 to -0.0000067721229 | <0.0001 |

In pregnancies with PE there was an inverse correlation between MoM values of biophysical and biochemical markers with gestational age at delivery (Figures 5.3 and 5.4).



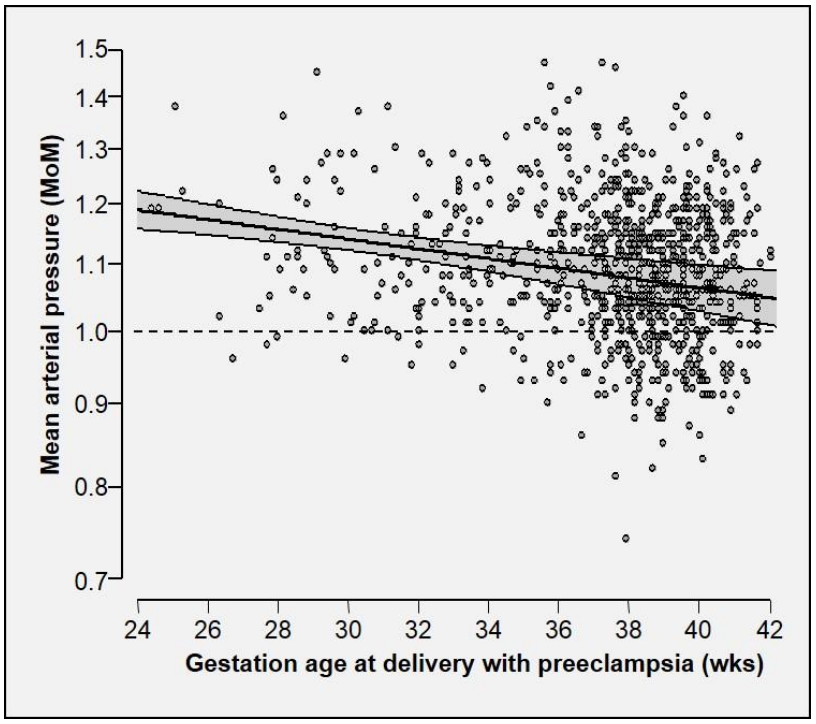




**Figure 5.3.** Scatter diagram and regression line with 95% confidence limits for the relationship between uterine artery pulsatility index (PI) multiple of the median (MoM) and mean arterial pressure (MAP) MoM and gestational age at delivery in pregnancies with preeclampsia.

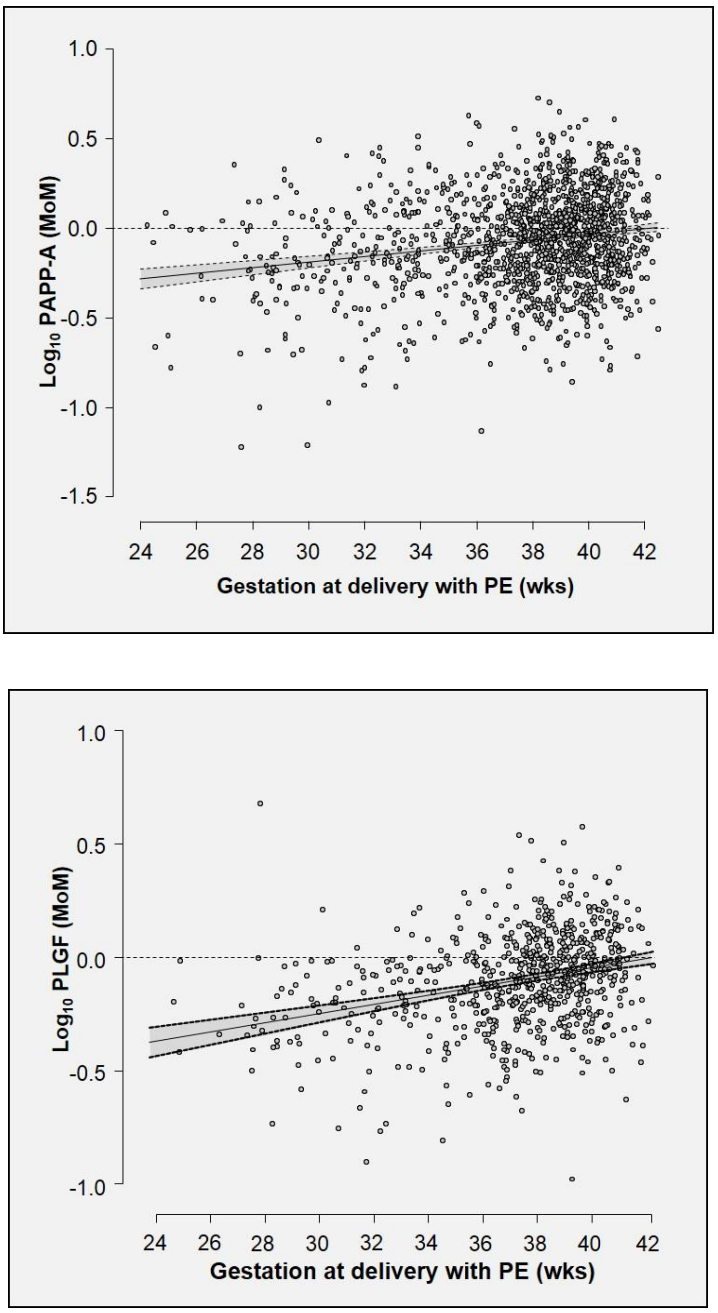



**Figure 5.4.** Scatter diagram and regression line with 95% confidence limits for the relationship between pregnancy associated plasma protein-A (PAPP-A) multiple of the median (MoM) and placental growth factor (PLGF) MoM and gestational age at delivery in pregnancies with preeclampsia.

The fitted regression models for $\log_{10}$ MoM values on gestational age at delivery are presented in Table 5.4 and the estimated parameters for the assumed bivariate Gaussian distributions for log MoM values are given in Table 5.5.

**Table 5.4.** Fitted regression model for marker $\log_{10}$ multiple of the median (MoM) values on gestation at time of delivery for pregnancies with preeclampsia.

| Marker | Intercept | SE | Slope | P |
|---|---|---|---|---|
| Uterine artery PI | 0.642102 | 0.038479 | -0.015173 | <0.0001 |
| Mean arterial pressure | 0.114859 | 0.014798 | -0.002115 | <0.0001 |
| Pregnancy associated plasma protein-A | -0.656448 | 0.078707 | 0.015555 | <0.0001 |
| Placental growth factor | 0.861296 | 0.089182 | 0.020221 | <0.0001 |

**Table 5.5.** Standard deviations (SD) and correlations, with 95% confidence limits, for $\log_{10}$ multiples of the median (MoM) biomarker values.

| | No preeclampsia | Preeclampsia |
|---|---|---|
| SD Uterine artery PI | 0.1242215 (0.122254 to 0.127840) | 0.1409539 (0.122250 to 0.158148) |
| SD MAP | 0.0386549 (0.037002 to 0.040257) | 0.0426263 (0.032693 to 0.053506) |
| SD PAPP-A | 0.2368016 (0.229462 to 0.237103) | 0.2679589 (0.252859 to 0.285103) |
| SD PLGF | 0.1764965 (0.173864 to 0.179168) | 0.2165872 (0.202972 to 0.229780) |
| Correlation Uterine PI and MAP | -0.0724816 (-0.075278 to -0.070882) | 0.0093204 (-0.004507 to 0.014433) |
| Correlation Uterine PI and PAPP-A | -0.156970 (-0.177317 to -0.145472) | -0.1766223 (-0.256403 to -0.099764) |
| Correlation Uterine PI and PLGF | -0.1322802 (-0.144440 to -0.111484) | -0.2113414 (-0.303681 to -0.154827) |
| Correlation MAP and PAPP-A | -0.0069674 (-0.020433 to 0.011444) | 0.0354576 (-0.091128 to 0.066178) |
| Correlation MAP and PLGF | -0.0346327 (-0.052455 to -0.018998) | 0.0128895 (-0.070326 to 0.091987) |
| Correlation PAPP-A and PLGF | 0.3009237 (0.293361 to 0.328260) | 0.3543634 (0.271487 to 0.421228) |

### 5.3.3 Performance of screening for preeclampsia

Estimated detection rates of PE requiring delivery before 34, 37 and 42 weeks' gestation, at false positive rates of 5% and 10% in screening by maternal factors, uterine artery PI, MAP, serum PAPP-A, PLGF and their combination are given in Table 5.6.

**Table 5.6.** Estimated detection rates of preeclampsia requiring delivery before 34, 37 and 42 weeks' gestation, at false positive rate (FPR) of 5% and 10%.

| Screening test | FPR (%) | PE <34 wks N=214 | | PE <37 wks N=568 | | PE <42 wks N=1,426 | |
|---|---|---|---|---|---|---|---|
| | | Risk cut-off | Detection | Risk cut-off | Detection | Risk cut-off | Detection |
| Maternal characteristics | 5.0 | 1:93 | 78 (35.5) | 1:35 | 186 (32.7) | 1:9 | 419 (29.4) |
| | 10.0 | 1:143 | 108 (50.5) | 1:51 | 246 (43.3) | 1:12 | 574 (40.3) |
| Uterine artery PI | 5.0 | 1:88 | 127 (59.3) | 1:31 | 227 (40.0) | 1:9 | 445 (31.2) |
| | 10.0 | 1:164 | 161 (75.2) | 1:52 | 313 (55.1) | 1:12 | 602 (42.2) |
| MAP | 5.0 | 1:88 | 125 (58.4) | 1:31 | 250 (44.0) | 1:8 | 532 (37.3) |
| | 10.0 | 1:159 | 156 (72.9) | 1:52 | 337 (59.3) | 1:12 | 763 (53.5) |
| PAPP-A | 5.0 | 1:88 | 93 (43.6) | 1:33 | 212 (37.3) | 1:9 | 449 (31.5) |
| | 10.0 | 1:151 | 117 (54.7) | 1:52 | 274 (48.2) | 1:12 | 601 (42.1) |
| PLGF | 5.0 | 1:95 | 127 (59.3) | 1:33 | 232 (40.8) | 1:9 | 415 (29.1) |
| | 10.0 | 1:170 | 155 (72.4) | 1:55 | 309 (54.4) | 1:12 | 572 (40.1) |
| Uterine PI and MAP | 5.0 | 1:96 | 171 (79.9) | 1:31 | 310 (54.6) | 1:7 | 498 (34.9) |
| | 10.0 | 1:197 | 192 (89.7) | 1:57 | 406 (71.5) | 1:12 | 807 (56.6) |
| PAPP-A and PLGF | 5.0 | 1:101 | 129 (60.3) | 1:34 | 243 (42.8) | 1:9 | 433 (30.4) |
| | 10.0 | 1:181 | 159 (74.3) | 1:56 | 317 (55.8) | 1:12 | 582 (40.8) |
| Uterine PI, MAP and PAPP-A | 5.0 | 1:105 | 175 (81.8) | 1:26 | 298 (52.5) | 1:7 | 514 (36.0) |
| | 10.0 | 1:216 | 198 (92.5) | 1:65 | 424 (74.6) | 1:12 | 811 (59.9) |
| Uterine PI, MAP and PLGF | 5.0 | 1:126 | 187(87.4) | 1:36 | 344 (60.6) | 1:8 | 536 (37.6) |
| | 10.0 | 1:261 | 205 (95.8) | 1:67 | 439 (77.3) | 1:12 | 755 (52.9) |
| Uterine PI, MAP, PAPP-A and PLGF | 5.0 | 1:128 | 200 (93.4) | 1:36 | 347 (61.1) | 1:8 | 539 (37.8) |
| | 10.0 | 1:269 | 206 (96.3) | 1:67 | 435 (76.6) | 1:12 | 764 (53.6) |

The comparison of the expected and observed number of cases with according to the estimated risk range is presented in Table 5.7.

**Table 5.7.** Accuracy of estimated risk for preeclampsia by a combination of maternal characteristics, uterine artery pulsatility index (PI), mean arterial pressure (MAP), serum pregnancy associated plasma protein-A (PAPP-A) and serum placental growth factor (PLGF).

| Estimated risk Range | Median (95% CI) | Observed | |
|---|---|---|---|
| | | Incidence | Risk |
| **PE <34 weeks** | | | |
| 1 in 2 to 1 in 50 | 1 in 29 (6-48) | 157 of 1,170 | 1 in 7.5 |
| 1 in 51 to 1 in 100 | 1 in 76 (53-98) | 22 of 1,241 | 1 in 56.4 |
| 1 in 101 to 1 in 300 | 1 in 197 (109-290) | 29 of 4,140 | 1 in 142.8 |
| Less than 1 in 300 | 1 in 3724 (452-41,342) | 6 of 51,121 | 1 in 8520.2 |
| **PE <37 weeks** | | | |
| 1 in 2 to 1 in 50 | 1 in 27 (5-48) | 399 of 4,582 | 1 in 11.5 |
| 1 in 51 to 1 in 100 | 1 in 74 (53-98) | 96 of 4,492 | 1 in 46.8 |
| 1 in 101 to 1 in 300 | 1 in 188 (108-288) | 63 of 12,518 | 1 in 198.7 |
| Less than 1 in 300 | 1 in 978 (340-5362) | 10 of 36,434 | 1 in 3643.4 |
| **PE <42 weeks** | | | |
| 1 in 2 to 1 in 10 | 1 in 7 (2-10) | 673 of 5,414 | 1 in 8.0 |
| 1 in 11 to 1 in 20 | 1 in 16 (11-20) | 364 of 7,283 | 1 in 20.0 |
| 1 in 21 to 1 in 50 | 1 in 34 (22-49) | 330 of 17,327 | 1 in 52.5 |
| Less than 1 in 50 | 1 in 99 (54-302) | 59 of 28,860 | 1 in 489.2 |

Table 5.8 shows the performance of screening for PE requiring delivery before 37 weeks' gestation by maternal factors, uterine artery PI, MAP, serum PAPP-A and serum PLGF at risk cut-off of 1:65 in women of Caucasian and Afro-Caribbean racial origin and according to obstetric history. In women of Afro-Caribbean racial origin, compared to Caucasians, and in nulliparous, compared to parous women, both the FPR and detection rates for PE are higher.

**Table 5.8.** Estimated detection rate (DR) of preeclampsia requiring delivery before 37 weeks' gestation and FPR, at risk cut-off of 1:65 in screening by maternal factors, uterine artery pulsatility index (PI), mean arterial pressure (MAP), serum pregnancy associated plasma protein-A (PAPP-A) and serum placental growth factor (PLGF) according to Caucasian and Afro-Caribbean racial origin and obstetric history.

| Study population | False positive rate (%) | Detection rate (%) |
|---|---|---|
| Total | 5,572 / 57,458 (9.7) | 432 / 568 (76.1) |
| Caucasian all | 3,012 / 42,514 (7.1) | 181 / 281 (64.4) |
| Caucasian nulliparous | 2,077 / 21,785 (9.5) | 130 / 187 (69.5) |
| Caucasian parous | 935 / 20,729 (4.5) | 51 / 94 (54.3) |
| Afro-Caribbean all | 2,007 / 9,268 (21.7) | 200 / 224 (89.3) |
| Afro-Caribbean nulliparous | 1,061 / 3,638 (29.2) | 93 / 96 (96.9) |
| Afro-Caribbean parous | 946 / 5,630 (16.8) | 107 / 128 (83.6) |

## 5.4 DISCUSSION

This study has established a new approach for early screening for PE by a combination of maternal characteristics and history with biophysical and biochemical markers. In this approach, which is based on a survival time model, the gestation at the time of delivery for PE is treated as a continuous rather than a categorical variable. As demonstrated by the MoM values of uterine artery PI, MAP and serum PAPP-A and PLGF in pregnancies with PE the distribution with gestational age is linear. Consequently, 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.

The major advantage of the new, compared to our previous models (Chapter 4), 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.

In early biochemical screening for PE several biochemical markers have been proposed, including maternal serum or plasma levels of soluble endoglin, inhibin-A,

activin-A, pentraxin-3 and P-selectin, which are increased and PAPP-A, PLGF and placental protein-13, which are decreased (Chapter 3). These markers are thought to be involved in placentation or in the cascade of events leading from impaired placentation to development of clinical symptoms of PE. In this study we examined only PAPP-A and PLGF because these are the only two that have been investigated extensively in screening for PE, they have both been shown to be useful in screening for aneuploidies (Wright *et al.,* 2010; Pandya *et al.,* 2012) and they are now part of the platform of automated machines that provide reproducible results within 30-40 minutes of sampling.

The findings demonstrate that in first-trimester screening for PE the performance of the test is better for early rather than late onset disease. This is particularly important because the objective of early screening is to identify the high-risk group that may benefit from therapeutic interventions that reduce the prevalence of PE. The prophylactic use of low-dose aspirin starting before 16 weeks' gestation is particularly effective in the prevention of preterm rather than term-PE (Roberge *et al.,* 2012a and 2012b). In screening for PE requiring delivery before 34 weeks the detection rate, at 10% FPR, was about 50% by maternal characteristics and this was improved to about 90% by the addition of biophysical markers and to about 75% by the addition of biochemical markers. The detection rate improved to more than 95% in screening by an algorithm combining maternal factors, biophysical markers and biochemical markers.

The new algorithm provides accurate patient-specific risks and in each range of estimated risks for PE by combined screening there was in general good agreement between the expected and observed number of affected pregnancies. The FPR and DRs of PE are influenced by the characteristics of the study population and for a given risk cut-off they are both higher in nulliparous than in parous women and in those of Afro-Caribbean than Caucasian racial origin. Consequently, comparison of the performance of screening using these algorithms between studies requires the appropriate adjustments for the characteristics of the population under investigation.

# CHAPTER 6

## CONCLUSIONS AND FUTURE STUDIES

## 6.1 CONCLUSIONS

### 6.1.1 Biochemical markers of preeclampsia

The results of the studies in Chapter 3 demonstrate that a series of biochemical markers, which are placental products involved either in placentation or in the cascade of events leading from impaired placentation to placental ischemia with release of inflammatory factors which cause endothelial dysfunction and consequent development of the clinical symptoms of the disease, are altered in pregnancies that subsequently develop PE from as early as 11-13 weeks' gestation. In pregnancies that subsequently develop PE there are altered maternal serum or plasma levels of PlGF, Activin A, Inhibin A, PP13, sEng, PTX-3 and P-selectin, but not PAI-2, Ang-2 or sFlt-1. The levels of PlGF, Inhibin A, PP-13 were significantly different in both early and late-PE but the levels of Activin A and P-Selectin were only altered in late-PE, whereas PTX-3 was different from controls only in early-PE but not in late-PE. The study also demonstrates that the level of these biochemical markers in maternal blood in unaffected pregnancies is affected by maternal demographic characteristics and consequently the measured concentration of these markers must be adjusted for these maternal and pregnancy characteristics before comparison with pathological pregnancies.

The biochemical markers also demonstrate a relationship with established markers of placentation reflected in uterine artery Doppler and maternal serum PAPP-A. In the case of serum PlGF, Activin-A and PP-13, there was a significant linear relationship with maternal serum PAPP-A whereas there was a significant relationship between PlGF, PP-13 and sEng with uterine artery PI suggesting a common pathogenesis of impaired trophoblastic relationship. These case-control studies demonstrate that the biochemical markers which provide a significant independent contribution to prediction of early-PE in addition to maternal factor derived *a priori* risk and uterine

artery PI and serum PAPP-A include PlGF, inhibin A and sEng. In case of late-PE, only PlGF and P-selectin provided significant contributions.

### 6.1.2 Multivariate model for early prediction of preeclampsia

The study in Chapter 4 demonstrates that effective screening for PE based on maternal characteristics and history necessitates the use of specific algorithms derived from multivariate logistic regression analysis. The study demonstrates that factors such as maternal weight and height, racial origin, method of conception, family history of PE and pre-existing chronic hypertension or diabetes mellitus define the *a priori* risk for early, intermediate and late-PE.

In general, the odds ratios for the factors in maternal history which defined the *a priori* risk for PE were inversely proportional to the gestation at delivery, with higher ratios for early disease compared to intermediate and late PE. Algorithms that combine the various maternal characteristics at 11-13 weeks could potentially identify 33%, 28% and 25% of pregnancies that subsequently develop early, intermediate and late PE, at the FPR of 5%.

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 case 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. Algorithms which combine maternal characteristics and biophysical and biochemical tests at 11-13 weeks could potentially identify about 90%, 80% and 60% of pregnancies that subsequently develop early, intermediate and late PE, at the FPR of 5%.

### 6.1.3 Competing risk model for early prediction of preeclampsia

This study in Chapter 5 establishes a new approach for early screening for PE by a combination of maternal characteristics and history with biophysical and biochemical markers. The specific advantages of this new approach are the use of Bayes theorem which combines *prior* information from maternal characteristics and the *posterior* risk is estimated by multiplying it with likelihoods associated with biomarker MoMs. In our previous approach to screening, we expressed the effects of variables as odds ratios for early, intermediate or late-PE. This led to arbitrary categorisation of the PE as two or three different diseases with different models for each. This alternative view assumes that PE is a spectrum disorder, the severity of which is reflected in gestational age at the time of delivery.

The competing risk approach takes into account the gestation at the time of delivery for PE and treats it as a continuous rather than a categorical variable. The effect of various risk factors is to modify the mean of the distribution of gestational age at delivery with PE. In pregnancies at low-risk for PE the distribution is shifted to the right with the implication that in most pregnancies delivery will actually 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 the risk for PE. It is also important to recognize that there are significant associations between all biophysical and biochemical markers in PE and unaffected pregnancies and therefore when combining the four biomarkers in calculating the patient-specific risk for PE the correlation factors are taken into account to provide accurate risk assessment for PE.

The findings demonstrate that in first-trimester screening for PE the performance of the test is better for early rather than late onset disease. In screening for PE requiring delivery before 34 weeks the DR, at 10% FPR, was about 50% by maternal characteristics and this was improved to about 90% by the addition of biophysical markers and to about 75% by the addition of biochemical markers. The detection rate

improved to more than 95% in screening by an algorithm combining maternal factors, biophysical markers and biochemical markers. Estimated DR of PE requiring delivery before 34, 37, and 42 weeks' gestation in screening by maternal factors are 93%, 61%, and 38%, respectively, at FPR of 5% and 96%, 77%, and 54%, respectively, at FPR of 10%.

In summary, this study demonstrates that effective screening for PE can be achieved in the first trimester of pregnancy. The value of this early screening is to identify pregnancies at high-risk which would benefit from pharmacological prevention or from close monitoring in specialist dedicated clinics to individualise management and select the ideal time for delivery.

## 6.2 FUTURE STUDIES

This thesis demonstrates that similar to first-trimester combined screening for fetal aneuploidies, effective screening for PE can also be achieved at 11-13 weeks' gestation by a combination of maternal factors and biochemical and biophysical markers. The major advantage of the proposed model is that not only it achieves an improved performance of screening compared to the model based on a series of maternal characteristics alone but 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.

The competing risk model for early prediction of PE requires prospective validation in multicentre studies which would also compare the performance of screening with the current method recommended by NICE. Such a multicentre study involving the use of the algorithm developed in this thesis will undertake screening at 11-13 weeks' gestation in 16,500 singleton pregnancies (**SPREE** - Screening programme for pre-eclampsia); this study has already received funding from the National Institute of Health Research in the UK and will be start in early in 2016.

The rationale for early prediction of PE is that pharmacological interventions in the high-risk groups, starting from the 12$^{th}$ week of pregnancy, could potentially reduce the prevalence of the disease. It is therefore necessary to investigate through randomised studies the value of drugs vs placebo in the high-risk groups identified by our method of screening in early pregnancy. One such study **(ASPRE -** Combined multi-marker screening and randomised patient treatment with aspirin for evidence-based pre-eclampsia prevention) involving several European centres aims to screen 33,680 singleton pregnancies at 11-13 weeks and randomise the high-risk group into either Aspirin 150 mg/day vs placebo. It is anticipated that aspirin will reduce the rate of preterm-PE and associated complications by 50%. The study is ongoing and recruitment will be completed by April 2016 with expected results by December 2016.

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# LIST OF PUBLICATIONS

This thesis is based on the following studies that have been published in peer-reviewed journals:

**Akolekar R**, Zaragoza E, Poon LCY, Pepes S, Nicolaides KH. 2008. Maternal serum placental growth factor (PlGF) at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy. *Ultrasound Obstet Gynecol* **32**:732-739.

**Akolekar R**, Minekawa R, Veduta A, Romero XC, Nicolaides KH. 2009. Maternal plasma Inhibin A at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy. *Prenat diag* **29**:753-760.

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