BIOPHYSICAL AND BIOCHEMICAL PREDICTION OF PREECLAMPSIA AT 20-24 WEEKS' GESTATION

Submitted by

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For the degree of International Doctor in Medicine

University of Granada

August 2016

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Editor: Universidad de Granada. Tesis Doctorales
Autora: Dahiana Marcela Gallo Gordillo
ISBN: 978-84-9163-028-9
URI: http://hdl.handle.net/10481/44469

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I confirm that Dr Dahiana Marcela Gallo Gordillo has carried out under my supervision the studies presented in the Thesis: Biophysical and Biochemical prediction of preeclampsia at 20-24 weeks' gestation.

I have read the Thesis and I am happy for this to be presented to the Tribunal for The Degree of International Doctor in Medicine

Professor Kypros Herodotou Nicolaides
London August 2016

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Granada August 2016

AKNOWLEDGEMENTS

English

I am very grateful to Professor Kypros Nicolaides for motivating and inspiring me to undertake the work for this thesis. He welcomed me to his unit, trained me in Fetal Medicine and research methodology and mobilised the necessary resources for the screening of the many thousands of women who participated in the studies. He supervised each step of data monitoring, analysis of data, writing of papers and writing of this thesis.

Professor Jesus Florido helped me since the first day in the application for the University of Granada to accept me as a candidate for The Degree of International Doctor in Medicine. He subsequently provided invaluable advice and support during the studies that formed the basis for this thesis.

Professor David Wright developed the mathematical framework for the studies. He introduced the competing risks model and applied Bayes theorem to estimate the patient-specific risk for preeclampsia.

Dr Francisca Molina has helped me to culminate my thesis and has been guidance in this process.

Many thanks also to all the research fellows of Harris Birthright Research Centre for Fetal Medicine who helped me in collecting the research data. Special thanks to my good friends Youssef Saiid, Ahmet Tayyar, Argyro Syngelaki and Santiago Garcia Tizon Larroca for the sleepless nights we were working together and for being there in the last three years.

Last but not the least; I would like to thank my parents and my brother for everything they have done to make me feel well even when I'm away from home.

AKNOWLEDGEMENTS

Spanish

Yo estoy muy agradecida con el Profesor Kypros Nicolaides por motivarme e inspirarme a realizar mi trabajo de tesis. El me recibió en su unidad, me entreno en medicina fetal, metodología de la investigación y movilizo todos los recursos necesarios para el tamizaje de miles de pacientes que participaron en los estudios. El superviso cada paso del monitoreo y análisis de datos, escritura de los artículos y escritura de la tesis.

El Profesor Jesús Florido me ayudo desde el primer día en la aplicación a la Universidad de Granada como candidata del título de doctor internacional en Medicina. Posteriormente me proporciono invaluable ayuda y soporte durante los estudios que forman la base de esta tesis.

El Profesor David Wright desarrollo el modelo matemático de los estudios. El introdujo el modelo de riesgos aplicando el teorema de Bayes para estimar los riesgos específicos de cada paciente para preeclampsia.

La Dra Francisca Molina me ha ayudado a culminar mi tesis y ha sido mi guía en este proceso

Mis agradecimientos también para todos los fellows del instituto de medicina fetal de Harris Birthright quienes me ayudaron a colectar los datos de investigación. Un especial agradecimiento a mis amigos Youssef Saiid, Ahmet Tayar, Argyro Syngelaki, Santiago Garcia Tizon Larroca y Ranjit Akolekar por todas las noches que pasamos sin dormir trabajando juntos en esta tesis y por estar allí en los últimos tres años.

Finalmente, quiero agradecer a mis padres y hermano por todas las cosas que han hecho para que yo me sintiera bien aun estando lejos de casa.

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Chapter 1 INTRODUCTION

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1.1 OVERVIEW

The French physician François Boissier de Sauvages de Lacroix in 1739 was the first to coin the term eclampsia, which he described as an acute form of convulsion, and contrasted with the chronic condition now known as epilepsy (Chesley, 1974). Proteinuria in the context of eclamptic seizures was first described in 1840 by Rayer, and high blood pressure as recorded by sphygmographic tracings shortly after. Vinay in 1894, reported that high blood pressure and proteinuria could occur in pregnant women, without eclamptic seizures and the term preeclampsia (PE) was born (Chesley, 1984).

Approximately 10% of pregnant women have blood pressure above normal at some point during pregnancy. PE, which is now known to be a multisystem disorder of pregnancy, complicates around 2-10% of pregnancies and is a major cause of perinatal and maternal morbidity and mortality (American College of Obstetricians and Gynecologists Committee on Practice Bulletins–Obstetrics, 2015; World Health Organization, 2011; Confidential Enquiry into Maternal and Child Health Perinatal Mortality, 2014).

Hypertension developing in the second half of pregnancy is subdivided according to the presence or absence of co-existing significant proteinuria into PE and gestational hypertension (GH). Evidence suggests that PE can be further subdivided into early-onset PE, requiring delivery before 34 weeks' gestation and late-onset PE, with delivery at or after 34 weeks, because the former is associated with a higher incidence of fetal growth restriction and adverse outcome. (Poon *et al*., 2014; Witlin *et al.*, 2000; Irgens *et al.*, 2001; Von Dadelszen *et al.*, 2003; Yu *et al*., 2008).

The underlying cause for PE is largely unknown but it is thought to be an inadequate remodelling of uterine spiral arteries in the placental bed due to superficial trophoblast invasion followed by placental hypoxia (Huppertz BI, 2014). These findings have been documented by histological and Doppler ultrasound studies of the uterine arteries (Campbell *et al*., 1983; Trudinger *et al*., 1985; Khong *et al.*, 1986; Papageorghiou *et al.*, 2002; Plasencia *et al.*, 2007). In normal pregnancy, the luminal diameter of the spiral arteries is greatly increased and the vascular smooth muscle is replaced by trophoblastic cells. In PE this process, which is genetically and immunologically governed, is deficient (Wilson *et al.*, 2003), and therefore there is decreased vascular capacitance and increased resistance in the uteroplacental circulation (Sagol *et al.*, 1999).

PE is a syndrome characterised by the development of hypertension and proteinuria during the second half of pregnancy. Along with detailed history taking at the booking visit to identify risk factors for PE, the measurement of BP during antenatal visits also constitutes the basis of screening for PE throughout pregnancy. There is evidence suggesting that raised BP in women developing PE can be observed as early as in the first-trimester of pregnancy (Poon *et al*., 2012; Moutquin *et al.*, 1985; Higgins *et al.*, 1997).

Over the years, efforts to diagnose the condition have been hampered by inability to predict which women are likely to be affected. However, extensive research in the last 20 years, mainly as a consequence of screening for aneuploidies, has identified a series of biophysical and biochemical markers of impaired placentation used in an attempt to predict at risk pregnancies (Wright *et al*., 2012; Akolekar *et al*., 2013).

Doppler ultrasound is a non-invasive assessment of placentation. While impedance to flow in uterine arteries decreases with gestation in normal pregnancies, in pregnancies destined to develop PE this impedance is increased (Martin *et al.*, 2001; Papageorghiou *et al*., 2002).

In addition, the maternal serum concentration of placental growth factor (PLGF), a glycoprotein synthesized by the placenta with angiogenic functions

is lower in PE than in normal pregnancies. The decrease in serum PLGF that is thought to be the consequence of placental hypoxia, precedes the clinical onset of the disease and is evident from the first trimester of pregnancy (Tsiakkas *et al*., 2015; Crovetto *et al*., 2015; Rizos *et al*., 2014).

Soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic factor that antagonizes PLGF and the vascular endothelial growth factor. In established PE serum sFlt-1 is increased and this increase precedes the development of the disease by about five weeks (Tsiakkas *et al*., 2015; Crovetto *et al*., 2015; Lai *et al*., 2014).

This thesis aims to develop a model for the prediction of PE based on maternal characteristics and medical history, mean arterial pressure (MAP), uterine artery pulsatility index and biochemical markers at 20-24 week's gestation.

1.2 DEFINIITION AND EPIDEMIOLOGY OF PREECLAMPSIA

1.2.1 Incidence of preeclampsia

Hypertensive disorders of pregnancy, including PE affects about 10% of all pregnant woman around the world and it is also responsible for about 15% of all maternal deaths in developed countries, 10% in Africa and Asia and 25% in Latin America and the Caribbean (World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy, 2011). Non-proteinuric GH occurs in approximately 8-10% of the unselected population but it is associated with a minimal increase in the risk of direct maternal, fetal or neonatal complications (Walker, 2000). On the other hand, the reported incidence of PE depends largely on the definition of the disease used. It complicates 2-10% of pregnancies, but the incidence rates are up to 3 times higher in some populations, which are likely to be the consequence of geographic, social, economic and racial differences (World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy, 1988). Thus, while less than 5% of all deliveries in developing countries are affected by PE or eclampsia (Villar *et al.*, 2001), the figure may be as high as 20% in some settings in Africa (Maharaj and Moodley, 1994).

The severity of PE ranges from a mild disorder with transient hypertension near the end of pregnancy, to a life threatening disorder with seizures (eclampsia) or HELLP (Hemolysis, Elevated liver enzymes and Low platelet) syndrome (Roberts *et al.,* 2001). PE accounts for at least 50,000 of the 585,000 maternal deaths per annum worldwide (Eclampsia Trial Collaborative Group, 1995; World Health Organization, 1996; Khan *et al.,* 2006), with some authors estimating as many as 200,000 maternal deaths being caused by the disease annually (Myers and Baker, 2002). Although worldwide up to 12% of all maternal deaths are caused by eclampsia (Murray and Lopez, 1998), in Colombia the disease accounts for about 40% of maternal deaths (López-Jaramillo *et al.,* 2001), highlighting differences of both incidence and severity of the disease around the world. Even in countries with low maternal mortality, PE and eclampsia account for as many as 20% of women dying during pregnancy (Wilson *et al*., 2002; Walker, 2000).

PE can lead to maternal and fetal problems, which are summarised in Table 1.1. Women with PE can have liver, kidneys, brain and clotting system abnormalities. As the placenta is involved, there are increased risks for the fetus. The most common abnormalities are poor growth, placental infarction and abruption as a result of inadequate blood supply through the damaged placenta. Rare but serious complications include the following: eclampsia (the occurrence of seizures superimposed on the syndrome of preeclampsia); stroke; hemolysis; elevated liver enzymes and low platelets.

Table 1.1 Maternal and fetal effects of preeclampsia.

Maternal	**Ophtalmological system**
	Cortical blindness
Central nervous system	Retinal edema
Local neurological effects	Retinal blindness
Seizures	
Headache	**Long term maternal effects**
Cerebral hemorrhage	Long-term risk of stroke
Cerebral edema	Long-term risk of hypertension
	Long-term risk of other
Cardiorespiratory system	cardiovascular disease
Circulatory collapse	
Pulmonary edema	
Adult respiratory distress syndrome	**Feto – Maternal**
	Placental abruption
Hepatic system	Placental infarction
Liver injury (hepatocellular necrosis)	Complications of operative delivery
HELLP syndrome	
	Fetal
Renal system	Growth restriction
Acute renal failure	Hypoxia
Proteinuria	Intrauterine fetal death
Oliguria	Premature delivery and its
	complications
Haematological system	Long-term risk of diabetes
Microangiopathic haemolysis	Long-term risk of cardiovascular
Thrombocytopenia	disease
Disseminated intravascular	
coagulation	

Fetal and neonatal outcomes related to PE vary around the world. Approximately 10-25% of fetal growth restriction and small for gestational age infants as well as 15-20% of all preterm births are attributable to PE. The associated complications of prematurity are substantial, including neonatal death and long-term neurodevelopmental delay (Jeyabalan, 2013; Roberts *et al.,* 2002). One quarter of stillbirths and neonatal deaths in developing countries are associated with PE / eclampsia. Infant mortality associated with PE is three times higher in low resource settings compared to high income countries, mainly due to the lack of neonatal intensive care facilities (Jeyabalan, 2013).

In addition to these short-term effects on maternal and neonatal health there is increasing evidence that there may be more lasting adverse cardiovascular effects. Epidemiological studies have shown that mothers who suffered with PE during pregnancy being at increased risk of premature cardiovascular disease (Christensen *et al*., 2016). Systematic reviews support the epidemiological findings and demonstrate approximately doubled risk of ischemic heart disease and cerebrovascular incidents (Bellamy *et al*., 2007; McDonald *et al*., 2008)

1.2.2 Definition

PE is a pregnancy-specific hypertensive disease with multisystem involvement and the lack of a complete understanding of aetiology and pathophysiology lead to a variation in definitions of the disease. PE is defined by the presenting symptoms and signs. Thus, most definitions rely on the presence of hypertension and proteinuria in previously normotensive women, after 20 weeks of gestation.

The lack of consensus in the definition of the disease may lead to an over-diagnosis of women who are not at risk of adverse perinatal events. The accepted definition of PE is that of the International Society for the Study of Hypertension in Pregnancy (ISSHP). The development of hypertension with a BP of 140/90 mmHg on two separate occasions four hours apart after 20 weeks of gestation in previously normotensive women with the presence of proteinuria defined as 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 (Davey and MacGillivray, 1988; Brown *et al.*, 2001). However, the definition has been modified recently and PE is diagnosed by hypertension and the coexistence of one or more of the following new-onset conditions: proteinuria (spot urine protein/creatinine >30 mg/ mmol [0.3mg/mg] or >300mg/day or at least 1g/L ['2+'] on dipstick testing), other maternal organ dysfunction as renal insufficiency (creatinine >90 umol/L; 1.02 mg/dL), liver involvement (elevated transaminases at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain), neurological complications (eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata), hematological complications (thrombocytopenia platelet count below 150,000/dL, DIC, hemolysis), uteroplacental dysfunction (fetal growth restriction) (Tranquili *et al*., 2014).

For the purpose of this thesis, the ISSHP classification of hypertensive disorders of pregnancy was chosen as it represents a consensus view of an appropriate definition for research use (Table 1.2). Early onset PE is defined as development of disease requiring delivery at <34 weeks of gestation; in intermediate disease delivery is at 34-37 weeks and in late disease delivery is at ≥37 weeks.

Table 1.2 The ISSHP classification for hypertensive disorders in pregnancy

1. **Chronic hypertension**
2. **Gestational hypertension**
3. **Preeclampsia de novo or superimposed on chronic hypertension**
4. **White coat hypertension**

1.3 ETIOLOGY AND PATHOPHYSIOLOGY OF PREECLAMPSIA

Although the cause of PE remains largely unknown there are four main aetiologic factors believed to be involved in the development of PE

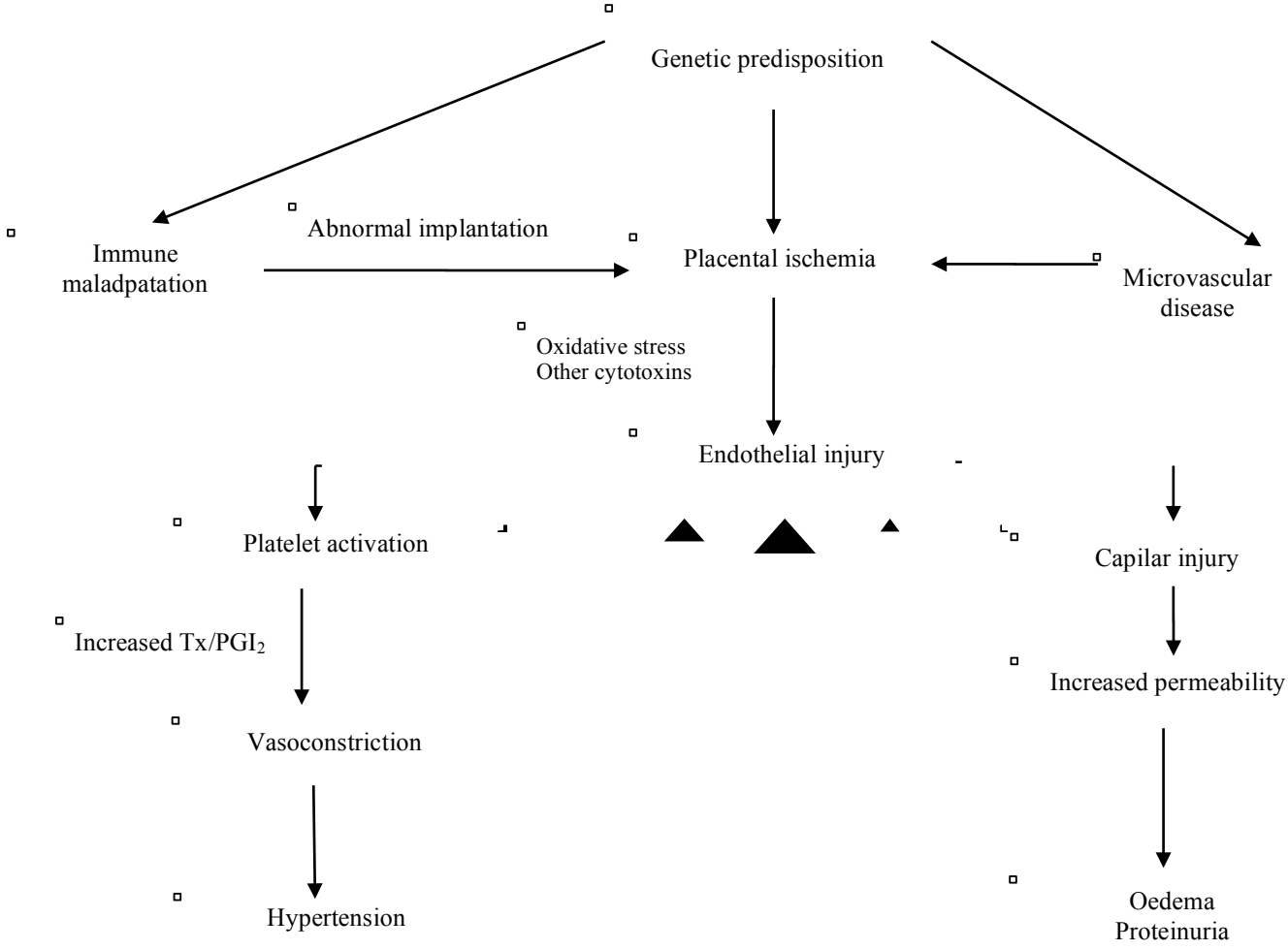


Figure 1.1. Proposed model for the pathophysiology of preeclampsia. Adapted from Wilson *et al.* (2003a) and Salas (1999).

1.3.1 Genetic predisposition

PE is thought to have a long-standing recognition of a familial component as there is a confirmed susceptibility on chromosome 10q22.1 (Oudejans *et al*., 2004). Haplotype analysis showed a parent of origin effect. A maximum allele sharing in the affected siblings was seen for maternally derived alleles in all families, but not for paternally derived alleles (Oudejans *et al*., 2004). A variety of genetic changes have been observed in association with PE, including mutations that affect endothelial function, vasoactive proteins, oxidative stress and immunological factors. Several studies reported associations between PE and polymorphisms of various genes such as angiotensinogen (Morgan *et al.*, 1999), tumour necrosis factor-α (TNF-α) (Heiskanen *et al.*, 2002), factor V Leiden (Bendetto *et al.*, 2002) and the 5,10-methylenetetrahydrofolate reductase genes (Schwahn *et al.*, 2001). However, no single gene is accountable for all the genetic risk in these women. Evidence suggest that there is a number of susceptibility genes, many of which interact with the maternal cardiovascular or haemostatic system, or with the regulation of maternal inflammatory responses. Genome-wide linkage studies have identified at least three preeclampsia loci showing substantial linkage: 2p12, 2p25, and 9p13. These loci segregate with different populations. Notably, these loci only explain a small percentage of the overall cases of PE. Moreover, although these linkage studies indicate maternal susceptibility, they do not exclude the additional involvement of fetal genes. (Laivuori *et al.*, 2003; Oudejans *et al*., 2004)

1.3.2 Immune maladaptation

Epidemiological studies support the concept of maternal fetal (paternal) immune maladaptation being centrally implicated in the causation of preeclampsia (Dekker *et al*., 2001; Wang *et al*., 2002; Dekker *et al*., 2003). Deposition of semen in the female genital tract provokes a cascade of cellular and molecular events that resemble a classic inflammatory response. The critical seminal factor seems to be seminal-vesicle-derived transforming growth factor 1 (TGF 1). It initiates an inflammatory reaction, allowing an increased

ability to sample and process paternal antigens, and a strong type- 2 immune reaction. By initiating a type-2 immune response towards paternal antigens, seminal TGF 1 may inhibit the induction of type-1 responses against the semi-allogenic that are thought to be associated with poor placental development and fetal growth (Robertson *et al*., 2002).

Evidence suggests that sperm exposure causes mucosal alloimmunisation (Peters *et al*., 2004). Limited sperm exposure is the most likely explanation for the high incidence of preeclampsia in teenagers. This hypothesis is supported by many studies, showing the protective effect of previous sperm exposure. Further evidence to support the hypothesis is that the risk for preeclampsia was three times higher in women conceiving via intracytoplasmic sperm injection (ICSI) with surgically obtained sperm (from men with complete azoospermia) than in those with standard in-vitro fertilisation and ICSI using sperm obtained by masturbation (Cedergren, 2004; Wang *et al*., 2002). Repeated intercourse with sustained antigen exposure (sperm cell) in the appropriate cytokine environment (TGF 1) is now thought to be essential in this partner-specific mucosal tolerance (Robertson *et al.,* 2002).

Preeclampsia is associated with a failure of cytotrophoblasts to mimic a vascular-adhesion phenotype. Initial vascular changes seem to precede endovascular trophoblast invasion, showing that interstitial trophoblast and decidual leucocytes (especially natural-killer cells) have a role in early disruption. These physiological changes create a low-resistance arteriolar system and no maternal vasomotor control, which allows the substantial increase in blood supply to the growing fetus. During the early stages of implantation, cytotrophoblast plugs might act as valves regulating blood flow in the intervillous space and protect the embryo from forceful maternal blood flow.

During early pregnancy, natural-killer cells in the uterus (probably derived from those in the blood) accumulate as a dense infiltrate around the invading cytotrophoblast cells. From mid-gestation onwards, these killer cells progressively disappear, which coincides with cytotrophoblast invasion, since human placentation is complete by about 20 weeks' gestation. Natural-killer cells affect both trophoblast invasion and vascular changes in the maternal placental bed. The uterine natural-killer cells produce several cytokines that are

implicated in angiogenesis and vascular stability, including vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and angiopoietin 2 (Croy *et al*., 2003; Van der Meer *et al*., 2004).

One of the major products of natural-killer cells is interferon (IFN). Animal studies (mainly in mice) have shown that proinflammatory IFN derived from uterine natural-killer cells is essential and acts physiologically in triggering pregnancy induced spiral artery modification (Croy *et al*., 2003). Release of IFN up regulates genes that stimulate 2- macroglobulin production. 2-macroglobulin regulates proteases, cytokines, and other molecules that signal vascular dilatation.

T cells were thought to be the unique cells needed for adaptive immune responses, absence of major T-cell interaction in preeclampsia seemed to negate the immune maladaptation hypothesis (Dekker *et al*., 1998). This concept was radically changed by the realization of the major role of decidual natural-killer cells, representing the predominant population of decidual lymphoid cells. Natural-killer cells function by cell killing or by cytokine production, which is enhanced by cytokines such as IFN , IFN , interleukin (IL) 2, IL12, and IL15 (Moffett-King *et al*., 2002). They express killer inhibitory and activatory receptors that recognise HLA- class-I molecules. HLA-G is important for activation of uterine natural-killer cells but being monomorphic cannot convey any partner-specific signal. By contrast, HLA-C loci are dimorphic for residues 77–80 and these two HLA-C groups interact with different natural-killer cell receptors.

1.3.3 Endothelial dysfunction

This vascular transformation is dependent on modulation of adhesion molecules (Damsky *et al.*, 1992). In PE, there is a loss of this modulation of adhesion molecules and an incomplete vascular invasion by the cytotrophoblasts (Zhou *et al.*, 1997). As a result of this, the spiral arteries remain muscular and undilated, thus reducing uteroplacental blood flow. There is secondary damage in many vessels showing acute atherosis with fatty change in the intimal cells, necrosis of

the vessel wall and luminal occlusion by aggregates of fibrin, platelets and lipid-laden macrophages (Pridjian and Puschett, 2002).

A generally accepted hypothesis for the aetiology of PE is impaired transformation of the spiral arteries. This is thought to be a two-stage placental disease. The first stage is the process that affects the spiral arteries which results in deficient placental perfusion. The second stage encompasses the effects of the placental ischaemia on both fetus and mother. The hypoxia is followed by the release of several biologically active placental factors into the maternal blood circulation. These factors are thought to cause maternal endothelial dysfunction and a systemic inflammatory reaction (Redman *et al.,* 1999; Sargent *et al.,* 2003).

The placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family. It is a dimeric protein mainly expressed in villous trophoblasts of the placenta (Ziche *et al.,* 1997) (Figure 1.2). The protein is expressed also slightly in some other organs (heart, lung and adipose tissue). PlGF is considered to be an angiogenic (Ziche *et al.,* 1997) factor and plays a role in endothelial cells mediating increased vascular permeability, which includes angiogenesis, vasculogenesis and growth of endothelial cells (Yamazaki *et al*., 2006; Maharaj *et al*., 2008)

Soluble fms-like tyrosine kinase-1 (sFlt-1) also known as soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) is a protein produced by the syncytiotrophoblast and is formed by alternative mRNA splicing of the membrane-bound form of VEGFR-1 or is released from the membrane by proteolytic cleavage (Kendall and Thomas 1993). 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).

Figure 1.2. Expression of PlGF, sVEGFR-1 and VEGF in the placental villus. ST = syncytiotrophoblasts; CT = cytotrophoblasts; EC = endothelial cells; Str = stroma (Elina Keikkala 2013).

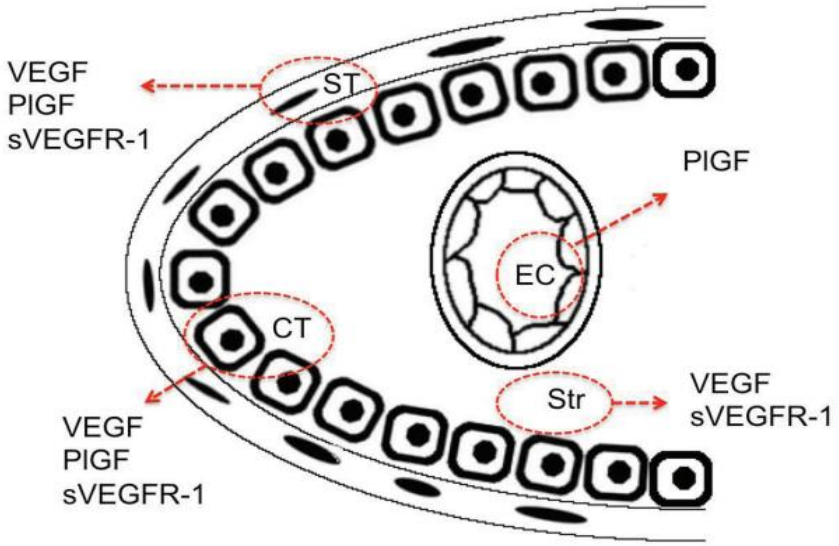
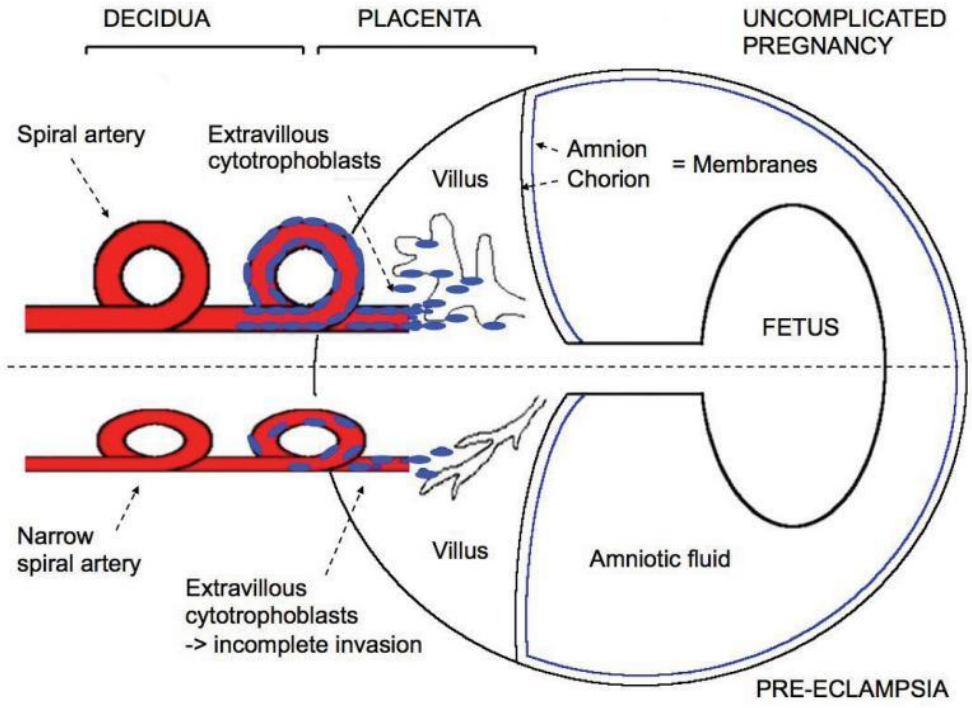


Figure 1.3. Spiral artery transformation in the preeclamptic and healthy placenta (Elina Keikkala 2013).



1.3.4 Oxidative stress

Oxidative stress plays a central role in the pathogenesis of PE. Maternal perfusion of the placenta does not occur until towards the end of the first trimester, when a rapid increase in local oxygen tension takes place, and the probable occurrence of a period of hypoxia–reperfusion until stability is reached (Williams *et al*., 2011). This is accompanied by increased expression and activity of such antioxidants as glutathione peroxidase, catalase and the

various forms of superoxide dismutase. If this antioxidant response is reduced, then the cascade of events leading to impaired placentation could be initiated (Burton *et al*., 2011). Genes involved in the generation or inactivation of reactive oxygen species, if defective, could increase endothelial dysfunction via lipid peroxidation, which has been a candidate causative agent for the endothelial damage of PE for more than 20 years (Perkins *et al*., 2006). Despite the strong correlation between oxidative stress and PE, only a small handful of genes have been investigated. Functional polymorphisms in the gene for microsomal epoxide hydrolase (EPHX) that catalyses the hydrolysis of certain oxides and may produce toxic intermediates that could be involved in PE, and glutathione S-transferase (GST), an antioxidant capable of inactivating reactive oxygen species, have shown associations (Williams *et al*., 2011).

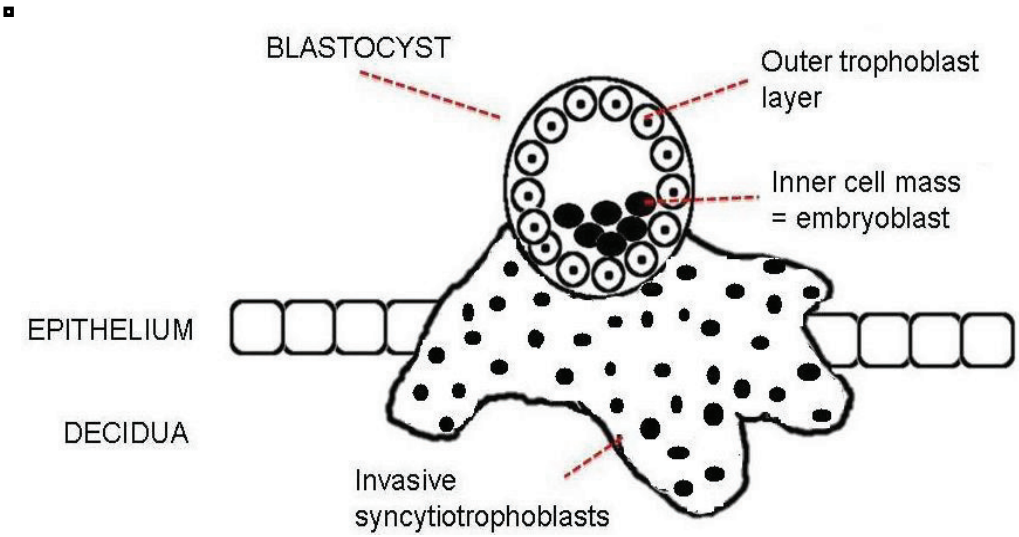
Postulated similarities between PE and atherosclerosis suggest that pathophysiological changes important in atherosclerosis may also have a role in PE. Oxidative stress, interacting with the dyslipidaemia of atherosclerosis, has been hypothesised to be important in the altered endothelial function leading to atherosclerosis (Witztum, 1994). 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. Short-lived reactive oxygen species interact with circulating lipids to form stable lipid peroxidation products which are capable of damaging cell structures (Witztum, 1994).

1.4 THE ROLE OF THE PLACENTA

1.4.1 Placentation in normal pregnancy

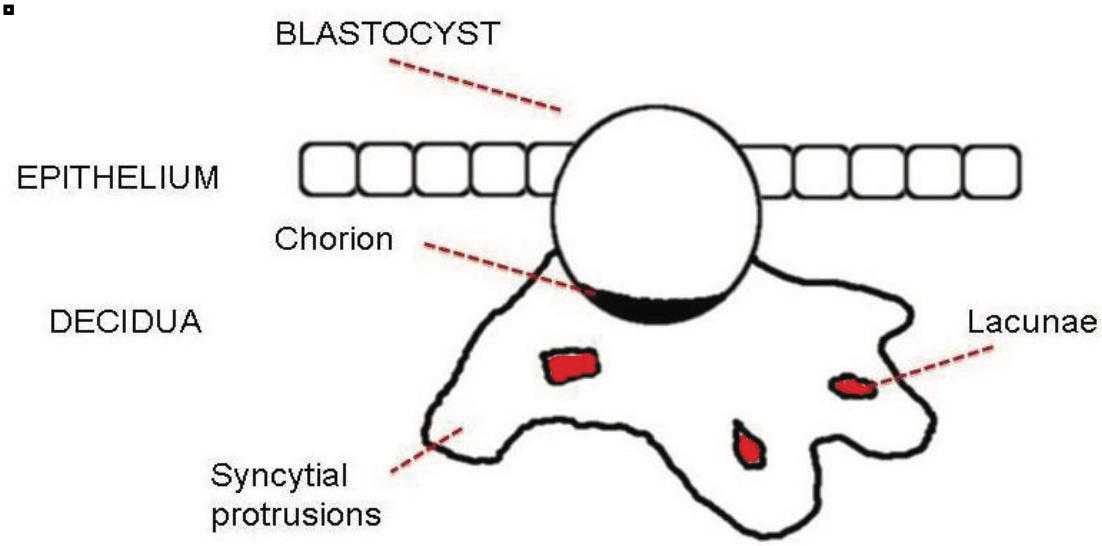
The development of the placenta starts at implantation of the blastocyst, an early embryonic structure, which attaches to the uterine epithelium. The blastocyst consists of an inner cell mass, called the embryoblast, and an outer trophoblast layer (Schoenwolf *et al*., 2009) (Figure 1.4). During the third gestational week, these trophoblasts invade the uterine endometrium. They differentiate into invasive syncytiotrophoblasts, multinucleated cells formed through fusion of several cytotrophoblasts (Figure 1.4).

Figure 1.4. Implantation of the blastocyst and early development of the placenta by the third week of gestation. (Modified from Schoenwolf et al 2009)



The syncytiotrophoblasts continue to invade deeper into the uterine endometrium, now termed decidua, and form lacunae, maternal fluid-filled spaces between syncytial protrusions. Maternal capillaries anastomose with these lacunae and fill them with maternal blood (Figure 1.5). Cytotrophoblasts remain at the embryonic side where they act as stem cells for the syncytiotrophoblasts. A new structure termed the chorion is now formed from the cytotrophoblast layer and extraembryonic mesodermal cells. The chorion is at the fetal surface of the placenta and is later covered by the amnion (Huppertz, 2008) (Figure 1.5)

Figure 1.5. Implantation of the blastocyst and early development of the placenta by the third week of gestation. Syncytiotrophoblasts invasion. (Modified from Schoenwolf et al 2009)



In the fourth gestational week the syncytial protrusions start to form the basic structure of the placenta, tree-like protrusions called villi. Cells within the villi differentiate into hematopoietic cells and form the first embryonic vessels lined with endothelial cells. By the end of the fourth week of gestation, the villi have developed enough to enable effective exchange of gases, nutrients and metabolites between maternal and fetal circulations (Huppertz, 2008; Schoenwolf *et al*., 2009). Additionally, part of the cytotrophoblasts differentiate into extravillous cytotrophoblasts, which invade through the endometrial stroma into the endometrial spiral arteries. They replace both the maternal endothelium and maternal vascular smooth muscle cells.

The spiral arteries now enlarge become low resistance vessels independent of maternal vasomotor control. This ascertains sufficient blood flow to the placenta throughout pregnancy (Huppertz, 2008; Schoenwolf *et al.,* 2009).

Different types of trophoblasts and their functions are listed in Table 1.3.

Table 1.3 Different types of trophoblasts and their function (Huppertz 2008)

Trophoblast type	**Weeks of gestation**	**Function**	**Differentiates into**
Trophoblast	3-4 weeks	Form outer blastocyst layer	Syncytiotrophoblasts Cytotrophoblasts
Syncytiotrophoblast	From 3rd week	Invade endometrium Formation of first lacunae	-
	From 4th week	Form syncytial protrusions Form outer layer of villous tree throughout pregnancy	-
Cytotrophoblast	3-4 weeks	Forms chorion with extraembryonic mesodermal cells	Villous cytotrophoblasts
Villous cytotrophoblast	From 5th week	Forms layer under syncytiotrophoblasts in placental villi Maintenance of syncytial layer of villi by differentiation	Syncytiotrophoblasts Extravillous cytotrophoblasts
Extravillous trophoblast	From 4th week	Invade endometrial stroma (decidua) Invade through maternal spiral arteries	-
	From 5th week	Replace maternal endothelium and smooth muscle cells in spiral arteries Transformation of spiral arteries to dilated tubes.	-

Throughout pregnancy the villi continue to mature and the placenta to develop. From the central to the distal part of the villous tree the number of cytotrophoblasts and the thickness of the syncytiotrophoblast layer decrease (Huppertz, 2008; Schoenwolf *et al.,* 2009). During the pregnancy, the weight of the placenta and the amount of cyto and syncytiotrophoblasts increase. The syncytiotrophoblasts remain dominant form and the proportion in relation to cytotrophoblasts increases as pregnancy advances between 13 and 31 weeks (Mayhew *et al*., 1999).

1.4.2 Placentation in preeclampsia

The placenta is well recognised as having a key role in the development of preeclampsia. This is known to be the case since PE occurs only during pregnancy, it resolves after delivery of the placenta, and it can occur in the absence of a viable fetus, for example in molar pregnancies. Blood supply to the placenta is via the spiral arteries, branches of the uterine arteries; placental development is a closely regulated process which is essential for normal fetal development.

The spiral arteries are remodelled in pregnancy in several stages, beginning at around the time of implantation. Remodelling transforms the arteries from low-flow, highly resistant vessels into the high-flow, low resistance vessels which are vital for normal placental development. Impaired remodelling of the spiral arteries is considered to be a key factor in the pathogenesis of PE (Steegers *et al.,* 2010). In PE, disturbance of spiral artery remodelling may occur as early as the time of implantation, offering a potential explanation for the fact that women with a history of sub-fertility or early miscarriage are at increased risk of the condition (Steegers *et al.,* 2010).

Intervillous flow, characterised by the appearance of connecting channels between the spiral arteries and the blastocyst, begins at 7-8 weeks' gestation.

Following this, the cytotrophoblastic cells of the developing placenta invade the decidual segments of the spiral arteries at around 10-12 weeks' gestation, and then the myometrial segments, at around 15-16 weeks' gestation. The trophoblast then invades both the endothelium and the highly muscular tunica media of the maternal spiral arteries.

In Preeclampsia, the cytotrophoblast invades the decidual portion of the spiral arteries, but invasion of the myometrial segments is impaired; the spiral arteries remain narrow, and blood supply to the fetus is restricted. The effects of this on the fetus become more significant as pregnancy progresses, since the uterine vasculature is unable to keep up with the increased amount of blood and nutrients necessary for fetal development.

Reduced perfusion of the placenta from the abnormal remodelling of the spiral arteries appears to be related to impaired placental development. In keeping with this theory, conditions associated with vascular insufficiency, including hypertension, diabetes, systemic lupus erythematosis (SLE) and renal disease all increase the risk of abnormal placentation and pre-eclampsia (Duckitt *et al.,* 2005).

Hypoperfusion of the developing placenta results in placental ischaemia; placental pathological findings indicative of ischaemia include atherosis, fibrinoid necrosis, thrombosis and placental infarction. The typical placental pathological appearances are not seen in all women with pre-eclampsia, but their presence does appear to correlate with disease severity (Salafia *et al*., 1998).

The interface between the placental and maternal components of pre-eclampsia development is thought to occur when the under-perfused, ischaemic placenta releases a variety of factors into the maternal circulation (Lee *et al.*, 2007).

1.5 PREDICTION OF PREECLAMPSIA

1.5.1 Background

The traditional method for 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, according to the American College of Obstetricians and Gynecologists (ACOG), taking a medical history to evaluate for risk factors (Table 1.4) is currently the best and only recommended screening approach for PE (ACOG, 2015). In the UK, the National Institute for Health and Clinical Excellence (NICE) has issued guidelines recommending that women should be considered to be at high-risk of developing PE if they have any one high-risk factor or any two moderate-risk factors (Table 1.5) (NICE, 2010). However, the performance of such approach, which essentially treats each risk factor as a separate screening test with additive detection rate (DR) and screen positive rate, is poor with DR of only 35% of all-PE and 40% of preterm-PE requiring delivery at <37 weeks' gestation, at false positive rate (FPR) of about 10% (Wright *et al.,* 2015)

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 Clinical risk factors for preeclampsia (American College of Obstetrics and Gynecologists 2015)

Primiparity
Previous preeclampsia
Chronic hypertension
Chronic renal disease
History of thrombophilia
Multiple pregnancy
In vitro fertilization
Family history of preeclampsia
Type I diabetes mellitus or type II diabetes mellitus
Obesity
Systemic lupus erythematosus
Advanced maternal age (older than 40 years)

Table 1.5 Recommendations on screening for preeclampsia (National Institute for Clinical Excellence 2010).

High risk factors
Hypertensive disease in a previous pregnancy
Chronic renal disease
Chronic hypertension
Diabetes mellitus
Autoimmune disease such as SLE or APS
Moderate risk factors
First pregnancy
Age ≥ 40 years
Body mass index ≥ 35 kg/m^2
Inter-pregnancy interval > 10 years
Family history of preeclampsia

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

1.5.2 Maternal demographic factors and obstetric history

Maternal age

Several studies reported increase in risk of PE with increasing maternal age (Saftlas *et al.*, 1990; Bianco *et al.*, 1996; Lawoyin and Ani, 1996; Mittendorf *et al.,* 1996; Chen *et al.*, 2000; Lee *et al.*, 2000; 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).

A systematic review 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*., 2009). In a large prospective observational cohort study of more than 120,000 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 (Wright *et al.,* 2015).

Parity

It is widely documented that women in their first pregnancy are more likely to suffer from PE. 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. 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, 1998).

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 or long interval between pregnancies (Robillard *et al.,* 1993). On the other hand, there are 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). In summary, the risk for PE is increased in nulliparous women (Wright *et al*., 2015) but further research into the mechanisms for this needs 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. This had led to the theory that prior exposure to paternal antigens has a protective role against preeclampsia.

The basis for this association is that reduced exposure to paternal antigen, by limited exposure to their sperm, conceiving after a short period of sexual relations or by alternatives techniques such as non-partner donor insemination or intracytoplasmic sperm injection (ICSI) results in an increased risk of PE (Marti and Herrmann 1977; Dekker, 2003; Dekker and Robillard, 2007).

Inter-pregnancy interval

Using a large registry in Norway, Skjaerven *et al*. evaluated the effects on the risk of PE of both the interbirth interval and a change of partner. There were over 550,000 women who had two or more singleton deliveries and 209,000 women who had three or more singleton deliveries. They found that the association between risk of PE and interval was more significant than the association with change of partner, with the risk in a second or third pregnancy directly related to the time elapsed since the previous delivery. Furthermore, when the interval was 10 years or more the risk of PE was about the same as that in nulliparous women. Further evidence for the importance of the inter-pregnancy interval comes from the study of Conde-Agudelo *et al*. (2000) which reported increased risks of PE in women with pregnancy intervals of more than 59 months compared with those with intervals of 18-23 months.

Parous women with history of previous preeclampsia

Previous history of PE is a strong risk factor for preeclampsia in subsequent pregnancies. Women who have PE in a first pregnancy are 7-10 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). 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 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 ($\leq$ 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 early

disease is 8%. (Langenveld *et al.,* 2011). An individual patient data meta-analysis examining the recurrence of hypertensive disorders in pregnancy reported that recurrence was 13.8% for PE and 8.6% for GH (Van Oostwaard *et al.*, 2015).

Assisted reproductive technologies

Assisted conception techniques have been shown to double the risk for PE (Shevell *et al.*, 2005; Maman *et al.*, 1998; Jackson *et al.*, 2004; Lambert-Messerlian *et al.*, 2006; Trogstad *et al.*, 2009). However, there is conflicting information as to whether both in-vitro fertilisation (IVF) and simple ovulation induction equally impact on the risk of PE. One large observational study has shown that it is IVF but not ovulation induction that increases the risk for PE (Shevell *et al.*, 2005) and one smaller case-control study has found that both techniques increase the likelihood of developing hypertension in pregnancy (Maman *et al.*, 1998). On the contrary, Trogstad *et al.* (2009) has reported that it is ovulation induction rather than IVF that increases the risk of PE by two-fold.

In a large prospective observational cohort study, more than 40,000 pregnancies were examined including 634 that conceived after IVF and 682 that conceived following ovulation induction. The results showed 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 with those having donor oocyte, there was an increased risk in those that had 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 another important risk factor for PE. Increased body mass index or increased abdominal circumference before pregnancy or in early pregnancy are well established risk factors for the condition (Eskenazi *et al.*, 1991; Stone *et al.*, 1994; Mittendorf *et al.*, 1996; Conde-Agudelo and Belizán, 2000; Mostello *et al.*, 2002). A pre-pregnancy body mass index (BMI) of more than 35 kg/m^2 increases the risk 3 to 5 fold as compared to those with a pre-pregnancy BMI of less than 24 kg/m^2 (Duckitt and Harrington 2005). Maternal obesity results in alteration of the plasma lipid profile with higher serum triglyceride and VLDL cholesterol, and lower HDL cholesterol concentrations than those observed in lean pregnant women. This pattern of dyslipidemia is similar to that of the “metabolic syndrome” described in the non-pregnant population (Sattar *et al*., 1997). Obesity is also associated with chronic low grade inflammation, a feature common to many of the other risk factors for the condition.

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.,* 2011).

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).

Racial origin

There is evidence that ethnic or racial origin is an important predictor of PE. The risk of PE is higher by 20-50% in black than in white women (Eskenazi *et al.,* 1991; Mittendorf *et al*., 1996; Sibai *et al.,* 1997; Knuist *et al*.,1998; Mostello *et al.,*2002; Caughey *et al.,* 2005). A large retrospective cohort study of 127,000 low risk pregnant women reported that rates of pre-eclampsia were higher among African- American women (5.2%, OR 1.41, 95% CI 1.25-1.62), and lower amongst Latin American (4.0%, OR 0.9, 95% CI 0.84-0.97) and Asian women (3.5%, OR 0.79, 95% CI 0.72- 0.88) compared to white women (Caughey *et al.,* 2005). Paternal ethnicity followed a similar pattern, with highest rates in African-American fathers, and lowest rates in Asian fathers. When maternal and paternal ethnic discordance were examined, the overall rate of PE was higher among mothers whose ethnicity differed from the father.

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 120,000 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 (Wright *et al.,* 2015).

Family history of preeclampsia

A family history of PE in first degree relatives has been shown to increase the risk of PE by 3-4 times (Duckitt and Harrington 2005). Arngrimsson *et al*. (1990) investigated the familial predisposition of PE by examining 94 families over at least three generations and demonstrated that the prevalence of PE in biological daughters is significantly higher than that in daughters-in-law (23% vs 10%). Cincotta and Brennecke (1998) demonstrated that a family history of PE in a mother, sister or both triples the risk of PE.

Cigarette smoking

Smoking cigarette in pregnancy is a controversial risk factor. Women who smoke during pregnancy seem to have less PE than non- smokers (Trogstad et al 2011). Smoking can, however, by no means be encouraged since it has numerous adverse effects on pregnancy which far outweigh any possible benefits (Andres and Day 2000). A large number of studies have suggested a reduction in the risk of PE with cigarette smoking in pregnancy. A systematic

review of 28 cohort and 7 case-control studies including more than 800,000 women has reported that cigarette smoking in pregnancy is associated with an overall 30% reduction in the risk of PE (Conde-Agudelo *et al.*, 2000). In addition, an inverse dose-response relationship has been observed. The risk for PE decreased as the number of cigarettes smoked daily during the pregnancy increased.

Pre-existing medical conditions

Pre-existing medical conditions such as insulin-dependent diabetes, autoimmune diseases and anti-phospholipid syndrome, chronic hypertension, renal disease diseases are clear risk factors of PE (Duckitt and Harrington 2005).

Chronic hypertension

Chronic hypertension increases the risk of PE approximately 10- fold as compared to controls. Women with chronic hypertension are also at risk for developing a severe form of the disease (Sibai et al 1995, McCowan et al 1996). Diastolic hypertension of 110 mmHg before 20 weeks' gestation increases the risk for PE 5-fold, preterm delivery (before 32 weeks' gestation) 7- fold and IUGR 7-fold (McCowan et al 1996).

Renal disease

Davies *et al.* (1970) have found that the prevalence of renal disease was higher in women who developed PE compared with those that did not (5.3% vs 1.8%). One study (Martinell *et al.*, 1990) compared women with renal disease (n=69), based on a history of urinary tract infections, with a prospective control population matched for age, parity, smoking and date of delivery and has demonstrated a near three-fold increase in the risk of developing PE in women with a history of urinary tract infections compared to controls (6.7% vs 2.6%).

Autoinmune 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).

Diabetes mellitus

The association of pre-gestational diabetes and PE is well recognized, and women with a history of diabetes have an up to 4-fold increased risk of development of PE compared to the general population (Garner *et al*., 1990; Ros *et al*., 1998; Lee *et al*., 2000).

1.5.3 Biophysical markers

Blood pressure

In PE, hypertension develops as a result of vasoconstriction and reduced peripheral vascular compliance (Salas, 1999). Although hypertension is only a secondary sign of PE, it is an important sign as it is an early indication of the disease. This highlights the importance of regular accurate monitoring of BP during antenatal care. Not only is BP monitoring useful in detecting PE, it also provides screening for pre-existing hypertension as pregnancy may often be the first opportunity for young women be in contact with the healthcare system. Accurate assessment of BP has been hindered by the considerable variability that BP exhibits within each individual. In particular, patients with higher BP tend to have higher variability and a more exaggerated response to stressful stimuli (Gordon *et al.*, 1976; Schulte *et al.*, 1984). During BP measurement at

rest the first recording is often the highest recording, which decreases as the patients become more familiar with the procedure (Wietlisbach *et al.*, 1988; Reeves, 1995; Huang and Morisky, 1999; Myers, 2006; Poon *et al.*, 2008a). It is therefore recommended by professional bodies that a series of BP measurements should be made until a pre-specified level of stability is achieved (National Heart Foundation of Australia, 2004; Pickering *et al.*, 2005).

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, blood pressure should be measured in both arms simultaneously and a series of four recordings are made at 1 min intervals. The final MAP should be calculated as the average of all four measurements.

Several studies have examined the use of MAP in the first and second trimesters as a screening test for subsequent development of hypertensive disorders. The studies reported widely contradictory results in the performance of screening, with detection rates of 8-93% and false positive rates of 2-55%, as a consequence of the varied methods in selection of the screened population, measurement of blood pressure, cut-offs used in defining the screen positive group and definitions of PE. The sample size ranged from 80 to 2,582, the incidence of PE was 3-53% and MAP was measured by either mercury sphygmomanometers or different types of automated devices at a wide range of gestations between 5 and 40 weeks (Friedman and Neff, 1977; Villar and Sibai, 1989; Rogers *et al*.,1994).

Uterine artery Doppler

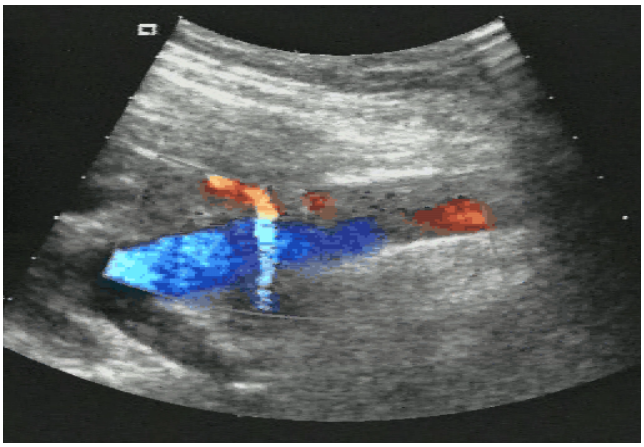
Doppler ultrasound provides a non-invasive method for the assessment of the uteroplacental circulation. Uterine artery Doppler is the most promising screening test for PE either alone or in combination with maternal history.
Several studies using colour Doppler imaging of the distal branches of uterine 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 uterine artery screening 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 vessels are identified using pulsed wave Doppler and distinguish 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 1.6. 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 1.6). 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 1.7). The Doppler gate is placed over the uterine artery, at an angle of less than 60°.

Figure 1.7. Transvaginal technique in obtaining uterine artery waveform lateral to uterine cervix.

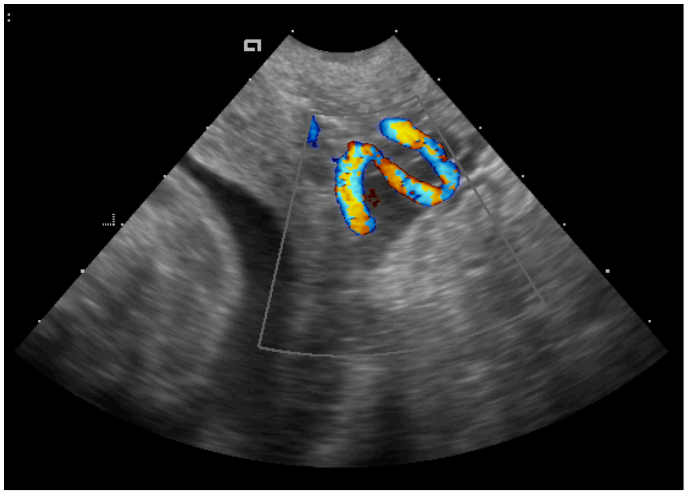
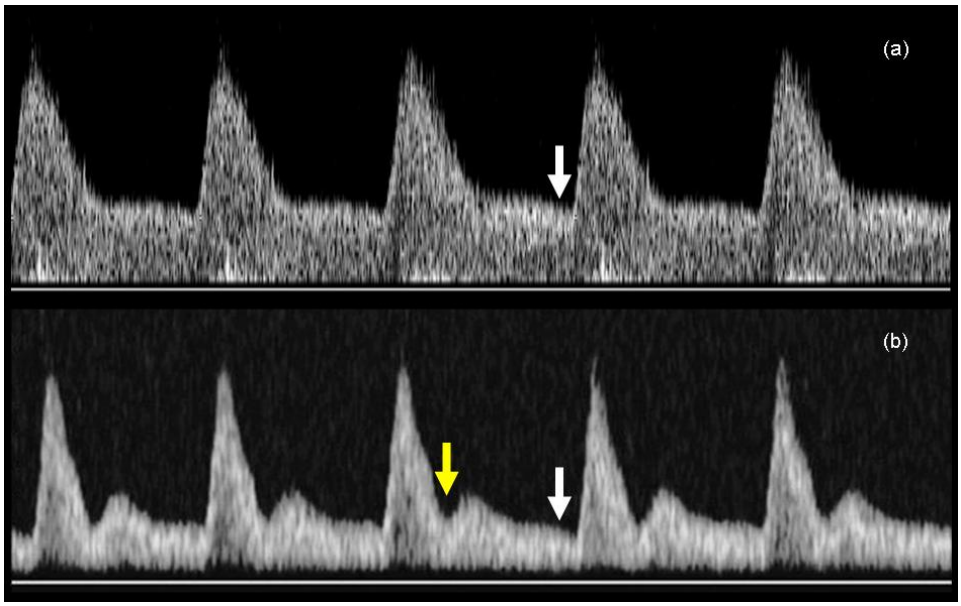


Figure 1.8. Pulsed wave signals of uterine artery blood flow (a) normal waveform: note the systolic upslope is less steep with an abundance of end-diastolic flow velocity (arrow); (b) abnormal waveform suggesting impaired placentation: note the steep systolic upslope with an early diastolic notch (yellow arrow) and reduced end-diastolic flow velocity (white arrow).



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

Resistance index = (PSV - MDV) / PSV, where PSV = Peak systolic velocity, MDV = minimum diastolic velocity

Pulsatility index = (PSV – MDV) / Mean velocity

Second trimester uterine artery Doppler

There is evidence that uterine artery Doppler in the mid trimester can detect 60% (24-89%) of PE at a FPR of 7% (4-14%). The discrepancies between Doppler studies may be a consequence of the differences in the use of Doppler technique for sampling (for example continuous-wave, pulsed-wave or colour Doppler to insonate the uterine arteries) and in the definition of abnormal flow velocity waveform (either resistance or pulsatility index above a certain cut-off or the presence of an early diastolic notch). A study in 157 high-risk women who underwent for uterine artery Doppler assessment at 24 weeks' gestation compared the velocity and impedance indices and concluded that uterine artery mean impedance indices perform better than velocity indices in the prediction of adverse pregnancy outcomes (Albaiges *et al*.,2003). 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.

A major second trimester screening study, involving 30,639 singleton pregnancies at 22-24 weeks' gestation, including 614 that developed PE reported that uterine artery Doppler is more effective in identifying early than late PE and PE associated with SGA than AGA fetuses (Yu *et al*., 2008). Uterine artery PI >95th percentile detected 77% of cases of early PE (requiring

delivery at <34 weeks), 36% of intermediate PE (requiring delivery at 34-37 weeks) and 22% of late PE delivering at >37 weeks. The respective percentages were 82%, 47% and 29% for those with PE and SGA, and 44%, 21% and 8% for those with SGA but without PE.

1.5.4 Biochemical markers

A wide range of potential biochemical markers have been investigated for the prediction of the disease (Table 1.7). Many such markers represent measurable manifestations of impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries and reduced placental perfusion that is thought to lead to placental ischemia and damage with the 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.

The most promising of all biochemical markers are placental growth factor (PlGF) and sFlt-1. There is evidence that placental insufficiency triggers an imbalance in the placental release to the maternal circulation with elevated concentrations of the anti-angiogenic sFlt-1 and decreased concentration of the pro-angiogenic PlGF (Herraiz *et al*., 2015).

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 as a multiple of the expected median (MoM) of the normal (Kagan *et al*., 2008a).

Table 1.7 Proposed maternal biochemical markers for the prediction of preeclampsia.

A disintegrin and metalloprotease 12	L-Arginine
Activin A	L-Homoarginine
Adiponectin	Leptin
Adrenomedullin	Magnesium
Alpha fetoprotein	Matrix metalloproteinase-9
Alpha-1-microglobulin	Microalbuminuria
Ang-2 angiopoietin-2	Microtransferrinuria
Antiphospholipid antibodies	N-Acetyl-*B*-glucosaminidase
Antithrombin III	Neurokinin B
Atrial natriuretic peptide	Neuropeptide Y
Beta2-microglobulin	Neutrophil gelatinase-associated
C-reactive protein	P-selectin
Calcium	Pentraxin-3
Cellular adhesion molecules	**Placenta growth factor**
Circulating trophoblast	Placental protein 13
Corticotropin release hormone	Plasminogen activator inhibitor-2
Cytokines	Platelet activation
Dimethylarginine (ADMA)	Platelet count
Endothelin	PAPA-A
Estriol	Prostacyclin
Ferritin	Relaxin
Fetal DNA	Resistin
Fetal RNA	Serum lipids
Free fetal haemoglobin	Soluble endoglin
Fibronectin	**Soluble fms-like tyrosine kinase**
Genetic markers	Thromboxane
Haptoglobin	Thyroid function
Hematocrit	Total proteins
Homocysteine	Transferrin
Human chorionic gonadotropin	Tumor necrosis factor receptor-1
Human placental growth hormone	Uric acid
Inhibin A	Urinary calcium to creatinine ratio
Insulin-like growth factor	Urinary kallikrein
Insulin-like growth factor binding protein	Vascular endothelial growth factor
Insulin resistance	Visfatin
Isoprostanes	Vitamin D

Placental growth factor

PLGF, a glycosylated dimeric protein, is a member of the vascular endothelial growth factor (VEGF) sub-family. 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.,* 2007). 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 (Torry *et al.,* 1998; Reuvekamp *et al.,* 1999; Livingston., 2001; Taylor *et al.,* 2003; Crispi *et al.,* 2006 Teixiera *et al.,* 2008). The decrease in serum PLGF and the separation in MoM values from normal is greater with earlier than later gestational age at which delivery for PE became necessary (Tsiakkas *et al.,* 2015).

Several studies, mainly in the second-trimester, reported that measurement serum PlGF may be useful in the prediction of PE (Table 1.8). 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 sFlt-1 (Stepan *et al.,* 2007).

Table 1.8. Studies of PlGF in the prediction of preeclampsia.

Author	GA	Preeclampsia			Non-preeclampsia	
		Definition	N	DR	n	FPR
Tidwell 2001	(16-20)	All PE	14	67%	25	11%
Su 2001	(14-19)	All PE	27	70%	277	30%
Madazli 2005	21-26	All PE	14	93%	108	6%
Espinoza 2007	24 (22-26)	All PE	110	69%	3186	49%
Stepan 2007	21 (19-24)	All PE	12	77%	38	38%
Diab 2008	23	All PE	33	88%	66	19%
Kusanovic 2009	22 (20-25)	All PE	62	52%	1560	24%
Kusanovic 2009	22 (20-25)	All PE	62	52%	1560	23%
Ghosh 2012	20-22	All PE	58	74%	1046	45%
McElrath 2012	17	All PE	139	53%	2014	40%
Necmiye 2013	15-19	All PE	13	62%	135	18%
Park 2014	24-27	All PE	8	63%	254	10%
Sibai 2008	12-20	Early-PE <27w	9	44%	690	10%
Crispi 2008	24	Early-PE <32w	10	84%	76	5%
Lambert-Messerlian 2009	(15-22)	Early-PE <32w	18	50%	225	10%
Ghosh 2012	22-24	Early-PE <32w	19	84%	1187	22%
Ghosh 2013	20-22	Early-PE <32w	11	82%	713	35%
Lambert Messerlian 2014	16 (15-20)	Early-PE <32w	20	28%	620	10%
Espinoza 2007	24 (22-26)	Early-PE <34w	25	80%	3186	49%
Espinoza 2007	24 (22-26)	Early-PE <34w	15	87%	3186	49%
Stepan 2007	21 (19-24)	Early-PE <34w	9	83%	38	38%
Diab 2008	23	Early-PE <34w	8	100%	66	24%
Kusanovic 2009	22 (20-25)	Early-PE <34w	9	100%	1613	4%
Kusanovic 2009	22 (20-25)	Early-PE <34w	9	100%	1613	3%
Villa 2013	19 (18-20)	Early-PE <34w	6	83%	79	9%
Wald 2012	(14-20)	Early-PE <36w	88	42%	275	10%
Sibai 2008	12-20	Early-PE <37w	76	21%	623	10%
Sibai 2008	(19-34)	Early-PE <37w	65	35%	589	10%
Myers 2013	15	Early-PE <37w	47	22%	3482	5%
Crispi 2008	24	Late-PE >32 w	19	0	76	5%
Lambert-Messerlian 2009	(15-22)	Late-PE ≥32w	26	NS	225	
Wald 2012	(14-20)	Late-PE ≥36w	88	18%	275	10%
Sibai 2008	(19-34)	Late-PE ≥37w	27	NS	600	10%

Vascular endothelial growth factor and soluble fmf-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 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.,* 2008a; Tache *et al.,* 2011). The sFlt-1 exerts anti-angiogenic effects by inhibiting biological activity of VEGF and PlGF (Kendall and Thomas, 1993). High 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).

Several studies have documented that serum concentration sFLT-1 is increased in the few weeks preceding the clinical onset of PE (Chaiworapongsa *et al.,* 2011; Rana *et al.,* 2012; Lai *et al.,* 2014). The main value of sFlt-1, is in the late second and third trimester to identify women that will develop PE requiring delivery within the subsequent four weeks. There is evidence that serum sFLT-1, measured in the second and third trimesters of pregnancy, improves the prediction of PE provided by maternal factors alone. In pregnancies that develop PE, serum sFLT-1 is increased and the separation in MoM values from normal is greater with earlier than later gestational age at which delivery for PE becomes necessary; consequently, the performance of

screening is superior for PE at <37 than PE at >37 weeks (Tsiakkas *et al.,* 2015).

Table 1.9. Studies of sFLT-1 in the prediction of preeclampsia.

Author	**GA**	**Preeclampsia**			**Non-preeclampsia**	
		Definition	**N**	**DR**	**N**	**FPR**
Stepan 2007	21 (19-24)	All PE	12	62%	38	30%
Diab 2008	23	All PE	33	96%	66	13%
Lim 2008	14-21	All PE	40	85%	100	45%
McElrath 2012	17	All PE	139	47%	2014	45%
Park 2014	24-27	All PE	8	75%	254	10%
Sibai 2008	12-20	Early-PE <27w	9	67%	684	10%
Crispi 2008	24	Early-PE <32w	10	37%	76	5%
Stepan 2007	21 (19-24)	Early PE <34w	9	67%	38	11%
Diab 2008	23	Early-PE <34w	8	100%	66	13%
Kusanovic 2009	22 (20-25)	Early-PE <34w	9	67%	1613	7%
Sibai 2008	(19-34)	Early-PE <37w	65	32%	584	10%
Sibai 2008	12-20	Early-PE <37w	76	18%	617	10%
Crispi 2008	24	Late-PE >32w	19	0	76	5%
Sibai 2008	(19-34)	Late-PE ≥37w	27	NS	600	10%

Combination of PLGF or sFlt-1 with other biomarkers

Table 2.0. Studies of PLGF or sFLT-1 in combination with other biomarkers in the prediction of preeclampsia.

Author	Biomarker	GA	Preeclampsia			Non-Preeclampsia	
			Definition	N	DR	n	FPR
Myers 2013	PLGF, MatCH	15	Early-PE <37w	47	45%	3482	5%
Espinoza 2007	PLGF, Ut Doppler	24	All PE	110	27%	3186	3.6%
Espinoza 2007	PLGF, Ut Doppler	24	Early-PE <34w	25	64%	3186	3.5%
Espinoza 2007	PLGF, Ut Doppler	24	Early-PE <34w	15	73%	3186	3.6%
Stepan 2007	PLGF, Ut-PI	21	All PE	12	77%	38	32%
Stepan 2007	PLGF, Ut-PI	21	Early-PE <34w	9	83%	38	24%
Crispi 2008	PLGF, Ut-PI	24	Early-PE <32w	10	89%	76	5%
Crispi 2008	PLGF, Ut-PI	24	Late-PE >32w	19	0	76	5%
Ghosh 2012	PLGF, Ut-PI	21	All PE	58	61%	1046	8%
Myers 2013	PLGF, Ut Doppler, MatCH	17	Early-PE <37w	47	47%	3482	5%
Rizos 2013	PLGF, Ut Doppler, BMI	21	All PE	12	45%	104	2.2%
Stepan 2007	sFlt1, Ut-PI	21	All PE	12	77%	38	27%
Stepan 2007	sFlt1, Ut-PI	21	Early-PE <34w	9	83%	38	11%
Crispi 2008	sFlt1, Ut-PI	24	Early-PE <32w	10	63%	76	5%
Crispi 2008	sFlt1, Ut-PI	24	Late-PE >32w	19	5.3%	76	5%
Crispi 2008	sFlt1, PLGF, Ut-PI	24	Early-PE <32w	10	89%	76	5%
Crispi 2008	sFlt1, PLGF, Ut-PI	24	Late-PE >32w	19	0	76	5%
Kienast 2015	sFlt1, PLGF, Ut-PI	22	All PE	48	65%	272	

1.6 PREVENTION OF PREECLAMPSIA

During the past decade, several randomized trials reported the use of various methods to reduce the rate or severity of PE such as protein or salt restriction; zinc, magnesium, fish oil, or vitamins C and E supplementation; the use of diuretics and other antihypertensive drugs; Aspirin or heparin to prevent PE. Even though these trials had limited sample sizes, results showed a minimum to no benefit.

1.6.1 Calcium

preeclampsia. In a large U.S. cohort of healthy primiparous women, calcium supplementation did not reduce incidence of PE (Levine *et al*., 1997). However, calcium supplementation might be expected to be of greater benefit in women who have a nutritional deficiency of calcium. A meta-analysis of 14 trials including 15,730 women reported a significant reduction in PE risk with calcium supplementation of > 1g/day (RR, 0.45; 95% CI, 0.31–0.65), with the greatest effect among women with low baseline calcium intake (RR, 0.36; 95% CI, 0.20–0.65) (Hofmeyr *et al*., 2014). Thus, calcium supplementation (1.5– 2 g) may be considered in pregnant women from populations with low baseline calcium intake (less than 600 mg/d) but this is not the case in developed countries. This limited evidence needs to be confirmed by larger and high-quality trials (Hofmeyr *et al*., 2014).

1.6.2 Folate

There are some data reporting a relationship between folate and the development of PE. Folate, an important methyl donor, is crucial for both DNA synthesis and conversion of homocysteine to methionine (Powers *et al.*, 1998). It is known that reduced folate intake or abnormal folate metabolism are associated with an increase in serum homocysteine (Ray and Laskin, 1999).

There is some data suggesting that homocysteine is increased in PE (Powers *et al.*, 1998; Raijmakers *et al.*, 2001; Mao *et al.*, 2010), thus there is a hypothetical possibility that folate supplementation could reduce the incidence of PE. Four retrospective cohort studies reported 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). However, two other studies failed to find a protective effect of folic acid supplementation (Timmermans *et al*., 2011; Li *et al*., 2013).

A recent meta-analysis of 13 trials including 2 randomized controlled trials (RCTs), 10 cohort studies, and 1 case–control study reported a pooled relative risk (RR, 0.62; 95% CI: 0.45–0.87) for the two RCTs, however, the pooled RR for nine cohort studies with available data on folic acid supplementation in pregnancy and gestational hypertension/preeclampsia was (RR, 0.92; 95% CI: 0.79–1.08) and the pooled RR for eight cohort studies with available data on folic acid supplementation and preeclampsia was (RR, 0.88; 95% CI: 0.76–1.02). Thus, whether folic acid supplementation in pregnancy can prevent the occurrence of gestational hypertension/preeclampsia remains uncertain (Hua *et al*., 2016).

1.6.3 Anti-oxidants

There is evidence that oxidative stress appears to contribute to the pathogenesis of

preeclampsia and it has been suggested that antioxidants may prevent preeclampsia.

Despite initial enthusiasm for using a combination of the anti- oxidants vitamin C and vitamin E for this purpose, large randomized, placebo-controlled trials conducted during pregnancy found that supplementation with vitamin C and vitamin E did not reduce the risk of pre- eclampsia or improve maternal and fetal outcomes in various populations (Poston *et al.,* 2006; Roberts *et al*., 2010; Rumbold *et al*., 2006; Spinnato *et al.*, 2007). A Cochrane systematic review of 15 randomized controlled trials including 20,748 women that used vitamin C and vitamin E for the prevention of preeclampsia found no benefit (RR, 0.94; 95% CI, 0.82–1.07) (Rumbold *et al*., 2008).

1.6.4 Heparin

Placental vascular lesions and incomplete transformation of uterine spiral arteries are found in pregnancies complicated by preeclampsia and small-for-gestational-age (SGA) (Brosens *et al*., 2011). Meta-analysis of randomized controlled trials (RCTs) have reported that, in pregnancies at high risk of PE, the prophylactic use of low-dose aspirin started ≤16weeks' gestation can reduce the prevalence of PE and SGA (Bujold *et al*., 2010; Roberge *et al*.,2012). The role of heparin in prevention of these conditions is becoming increasingly apparent, mainly due to its antithrombotic and anti-inflammatory effects, similar to those of aspirin (Mastrolia *et al.,* 2015).

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 a recent 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) and in delivery of SGA neonates (RR 0.54, 95% CI 0.32 to 0.91) (Roberge *et al*., 2016). Based on the limited evidence, the addition of LMWH to low-dose aspirin could reduce the prevalence of PE and SGA in women with a history of PE. However, a well-conducted future trial should be performed before a clinical application.

1.6.5 Aspirin

It has been hypothesized that alterations in systemic prostacyclin–thromboxane balance contributes to PE. Furthermore, inflammation is increased in preeclampsia (Redman *et al*.,2005). Aspirin (acetylsalicylic acid) is an anti-inflammatory agent that blocks the production of thromboxanes and has been studied in several trials for the prevention of preeclampsia, both in high-risk and low risk groups.

For women at high risk of preeclampsia, there are early trials suggested that daily aspirin had a significant protective effect (Wallenburg *et al*., 1986; Schi *et*

al., 1989). These initially promising findings were not confirmed in three large randomized controlled trials (Italian study of aspirin in pregnancy, 1993; CLASP Collaborative Group, 1994; Caritis *et al.,*1998). All three studies found a non-significant trend toward a lower incidence of preeclampsia in the aspirin-treated groups with no major adverse effects. A subsequent comprehensive meta-analysis of antiplatelet agents to prevent preeclampsia that included more than 30,000 women from 31 trials at varying risk statuses suggested that antiplatelet agents have a modest benefit, with a relative risk (RR) of preeclampsia of 0.90 (95% confidence interval [CI], 0.84–0.97) for aspirin-treated participants (Duley *et al*., 2007).

A follow-up Cochrane meta-analysis of 59 trials with more than 37,000 women found a 17% reduction in risk of preeclampsia associated with use of anti-platelet agents, with a significant increase in absolute risk reduction in women who are at high risk of the disease (Knight *et al*., 2007).

In most studies that evaluated aspirin for the prevention of PE, the initiation of treatment was at or after 16 weeks' gestation. Examination of a small number of randomised trials of low-dose aspirin in women at high risk for PE suggests that the effectiveness of therapy is related to the gestational age at the initiation of treatment. A meta-analysis by Bujold *et al* reported that low-dose aspirin started at 16 weeks or earlier was associated with a significant reduction in the relative risk (RR) for PE (0.47, 95% CI 0.34 to 0.65) and fetal growth restriction (FGR; 0.44, 95% CI 0.30 to 0.65). In contrast, aspirin started after 16 weeks did not have a significant benefit (PE: RR 0.81, 95% CI 0.63 to 1.03; FGR: RR 0.98, 95% CI 0.87 to 1.10). More detailed analyses of these data on PE demonstrated that low-dose aspirin started at or before 16 weeks' gestation was particularly effective in preventing preterm PE rather than term PE (RR: 0.11,95% CI 0.04 to 0.33 vs RR: 0.98, 95% CI 0.42 to 2.33)

The small number and small size of individual trials preclude definitive conclusions to be drawn regarding the effectiveness of aspirin starting before 16 weeks and the results need to be examined in a prospective major randomised trial.

1.6.6 Dietary salt intake

One systematic review of all the trials that studied sodium restriction in 603 women found no significant benefits (RR, 1.11) (Duley *et al.,* 2005). However, the trials may not have had adequate power to detect a benefit. Similarly, metaanalysis of approximately 7,000 randomized patients from clinical trials suggested that diuretics did not reduce the incidence of preeclampsia (Collins *et al*., 1985).

1.6.7 Lifestyle modifications

Although bed rest has been suggested as a preventive strategy, the evidence for this is scarce (Meher *et al.,* 2006). The only two studies located that evaluated bed rest as a preventive strategy were both small (32 participants and 72 participants) and did not evaluate perinatal and maternal morbidity and mortality and adverse effects of bed rest. However, regular exercise has been hypothesized to prevent preeclampsia by improving vascular function (Weissgerber *et al*., 2004; Yeo *et al*., 2001). In women who are not pregnant, moderate exercise has been shown to reduce hypertension and cardiovascular disease. Thirty minutes of moderate exercise on most days is currently recommended during normal pregnancy (Zavorsky *et al*., 2011). Moderate exercise also has been hypothesized to stimulate placental angiogenesis and improve maternal endothelial dysfunction. Several small clinical trials have evaluated the utility of modest exercise for the prevention of preeclampsia, but the CIs were too wide to make any reliable conclusions about the efficacy (Meher *et al*., 2006). Large randomized controlled clinical trials are needed that can evaluate whether moderate exercise can reverse markers of endothelial dysfunction and prevent adverse pregnancy outcomes.

1.7 AIMS OF THE THESIS

The aims of the studies in this thesis are:

1. To develop a model for prediction of PE using combined screening by maternal characteristics and medical history, uterine artery PI, MAP, serum PLGF and sFLT-1 at 20-24 weeks' gestation.

2. To explore the possibility of carrying out routine screening for preterm-PE by maternal factors and MAP in all pregnancies and reserving measurements of UTPI and PLGF only for a subgroup of the population selected on the basis of the risk derived from screening by maternal factors and MAP alone.

3. To determine maternal characteristics affecting uterine artery PI in normal pregnancies at 20-24 weeks' gestation and examine in pregnancies with PE the relation between uterine artery PI MoM and severity of disease.

4. To assess the performance of screening for PE by MAP at 11-13 and at 20-24 weeks' gestation.

Chapter 2 PUBLISHED STUDIES

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Study 1 Prediction of preeclampsia by mean arterial pressure at 11-13 and 20-24 weeks' gestation.

Study 2 Prediction of preeclampsia by uterine artery Doppler at 20-24 weeks' gestation.

Study 3 Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19-24 weeks' gestation

Study 4 Contingent screening for preterm preeclampsia

Study 1

Gallo DM, Poon LC, Fernandez M, Wright D, Nicolaides KH. Prediction of Prediction of preeclampsia by mean arterial pressure at 11-13 and 20-24 weeks gestation. Fetal Diagn Ther 2014;36:28-37.

Impact Factor: 2.939

Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2014;36:28–37
DOI: 10.1159/000360287

Received: December 8, 2013
Accepted: January 28, 2014
Published online: April 15, 2014

Prediction of Preeclampsia by Mean Arterial Pressure at 11–13 and 20–24 Weeks' Gestation

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Key Words
Preeclampsia · Mean arterial pressure · First trimester · Second trimester · Screening · Pyramid of pregnancy care

Abstract
Objectives: To assess the performance of screening for preeclampsia (PE) by mean arterial pressure (MAP) at 11–13 and at 20–24 weeks' gestation. ***Methods:*** MAP was measured at 11–13 and 20–24 weeks in 17,383 singleton pregnancies, including 70 with early PE, requiring delivery <34 weeks' gestation, 143 with preterm PE, delivering <37 weeks and 537 with total PE. MAP was expressed as multiple of the median (MoM) after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes. The performance of screening for PE by maternal characteristics and MAP MoM at 11–13 weeks (MAP-1), MAP MoM at 20–24 weeks (MAP-2) and their combination was evaluated. ***Results:*** In screening by maternal characteristics and MAP-1, at a false-positive rate (FPR) of 10%, the detection rates (DR) of early PE, preterm PE and total PE were 74.3, 62.9 and 49.3%, respectively; the DR at FPR of 5% were 52.9, 42.7 and 35.8%. In screening by MAP-1 and MAP-2 the DR at FPR of 10%, were 84.3, 65.7 and 52.5%; the DR at FPR of 5% were 60.0, 49.7 and 37.6%, respectively. ***Conclusions:*** Performance of screening for PE by MAP is best when measurements are taken at both 11–13 and 20–24 weeks' gestation than at only one of these gestational ranges.

Introduction

Preeclampsia (PE) affects about 2% of pregnancies and is a major cause of maternal and perinatal morbidity and mortality [1–3]. Consequently, extensive research in the last decade has focused on prediction of pregnancies at high risk for PE with the objectives of, firstly, undertaking pharmacological interventions to prevent the development of the disease and, secondly, for those who develop PE to diagnose the condition at its early stages and improve outcome by close monitoring for timely delivery.

An important component of various biophysical and biochemical markers used in screening for PE is mean arterial pressure (MAP) [4, 5]. We have previously proposed that MAP should be measured by validated automated devices, that two measurements should be taken from each arm and the average of the four should be used and that the MAP should be expressed as multiple of the median (MoM) after adjustment for maternal characteristics [6]. We have also proposed that in screening for PE, gestation at the time of delivery for PE is treated as a continuous rather than categorical variable [7]. This approach, which is based on a survival time model, assumes that if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on a competition between delivery before or after development of PE.

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The effect of variables from maternal characteristics and history and biomarkers is to modify the mean of the distribution of gestational age at delivery with PE, so that 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 high-risk pregnancies the distribution is shifted to the left. We estimated that screening at 11–13 weeks' gestation by a combination of maternal characteristics and MAP would detect, at a false-positive rate (FPR) of 10%, about 73% of pregnancies that would develop early PE, requiring delivery <34 weeks' gestation, 59% of cases with preterm PE, delivering <37 weeks and 54% of all cases of PE [7].

The objective of this screening study in singleton pregnancies examined at both 11–13 and 20–24 weeks, were, firstly, to examine the maternal characteristics that affect MAP in normal pregnancies and, secondly, to compare the performance of screening for PE by MAP in the first and second trimesters of pregnancy.

Methods

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending their first- and second-trimester routine ultrasound examinations between 2006 and 2013 at three hospitals in and around London (King's College Hospital; University College London Hospital; Medway Maritime Hospital, Kent). The first-trimester visit, at 11–13 weeks' gestation, included recording of maternal characteristics and medical history, measurement of serum-free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A (PAPP-A) and an ultrasound scan to, firstly, confirm gestational age from the measurement of the fetal crown-rump length (CRL) [8], secondly, diagnose any major fetal abnormalities and, thirdly, measure fetal nuchal translucency thickness as part of combined screening for aneuploidies [9]. The second-trimester visit, at 20–24 weeks' gestation, included ultrasound examination for assessment of fetal growth and anatomy. In both visits we measured maternal MAP. Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the ethics committee of each participating hospital.

Entry Criteria

The entry criteria for the study were singleton pregnancies with measurements of MAP at 11–13 and/or 20–24 weeks' gestation that resulted in live birth or stillbirth of phenotypically normal babies at or after 24 weeks' gestation.

In the study comparing the performance of screening for PE by MAP in the first and second trimesters of pregnancy, we used data from 17,383 cases with measurements of MAP at both 11–13 weeks' gestation (MAP-1) and 20–24 weeks (MAP-2). The 17,383 cases included 537 (3.1%) who developed PE, 527 (3.0%) who developed gestational hypertension (GH), 891 (5.1%) delivering small for gestational age (SGA) neonates (without hypertension in pregnancy) and 15,428 (88.8%) cases who were unaffected by these outcomes.

In the estimation of MoM values for MAP-1, we used data from 60,835 pregnancies, including the 17,383 cases with recordings of both MAP-1 and MAP-2. The 60,835 cases included 1,496 (2.5%) who developed PE, 1,497 (2.5%) who developed GH, 2,994 (4.9%) delivering SGA neonates and 54,848 (90.2%) cases who were unaffected by these outcomes.

In the estimation of MoM values for MAP-2 we used data from 19,278 pregnancies, including the 17,383 cases with recordings of both MAP-1 and MAP-2. The 19,278 cases included 587 (3.0%) who developed PE, 592 (3.1%) who developed GH, 1,028 (5.3%) delivering SGA neonates and 17,071 (88.6%) cases who were unaffected by these outcomes.

Maternal History and Characteristics

Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilisation), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), history of type 1 or 2 diabetes mellitus (yes or no), history of systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS) (yes or no), family history of PE in the mother of the patient (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks), previous pregnancy with PE (yes or no), previous pregnancy with SGA babies (yes or no) and inter-pregnancy interval. The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were recorded.

Mean Arterial Pressure

The MAP was measured by validated automated devices (3BTO-A2; Microlife, Taipei, Taiwan), which were calibrated before and at regular intervals during the study. The recordings were made by doctors who had received appropriate training on the use of these machines. The women were in the sitting position, their arms were supported at the level of the heart, and a small (22 cm), normal (22–32 cm), or large (33–42 cm) adult cuff was used depending on the mid-arm circumference. After rest for 5 min, two recordings of blood pressure (BP) were made in both arms simultaneously. We calculated the final MAP as the average of all four measurements [6].

Outcome Measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with preexisting or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or non-proteinuric GH.

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [10]. The systolic BP should be ≥140 mm Hg and/or the diastolic BP should be ≥90 mm Hg on at least two occasions 4 h apart developing after 20 weeks' gestation in previously normotensive women and there should be proteinuria (≥300 mg in 24 h or two readings of at ≥2+ 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 af-

Fetal Diagn Ther 2014;36:28–37
DOI: 10.1159/000360287

Table 1. Characteristics of the study population

Characteristic	MAP at 11–13 weeks		MAP at 20–24 weeks		MAP at 11–13 and 20–24 weeks	
	normal (n = 54,848)	PE (n = 1,496)	normal (n = 17,071)	PE (n = 587)	normal (n = 15,428)	PE (n = 537)
Maternal age, years, median (IQR)	31.3 (27.0–35.0)	31.3 (26.6–35.7)	31.0 (26.4–34.7)	31.0 (26.7–34.7)	31.0 (26.4–34.7)	31.1 (26.8–34.8)
Maternal weight at 11–13 weeks, kg, median (IQR)	65.7 (58.9–75.5)	72.0 (63.0–85.6)*	–	–	66.7 (59.1–77.0)	73.0 (63.0–87.0)*
Maternal weight at 20–24 weeks, kg, median (IQR)	–	–	71.0 (63.6–81.3)	77.0 (67.4–90.1)*	71.0 (63.6–81.5)	77.0 (67.6–90.8)*
Maternal height, cm, median (IQR)	164 (160–168)	163 (158–167)*	164 (160–168)	163 (158–167)*	164 (160–169)	163 (159–167)*
GA at screening at 11–13 weeks, median (IQR)	12.7 (12.3–13.1)	12.6 (12.3–13.0)	–	–	12.7 (12.3–13.1)	12.6 (12.3–13.01)
GA at screening at 20–24 weeks, median (IQR)	–	–	22.3 (21.9–23.0)	22.3 (22.0–23.0)	22.3 (21.9–22.9)	22.1 (21.9–23.0)
Racial origin, n (%)						
Caucasian	40,344 (73.6)	814 (54.4)*	11,331 (66.4)	266 (45.3)*	10,468 (67.9)	251 (46.7)*
Afro–Caribbean	8,698 (15.9)	529 (35.4)*	4,137 (24.2)	269 (45.8)*	3,523 (22.8)	244 (45.4)*
South Asian	2,872 (5.2)	89 (5.9)	721 (4.2)	28 (4.8)	649 (4.2)	20 (3.7)
East Asian	1,514 (2.8)	27 (1.8)*	372 (2.2)	11 (1.9)	338 (2.2)	10 (1.9)
Mixed	1,420 (2.6)	37 (2.5)	510 (3.0)	13 (2.2)	450 (2.9)	12 (2.2)
Past obstetric history, n (%)						
Nulliparous	27,037 (49.3)	885 (59.2)*	7,818 (45.8)	317 (54.0)*	7,045 (45.7)	290 (54.0)*
Parous with no prior PE and SGA	24,689 (45.0)	358 (23.9)*	8,183 (47.9)	159 (27.1)*	7,431 (48.2)	148 (27.6)*
Parous with prior PE no SGA	1,274 (2.3)	169 (11.3)*	428 (2.5)	70 (11.9)*	393 (2.5)	64 (11.9)*
Parous with prior SGA no PE	1,694 (3.1)	40 (2.7)	581 (3.4)	23 (3.9)	506 (3.3)	18 (3.4)
Parous with prior PE and SGA	154 (0.3)	44 (2.9)*	61 (0.4)	18 (3.1)*	53 (0.3)	17 (3.2)*
Inter-pregnancy interval, months, median (IQR)	28.6 (17.5–45.3)	34.9 (19.0–61.6)*	30.4 (17.9–46.7)	36.0 (20.0–63.1)*	30.0 (18.0–47.0)	35.3 (19.2–62.5)*
Cigarette smoker, n (%)	4,906 (8.9)	106 (7.1)*	1,633 (9.6)	33 (5.6)*	1,494 (9.7)	31 (5.8)*
Patients' mother had PE, n (%)	2,066 (3.8)	117 (7.8)*	631 (3.7)	36 (6.1)*	568 (3.7)	34 (6.3)*
Conception, n (%)						
Spontaneous	52,907 (96.5)	1,414 (94.5)*	16,539 (96.9)	565 (96.3)	14,964 (97.0)	516 (96.1)
Ovulation drugs	661 (1.2)	25 (1.7)	197 (1.2)	11 (1.9)	156 (1.0)	11 (2.0)*
In vitro fertilisation	1,280 (2.3)	57 (3.8)*	335 (2.0)	11 (1.9)	308 (2.0)	10 (1.9)
Chronic hypertension, n (%)	528 (1.0)	179 (12.0)*	201 (1.1)	88 (15.0)*	180 (1.1)	80 (14.9)*
No medication	269 (0.5)	78 (5.2)*	93 (0.5)	40 (6.8)*	98 (0.6)	39 (7.3)*
Medication	259 (0.5)	101 (6.8)*	108 (0.6)	48 (8.2)*	82 (0.5)	41 (7.6)*
Preexisting diabetes mellitus, n (%)	377 (0.7)	37 (2.4)*	137 (0.8)	9 (1.6)	127 (0.8)	8 (1.5)
Type 1	196 (0.4)	17 (1.1)*	67 (0.4)	1 (0.2)	61 (0.4)	1 (0.2)
Type 2	181 (0.3)	20 (1.3)*	70 (0.4)	8 (1.4)*	66 (0.4)	7 (1.3)*
SLE/APS, n (%)	108 (0.2)	10 (0.6)*	28 (0.2)	3 (0.5)	26 (0.2)	3 (0.6)
Gestation at delivery, weeks, median (IQR)	40.1 (39.1–41.0)	36.4 (38.5–40.0)*	40.1 (39.1–40.9)	38.5 (36.7–40.0)*	40.1 (39.1–40.9)	38.5 (36.7–40.0)*
Birth weight, g, median (IQR)	3,440 (3,140–3,750)	2,940 (2,258–3,420)*	3,430 (3,130–3,744)	2,988 (2,315–3,432)*	3,433 (3,135–3,750)	3,000 (2,325–3,475)*
Birth weight centile, median (IQR)	49.6 (26.9–74.9)	25.6 (6.8–59.3)*	49.3 (26.7–74.6)	29.4 (7.1–61.7)*	49.6 (27.0–75.0)	29.8 (7.6–62.2)*

* Significant p value <0.05.

ter 20 weeks' gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

The definition of SGA was birth weight below the 5th percentile of a reference range derived from our population [11].

Statistical Analysis

Comparisons of maternal characteristics between the outcome groups were by χ^2 test or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables.

The distribution of MAP-1 and MAP-2 were made gaussian after logarithmic transformation. Backward stepwise multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of the $\log_{10}$ MAP, adjusting for the adverse pregnancy outcomes as specified (PE, GH and SGA). Variables were excluded from the model if the p value was >0.05 or if their effect size was less than one tenth of the $\log_{10}$ MoM standard deviation. Gestational age for MAP-1 was centred by subtracting 77 days and for MAP-2 by subtracting 133 days, maternal weight was centred by subtracting 69 kg and maternal height was centred by subtracting 164 cm. The distribution of MAP-1 and MAP-2 was then expressed as MoM in all cases, correcting for the significant predictors as defined in the multiple regression.

A competing risk model was used to combine the prior information from maternal characteristics with MAP MoM values [7, 12]. The distribution of gestational age at delivery with PE was defined by two components: firstly, the prior distribution based on maternal characteristics [7] and, secondly, the distribution of MAP MoM values with gestational age in pregnancies affected by PE. In the cases of PE, regression analysis was used to determine the relationship between $\log_{10}$ MoM values with gestational age at delivery.

The risk for early PE (<34 weeks), preterm PE (<37 weeks) and total PE in screening by maternal characteristics, MAP-1, MAP-2 and their combination was estimated for each pregnancy and the detection rate (DR) of early PE, preterm PE and total PE, at fixed FPR of 5 and 10% were calculated. The performance of screening for PE by MAP-1 MoM, MAP-2 MoM and their combination was compared by the areas under receiver operating characteristic (AUROC) curve analysis.

R statistical software [13], SPSS Version 20.0 (IBM SPSS Statistics for Windows, Armonk, N.Y., USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) were used for the data analyses.

Results

The characteristics of the study populations with measurements of MAP-1, MAP-2 and both MAP-1 and MAP-2 are presented in table 1. In the PE group, compared to the normal group, there was a higher median maternal weight, a longer inter-pregnancy interval, a higher prevalence of Afro-Caribbean racial origin, personal history of PE with and without associated SGA, family history of PE, women who conceived with ovulation drugs, history of chronic hypertension and preexisting diabetes mellitus, and there was a lower maternal height and a lower prevalence of smokers. The median gestational age at delivery and neonatal birth weight were significantly lower in the PE group than in the normal group.

Normal Pregnancy Outcome

Multiple regression analysis demonstrated that for the prediction of both $\log_{10}$ MAP-1 and $\log_{10}$ MAP-2 significant independent contributions were provided by gestational age at screening, maternal weight and height, Afro-Caribbean racial origin, family history of PE, prior history of PE, cigarette smoking and chronic hypertension (tables 2, 3). The biggest effects on both MAP-1 and MAP-2 were provided by maternal weight (around 14% increase for change in weight from 50 to 100 kg) and history of chronic hypertension (around 12% increase), whereas the effects of the other factors were less than 3%.

In each patient we used the models in tables 2 and 3 to derive the expected $\log_{10}$ MAP-1 and $\log_{10}$ MAP-2, and then expressed the observed values as MoM of the expected. The median of MAP-1 and MAP-2 are presented in table 4. In the normal group, the median MAP-2 was 0.8 mm Hg (95% CI 0.6–1.0) and 0.9% (95% CI 0.1–6.0) lower than MAP-1 ($p < 0.0001$).

PE Group

In the PE group, compared to the normal group, the median MAP-1 and MAP-2, expressed as mm Hg or MoM, were significantly increased (table 4). There was a significant inverse association between gestational age at delivery with both MAP-1 $\log_{10}$ MoM ($r = -0.190$, $p < 0.0001$; fig. 1) and MAP-2 $\log_{10}$ MoM ($r = -0.259$, $p < 0.0001$; fig. 1). The fitted regression models for $\log_{10}$ MoM values on gestational age at delivery are presented in table 5 and the estimated parameters for the assumed multivariate gaussian distributions for $\log_{10}$ MoM values are given in table 6.

The DR of early PE, preterm PE and total PE, at fixed FPR of 5 and 10%, in screening by maternal characteristics, MAP-1, MAP-2 and their combination are given in table 7 and illustrated in figure 2. In the prediction of early PE, the AUROC for maternal characteristics with MAP-1, maternal characteristics with MAP-2 and the combination of all were significantly higher than the AUROC for maternal characteristics alone ($p = 0.001$; $p = 0.002$; $p = 0.001$) (table 7). The AUROC for the combination of all was not significantly different from the AUROC for maternal characteristics with MAP-1 (p =

Table 2. Fitted regression model for $\log_{10}$ MAP at 11–13 weeks

	Estimate	Standard error	LCL	UCL	p
Constant	1.93382	0.00106	1.93174	1.93590	<0.00001
(Gestation at screening – 77 days)	0.00054736	0.00017672	0.00020098	0.00089374	0.002
(Gestation at screening – 77 days)^2	–0.000034347	0.000007126	–0.000048315	–0.000020380	0.00001
(Weight – 69 kg)	0.0012177	0.0000143	0.0011897	0.0012457	<0.00001
(Weight – 69 kg)^2	–0.0000098635	0.0000004797	–0.0000108038	–0.0000089232	<0.00001
(Height – 164 cm)	–0.00022305	0.00002462	–0.00027130	–0.00017480	<0.00001
Afro-Caribbean racial origin	–0.0036266	0.0004170	–0.0044439	–0.0028094	<0.00001
Smoker	–0.0080903	0.0005249	–0.0091191	–0.0070615	<0.00001
History of chronic hypertension	0.051581	0.001390	0.048857	0.054305	<0.00001
Patient's mother had PE	0.0063512	0.0007766	0.0048291	0.0078732	<0.00001
Parous with no previous PE	–0.0050264	0.0003150	–0.0056437	–0.0044090	<0.00001
Parous with previous PE	0.0088850	0.0009070	0.0071072	0.0106628	<0.00001

LCL = Lower confidence limit; UCL = upper confidence limit.

Table 3. Fitted regression model for $\log_{10}$ MAP at 20–24 weeks

	Estimate	Standard error	LCL	UCL	p
Constant	1.92843	0.00301	1.93063	1.94245	<0.00001
(Gestation at screening – 133 days)	–0.00014481	0.00003801	–0.00021931	–0.00007032	0.00014
(Weight – 69 kg)	0.001392712	0.000029353	0.001335181	0.001450243	<0.00001
(Weight – 69 kg)^2	–0.0000126	0.0000008	–0.0000142	–0.0000110	<0.00001
(Height – 164 cm)	–0.0002180270	0.0000423726	–0.0003010774	–0.0001349767	<0.00001
Afro-Caribbean racial origin	–0.00550050	0.00061446	–0.00670484	–0.00429616	<0.00001
Smoker	–0.0008325	0.0008735	–0.0025446	0.0008797	0.04033
History of chronic hypertension	0.0449005	0.0021246	0.0407363	0.0490647	<0.00001
Patient's mother had PE	0.005253	0.001329	0.002648	0.007858	0.00008
Parous with no previous PE	–0.0071593	0.0005381	–0.0082139	–0.0061046	<0.00001
Parous with previous PE	0.0056815	0.0014763	0.0027880	0.0085750	0.00001

LCL = Lower confidence limit; UCL = upper confidence limit.

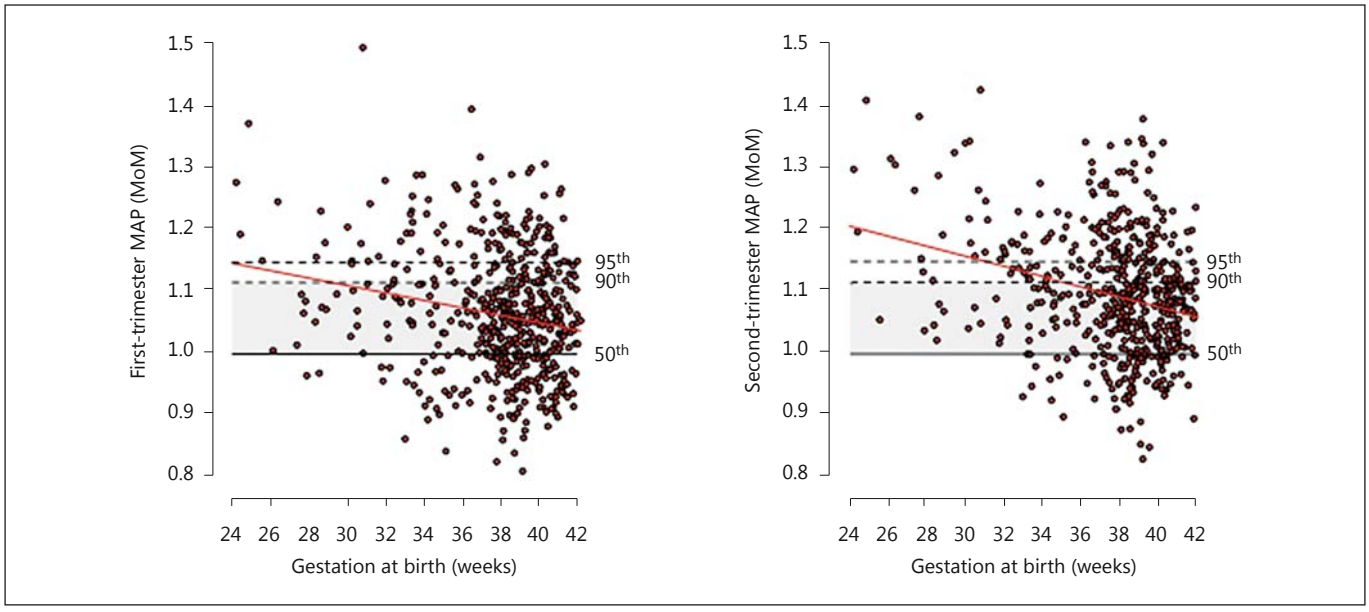
Table 4. First- and second-trimester MAP in outcome groups

	Normal (n = 15,428)	PE (n = 537)	p
MAP at 11–13 weeks, mm Hg, median (IQR)	84.7 (79.7–90.3)	92.5 (86.6–100.3)	<0.0001
MAP at 20–24 weeks, mm Hg, median (IQR)	83.9 (79.0–89.4)	91.7 (86.0–98.3)	<0.0001
MAP at 11–13 weeks, MoM, median (IQR)	0.995 (0.943–1.053)	1.055 (0.993–1.125)	<0.0001
MAP at 20–24 weeks, MoM, median (IQR)	0.999 (0.946–1.056)	1.060 (0.997–1.130)	<0.0001

Comparisons between outcome groups were by Mann-Whitney U test.

Table 5. Fitted regression model for marker $\log_{10}$ MoM values of MAP on gestation at time of delivery for pregnancies with PE

Marker	Intercept	Standard error	p	Slope	Standard error	p
MAP at 11–13 weeks	0.094903	0.011256	<0.0001	–0.0017948	0.0002974	<0.0001
MAP at 20–24 weeks	0.14474	0.01755	<0.0001	–0.0031737	0.0004626	<0.0001



Color version available online

Fig. 1. Relationship between gestational age at delivery and first-trimester (left) and second-trimester (right) MAP MoM in women who developed PE. The three horizontal lines represent the 50th, 90th and 95th percentiles of MAP MoM.

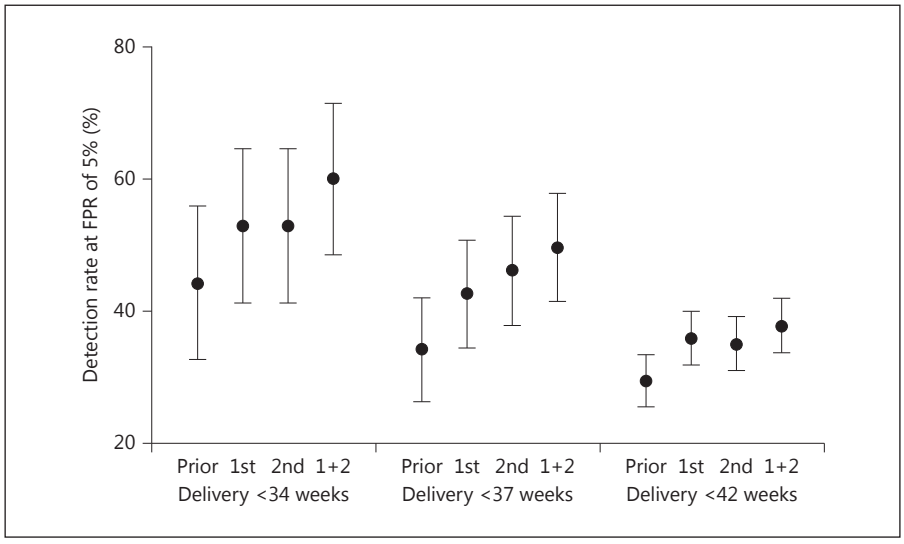


Fig. 2. Estimated DR, with 95% CIs, of PE requiring delivery at <34, <37 and <42 weeks' gestation, at FPR of 5%, in screening by maternal characteristics and history (prior), MAP at 11–13 weeks' gestation (1), MAP at 20–24 weeks (2) and their combination (1 & 2).

Table 6. Standard deviations (SD) and correlations, with 95% CIs, for $\log_{10}$ MoM for MAP

	Normal outcome	PE
SD MAP at 11–13 weeks (MAP-1)	0.036805 (95% CI 0.036506, 0.037108)	0.039748 (95% CI 0.038372, 0.041226)
SD MAP at 20–24 weeks (MAP-2)	0.035112 (95% CI 0.034603, 0.035638)	0.040725 (95% CI 0.038521, 0.043198)
Correlation MAP-1 and MAP-2	0.44381 (95% CI 0.43707, 0.45051)	0.46898 (95% CI 0.42844, 0.50763)

Table 7. Estimated detection rates of PE requiring delivery before 34, 37 and 42 weeks' gestation at FPRs of 5 and 10%

Screening test	PE <34 weeks (n = 70)			PE <37 weeks (n = 143)			PE all (n = 537)		
	AUROC	detection rate (95% CI)		AUROC	detection rate (95% CI)		AUROC	detection rate (95% CI)	
		FPR 5%	FPR 10%		FPR 5%	FPR 10%		FPR 5%	FPR 10%
Maternal characteristics	0.831 (0.825–0.837)	44.3 (32.6–55.9)	55.7 (44.1–67.4)	0.800 (0.794–0.807)	34.3 (26.5–42.0)	45.5 (37.3–53.6)	0.761 (0.754–0.768)	29.4 (25.6–33.3)	40.8 (36.6–44.9)
Maternal characteristics plus MAP at 11–13 weeks	0.887 (0.882–0.892)[a]	52.9 (41.2–64.6)	74.3 (64.0–84.5)	0.862 (0.856–0.867)[a]	42.7 (34.6–50.8)	62.9 (55.0–70.9)	0.803 (0.797–0.809)[a]	35.8 (31.7–39.8)	49.3 (45.1–53.6)
MAP at 20–24 weeks	0.886 (0.881–0.892)[a]	52.9 (41.2–64.6)	71.4 (60.8–82.0)	0.867 (0.861–0.872)[a]	46.2 (38.0–54.3)	60.1 (52.1–68.2)	0.802 (0.795–0.808)[a]	35.0 (31.0–39.0)	47.7 (43.4–51.9)
MAP at 11–13 weeks + 20–24 weeks	0.893 (0.888–0.899)[a]	60.0 (48.5–71.5)	84.3 (75.8–92.8)	0.880 (0.875–0.885)[a]	49.7 (41.5–57.8)	65.7 (58.0–73.5)	0.813 (0.806–0.819)[a, b]	37.6 (33.5–41.7)	52.5 (48.3–56.7)

Comparison of AUROC: [a] significantly higher than screening by maternal characteristics alone; [b] significantly higher than screening by maternal characteristics and either MAP at 11–13 weeks or MAP at 20–24 weeks.

0.358) or maternal characteristics with MAP-2 (p = 0.220). In the prediction of preterm PE, the AUROC for maternal characteristics with MAP-1, maternal characteristics with MAP-2 and the combination of all were significantly higher than the AUROC for maternal characteristics alone (p = 0.001; p < 0.001; p < 0.001). The AUROC for the combination of all was not significantly different from the AUROC for maternal characteristics with MAP-1 (p = 0.062) or maternal characteristics with MAP-2 (p = 0.120). In the prediction of total PE, the AUROC for maternal characteristics with MAP-1, maternal characteristics with MAP-2 and the combination of all were significantly higher than the AUROC for maternal characteristics (p < 0.001; p < 0.001; p < 0.001). The AUROC for the combination of all was significantly higher than the AUROC for maternal characteristics with MAP-1 (p = 0.039) and maternal characteristics with MAP-2 (p = 0.021).

Discussion

Principal Findings of This Study

In normal singleton pregnancies, MAP is affected by maternal characteristics and medical history. At both 11–13 and 20–24 weeks' gestation, MAP decreases with gestational age and height, increases with maternal weight, it is higher in women with chronic hypertension and in those with a personal or family history of PE and lower in women of Afro-Caribbean racial origin, in smokers and in parous women with no previous PE. Consequently, the measured MAP must be adjusted for these variables and expressed as a MoM before valid comparisons can be carried out between normal and pathological pregnancies.

In pregnancies that develop PE, MAP MoM at 11–13 and 20–24 weeks' gestation is higher than in normal pregnancies and the increase is inversely related to the gestational age at delivery. We used a survival time model in screening for PE by a combination of maternal characteristics and history with MAP. In this model the gestation at the time of delivery for PE, for maternal and or fetal indications, is treated as a continuous rather than a categorical variable.

The observed performance of screening for PE by MAP at 11–13 weeks is similar to the modelled one in our previous study [7] and it is also similar to that of MAP at 20–24 weeks. Prediction of PE by MAP is best when measurements are taken both at 11–13 and at 20–24 weeks, than at only one of these gestational ranges.

Table 8. Previous studies reporting the performance of screening for PE by MAP

Author	Device	n	Definition PE	PE, %	GA, weeks	Cut-off, mm Hg	DR, %	FPR, %
Fallis et al., 1963 [14]	not specified	113	1	35	<24	90	82	12
Page and Christianson, 1976 [15]	not specified	14,833	2	3	20–24	90	44	13
Friedman and Neff, 1977 [16]	not specified	22,582	1	12	17–26	90	64	37
Robrecht et al., 1980 [17]	not specified	285	3	20	14–28	85	38	5
Öney et al., 1983 [18]	mercury sphygmomanometer	200	2	15	18–26	90	93	34
Mahanna et al., 1983 [19]	automated (Bosch)	210	1	5	12–40	90	90	8
Moutquin et al., 1985 [20]	automated (Dinamap 845)	983	4	8	9–12	90	62	38
					21–24	90	56	22
Villar and Sibai, 1989 [21]	not specified	700	5	20	13–27	90	8	8
Ales et al., 1989 [22]	automated (ultrasound device)	730	6	5	15–23	85	88	16
Conde-Agudelo et al., 1993 [23]	automated (Dinamap 845)	580	2	15	20–40	85	48	40
Kyle et al., 1993 [24]	automated (TM2420)	145	7	12	18	85	24	10
Rogers et al., 1994 [25]	automated (Dinamap 845)	220	8	15	18–26	68	93	39
Atterbury et al., 1996 [26]	mercury sphygmomanometer	114	9	–	18–22	85	39	11
Higgins et al., 1997 [27]	automated (Spacelab 90207)	1,048	10	8	18–24	91	13	2
Caritis et al., 1998 [28]	not specified	2,503	2	19	13–26	85	66	42
Shaarawy and Abdel-Magid, 2000 [29]	not specified	80	2	20	20	80	48	55
Stamilio et al., 2000 [30]	not specified	1,998	2	3	13	90	35	12
Brown et al., 2001 [31]	automated (Spacelab 90207)	286	2	53	18–30	79	65	28
Iwasaki et al., 2002 [32]	automated (BP-203RV)	1,599	2	3	5–13	92	9	20
Ebeigbe et al., 2004 [33]	not specified	1,200	2	9	<20	90	23	8
Onwudiwe et al., 2008 [34]	automated (3BTO-A2, Microlife)	3,359	2	3	22–24	90	46	10

Definitions of hypertensive disease in pregnancy: 1 = Not defined. 2 = Systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg on 2 occasions 4 (or 6) h apart after 20 (or 24) weeks' gestation, together with proteinuria (≥300 mg/dl in a 24-hour urine collection or ≥2+ on dipstick in ≥2 random urine specimens). 3 = Systolic BP ≥135 mm Hg and diastolic BP ≥85 mm Hg and increase in diastolic BP >20 mm Hg on 2 occasions. 4 = Systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg after 20 weeks' gestation that disappears at the post-partum visit, together with proteinuria (≥1+ on dipstick) and/or oedema (weight gain of >1 kg/week), or elevated serum urate (≥4.6 mg/dl). 5 = Systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg on 2 occasions 6 h apart, together with proteinuria (not specified). 6 = Systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg or MAP ≥107 mm Hg, or an increase in systolic BP ≥30 mm Hg and in diastolic BP ≥15 mm Hg on 2 occasions 6 h apart after 24 weeks' gestation, together with proteinuria (≥300 mg/dl in a 24-hour urine collection or ≥2+ on dipstick in random urine specimen). 7 = Increase in diastolic BP from the booking reading in the first half of pregnancy by ≥25 mm Hg, to a maximum of ≥90 mm Hg, together with proteinuria (≥1+ on dipstick in ≥2 random urine specimens). 8 = Systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg on 2 occasions 4 h apart, together with proteinuria (≥2+ on dipstick in ≥2 random urine specimens). 9 = Systolic BP >170 mm Hg and/or diastolic BP >110 mm Hg on 2 occasions 6 h apart, together with proteinuria (>2+ in a random urine sample) or oliguria, cerebral or visual disturbances, pulmonary oedema, epigastric or upper-right quadrant pain, elevated liver enzymes, and/or thrombocytopenia. 10 = Diastolic BP ≥110 mm Hg or 2 consecutive diastolic BP readings of ≥90 mm Hg 4 h apart, together with proteinuria (≥1+ on dipstick in random urine specimens 4 h apart or ≥300 mg/dl in a 24-hour urine collection) (≥300 mg/dl in a 24-hour urine collection or ≥1+ on dipstick in ≥2 random urine specimens).

Comparison of the Findings with Previous Studies in the Literature

Several studies have examined the use of MAP in the first and second trimesters as a screening test for subsequent development of hypertensive disorders in pregnancy and the findings of all such studies are summarized in table 8 [14–34]. The studies reported widely contradictory results in the performance of screening, with DR of 8–93% [18, 21] and FPRs of 2–55% [27, 29], as a consequence of the varied methods in selection of the screened population, measurement of BP, cut-offs used in defining the screen-positive group and definitions of PE. The sample size ranged from 80 to 2,582 [16, 29], the incidence of PE was 3–53% [15, 31] and MAP was measured by either mercury sphygmomanometers or different types of automated devices at a wide range of gestations between 5 and 40 weeks [23, 32].

In this study we used a standardized approach for measurement of MAP in a large number of pregnant women during two hospital visits at which an ultrasound examination is carried out routinely, adjusted the measured MAP to correct for maternal characteristics, used the definition of PE proposed by the International Society for the Study of Hypertension in Pregnancy (ISSHP) and reported the relation between MAP MoM and gestational age at delivery for PE, rather than erroneously considering the disease as being homogeneous across all gestational ages.

The finding that the performance of screening for PE by MAP at 11–13 and 20–24 weeks was similar is compatible with the results of previous longitudinal studies which reported that in pregnancies developing PE the MAP was increased from the first trimester and the deviation from normal increased only after 31 weeks [20, 21].

Limitations of the Study

In previous studies we combined data from maternal characteristics and history with the measurements of MAP, uterine artery pulsatility index and maternal serum placental growth factor and PAPP-A at 11–13 weeks' gestation to establish an algorithm for effective screening for PE [4, 5]. This study was limited to defining the factors affecting MAP, describing the relation of MAP MoM with gestation at birth in pregnancies complicated by PE and examining the performance of screening by maternal characteristics and history with MAP at 11–13 and 20–24 weeks. The development of an algorithm combining MAP with other biomarkers will be the subject of future studies.

Implications for Practice

In the traditional pyramid of pregnancy care, women are examined every 4 weeks until 28 weeks, then every 2 weeks until 36 weeks and finally every 1 week until delivery with the aim of diagnosing complications when they occur [35]. Extensive research in the last 20 years has led to the proposal that the traditional pyramid of care should be inverted with the main emphasis placed in the first rather than the third trimester of pregnancy and the objective of predicting and preventing complications [36]. It is proposed that women should be examined in essentially three integrated clinics, at 11–13, 20–24 and 32–34 weeks' gestation, to initially define and subsequently modify their individual risk for a wide range of pregnancy complications.

In the context of PE, the rationale of screening at 11–13 weeks is to define the high-risk group which could benefit from prophylactic treatment with low-dose aspirin [37, 38]. The objective of screening at 20–24 weeks is to improve the prediction provided by the first-trimester assessment and identify the group in need for closer surveillance of the maternal and fetal condition and thereby define the best time for delivery.

Acknowledgments

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No. 1037116). We are grateful to Alan Wright for providing some statistical support.

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Study 2

Gallo DM, Poon LC, Akolekar R, Syngelaki A, Nicolaides KH. Prediction of preeclampsia by uterine artery Doppler at 20-24 weeks' gestation. Fetal Diagn Ther 2013;34:241-7.

Impact Factor: 2.939

Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther
DOI: 10.1159/000356171

Received: September 12, 2013
Accepted after revision: October 1, 2013
Published online: November 2, 2013

Prediction of Preeclampsia by Uterine Artery Doppler at 20–24 Weeks' Gestation

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Key Words
Preeclampsia · Uterine artery Doppler · Second trimester · Pyramid of pregnancy care

Abstract
Objectives: To determine maternal characteristics affecting uterine artery pulsatility index (PI) in normal pregnancies at 20–24 weeks' gestation and examine in pregnancies with preeclampsia (PE) the relation between uterine artery PI multiple of the median (MoM) and severity of disease. ***Methods:*** Uterine artery PI was measured at 20–24 weeks in 50,490 singleton pregnancies, including 1,442 (2.9%) that developed PE. Uterine artery PI was expressed as MoM after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes. In PE, the correlation between uterine artery PI MoM with gestational age at delivery and birth weight Z-score was determined. ***Results:*** In the normal group there were significant independent contributions to uterine artery PI from gestational age, racial origin and prior history of PE, and/or small for gestational age (SGA). In the PE group, there was an inverse significant association between uterine artery PI MoM and both gestational age at delivery and birth weight Z-score ($p < 0.0001$). Uterine artery PI was above the 95th percentile (1.509 MoM) in 72.7, 36.1 and 14.9% of cases of PE requiring delivery at <34, 34–37 and ≥38 weeks, respectively, and the percentages for PE with SGA were 80.2, 55.6 and 37.4%. ***Conclusions:*** In a normal pregnancy, uterine artery PI is affected by maternal characteristics, and in PE, uterine artery PI MoM is related to the severity of the disease.

Introduction

Preeclampsia (PE), which is a major cause of maternal and perinatal morbidity and mortality [1–3], is the consequence of impaired placentation manifested in increased impedance to flow in the uterine arteries in the first, second and third trimesters of pregnancy [1–11]. Several uterine artery Doppler studies have reported that in pregnancies that develop PE, especially in those requiring early delivery and in those associated with birth of small for gestational age (SGA) neonates, the pulsatility index (PI) is increased [7–11].

Most of the Doppler studies were carried out during the second trimester and the high-risk group was identi-

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fied by values in PI, or some other index of impedance to flow, above a certain cutoff. For example, in the largest study, which involved 30,639 singleton pregnancies examined at 22–24 weeks' gestation, the uterine artery PI of 1.58, which was the 95th percentile of the normal range, was used [9]. In our recent studies, however, we adopted an approach, widely used in biochemical screening, of expressing the measured PI as a multiple of the median (MoM) after adjustment for those maternal characteristics that influence the measurement in the normal outcome group. In normal pregnancies at 11–13 weeks' gestation, uterine artery PI decreases with gestational age, increases with maternal weight and is higher in women of Afro-Caribbean origin than in other racial groups [7]. At 30–33 weeks, uterine artery PI increases with maternal age and weight, decreases with height, and is higher in women of Afro-Caribbean racial origin than in other racial groups [10].

The objectives of this screening study were (1) to determine the maternal characteristics that affect uterine artery PI in normal pregnancies at 20–24 weeks' gestation, and (2) to examine in pregnancies with PE the relation between uterine artery PI MoM and the severity of the disease, defined by the gestational age at delivery and the presence of fetal growth restriction.

Methods

The study population consisted of singleton pregnancies undergoing a routine ultrasound examination at 20–24 weeks' gestation, which was preceded by combined screening for aneuploidies at 11–13 weeks' gestation between 2006 and 2013 at three hospitals in and around London (King's College Hospital, University College London Hospital, Medway Maritime Hospital in Kent) [12]. All women delivered alive or dead phenotypically normal babies at or after 24 weeks' gestation. Gestational age at screening was calculated from the measurement of the fetal crown-rump length at 11–13 weeks [13].

The scan included examination of the fetal anatomy and growth by transabdominal sonography and measurement of uterine artery PI by transvaginal sonography [8]. The scans were carried out by sonographers who had received the Certificate of Competence in Doppler of The Fetal Medicine Foundation (http://www.fetalmedicine.com). Women with a mean uterine artery PI greater than 1.6 were followed up with growth scans, blood pressure measurements and urinalysis for protein at 28, 32 and 36 weeks. Women with normal uterine artery Doppler received routine antenatal care.

We prospectively examined 53,160 singleton pregnancies. We excluded 2,670 (5.0%) pregnancies because they had missing outcome data (n = 2,269), there was a major fetal defect or aneuploidy (n = 304), the pregnancy resulted in miscarriage between 20 and 24 weeks' of gestation (n = 87) or the pregnancy was terminated for psychosocial reasons (n = 10). In the remaining 50,490 cases, there were 1,442 (2.9%) that developed PE, 1,437 (2.8%) with gestational hypertension, 2,595 (5.1%) that delivered SGA neonates (without hypertension in pregnancy), 2,629 (5.2%) that delivered large for gestational age neonates and 42,387 (83.0%) that were unaffected by these outcomes.

Maternal History and Characteristics

Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilisation), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), history of type 1 or 2 diabetes mellitus (yes or no), history of systemic lupus erythematosus or antiphospholipid syndrome (yes or no), family history of PE in the mother of the patient (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks), previous pregnancy with PE (yes or no), previous pregnancy with SGA babies (yes or no), and interpregnancy interval (from the previous delivery or miscarriage to the estimated date of conception of the current pregnancy). The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were recorded.

Outcome Measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. 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 non-proteinuric gestational hypertension.

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [14]. The systolic blood pressure should be 140 mm Hg or more and/or the diastolic blood pressure should be 90 mm Hg or more on at least two occasions 4 h apart developing after 20 weeks' gestation in previously normotensive women, and there should be proteinuria of 300 mg or more in 24 h 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' gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

The neonatal birth weight was expressed as a Z-score (difference between observed and expected divided by fitted SD) and percentile corrected for gestational age of a reference range derived from our population [15]. The definitions of SGA and large for gestational age were birth weight below the 5th percentile and above the 95th percentile, respectively.

Statistical Analysis

Comparisons of maternal characteristics between the outcome groups were made using a χ^2 test or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables. The distribution of the mean uterine artery PI was made gaussian after logarithmic transformation. Backward stepwise multiple regression analysis was used to determine which of the factors

Table 1. Characteristics in the study population

	Normal (n = 42,387)	PE (n = 1,442)	p
Maternal age, years	30.8 (26.2–34.6)	31.0 (26.2–35.6)	0.01
Maternal weight, kg	69.8 (63.0–79.6)	76.4 (67.2–90.0)	<0.0001
Maternal height, cm	164 (160–169)	163 (159–168)	<0.0001
Gestation at screening, weeks	22.3 (22.0–22.7)	22.3 (22.0–22.9)	0.202
Racial origin			
Caucasian	30,258 (71.4)	782 (54.2)	<0.0001
Afro-Caribbean	8,024 (18.9)	544 (37.7)	<0.0001
South Asian	1,978 (4.7)	60 (4.2)	0.405
East Asian	1,062 (2.5)	22 (1.5)	0.023
Mixed	1,065 (2.5)	34 (2.4)	0.777
Past obstetric history			
Nulliparous	21,731 (51.3)	873 (60.5)	<0.0001
Parous with no prior PE and SGA	18,101 (42.7)	313 (21.7)	<0.0001
Parous with prior PE no SGA	1,015 (2.4)	176 (12.2)	<0.0001
Parous with prior SGA no PE	1,398 (3.3)	42 (2.9)	0.464
Parous with prior PE and SGA	142 (0.3)	38 (2.6)	<0.0001
Interpregnancy interval, months	29.1 (17.4–46.7)	33.5 (18.6–61.7)	<0.0001
Cigarette smoker	4,157 (9.8)	101 (7.0)	0.0005
Patients' mother had PE	1,659 (3.9)	112 (7.8)	<0.0001
Conception			
Spontaneous	41,020 (96.8)	1,364 (94.6)	<0.0001
Ovulation drugs	435 (1.0)	20 (1.4)	0.231
In vitro fertilisation	932 (2.2)	58 (4.0)	<0.0001
Chronic hypertension	473 (1.1)	182 (12.6)	<0.0001
No medication	229 (0.5)	75 (5.2)	<0.0001
Medication	244 (0.6)	107 (7.4)	<0.0001
Pre-existing diabetes mellitus	253 (0.6)	33 (2.3)	<0.0001
Type 1	117 (0.3)	14 (1.0)	<0.0001
Type 2	136 (0.3)	19 (1.3)	<0.0001
Systemic lupus erythematosus/APS	79 (0.2)	10 (0.7)	<0.0001
Gestation at delivery, weeks	40.1 (39.1–40.9)	38.5 (36.5–40.0)	<0.0001
Birth weight, g	3,400 (3,111–3,685)	2,968 (2,293–3,420)	<0.0001
Birth weight centile	46.7 (25.3–69.8)	26.7 (7.1–61.9)	<0.0001

Values represent medians (interquartile range) or n (%). Significance set at $p < 0.05$. APS = Antiphospholipid syndrome.

amongst the maternal characteristics and gestation were significant predictors of the $\log_{10}$ uterine artery PI, adjusting for the adverse pregnancy outcomes as specified (PE, gestational hypertension, SGA and large for gestational age). Variables were not considered to be significant predictors if the p value was >0.05 or if the ratio of the regression coefficients to the SD of the uterine artery PI $\log_{10}$ MoM was less than 0.1. Gestational age at screening was centred by subtracting 22 weeks, maternal weight was centred by subtracting 70 kg and maternal height was centred by subtracting 165 cm. The distribution of $\log_{10}$ uterine artery PI was then expressed as MoM in all cases, correcting for the significant predictors as defined in the multiple regression. Linear regression analysis was used to determine the significance of association between uterine artery PI $\log_{10}$ MoM with gestational age at delivery and birth weight Z-score in the cases of PE. The proportions of SGA in cases of PE with uterine artery PI MoM above the 90th and 95th percentiles were determined.

The statistical software package SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0; IBM, Armonk, N.Y., USA) was used for the data analyses.

Results

The characteristics of the study population are presented in table 1. In the PE group, compared to the normal group, there was a higher median maternal age and

Table 2. Fitted regression model for $\log_{10}$ uterine artery PI at 20–24 weeks

Coefficient	Estimate	Standard error	LCL	UCL	p
Intercept	0.0126703	0.00063842	0.011419	0.013922	<0.0001
(GA – 22 weeks)	–0.013572	0.00055559	–0.014661	–0.012483	<0.0001
Racial origin					
Afro-Caribbean	0.013824	0.0012790	0.011318	0.016331	<0.0001
South Asian	–0.011404	0.0023909	–0.016091	–0.0067181	<0.0001
East Asian	–0.014171	0.0032668	–0.020574	–0.0077678	<0.0001
Past obstetric history					
Parous – previous SGA no PE	0.0078398	0.0027477	0.0024542	0.013225	0.004
Parous – previous PE no SGA	0.015575	0.0029777	0.0097385	0.021411	<0.0001
Parous – previous PE and SGA	0.042149	0.0073388	0.027765	0.056533	<0.0001

GA = Gestational age; LCL = lower confidence limit; UCL = upper confidence limit.

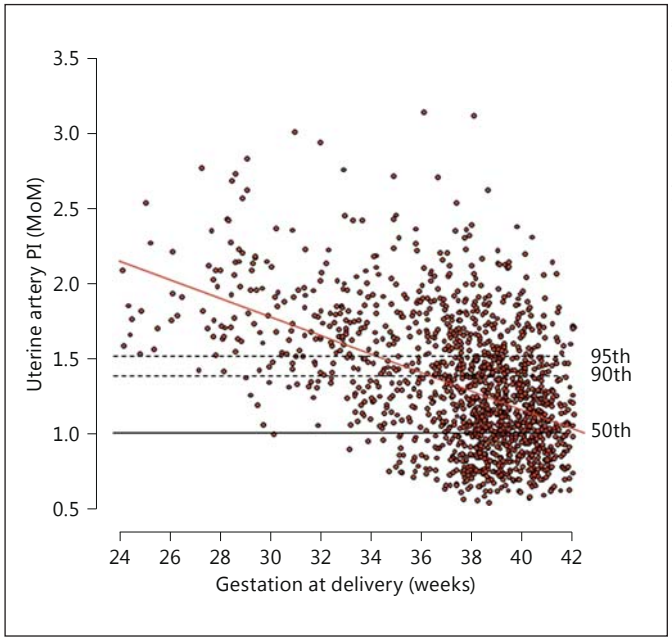

Fig. 1. Relationship between gestational age at delivery and mean uterine artery PI MoM in women who developed PE. The three horizontal lines represent the 50th, 90th and 95th percentiles of mean uterine artery PI MoM.

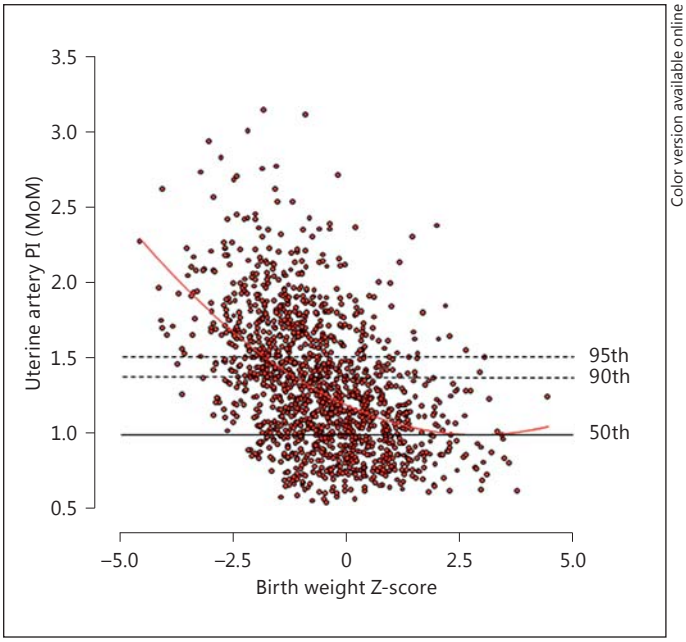

Fig. 2. Relationship between birth weight Z-score and mean uterine artery PI MoM in women who developed PE. The three horizontal lines represent the 50th, 90th and 95th percentiles of mean uterine artery PI MoM.

weight; a longer interpregnancy interval; a higher prevalence of Afro-Caribbean racial origin, personal history of PE with and without associated SGA, family history of PE, women who conceived with in vitro fertilisation, history of chronic hypertension, pre-existing diabetes mellitus and systemic lupus erythematosus or antiphospholipid syndrome; a lower maternal height, and a lower prevalence of East-Asian racial origin and smokers. The median gestational age at delivery and neonatal birth weight were significantly lower in the PE group than in the normal group.

Multiple regression analysis demonstrated that for the prediction of the mean $\log_{10}$ uterine artery PI, significant independent contributions were provided by

Table 3. Fitted regression model for $\log_{10}$ MoM uterine artery PI at 20–24 weeks' gestation at the time of delivery and neonatal birth weight Z-score for pregnancies with PE

	Coefficient	Standard error	LCL	UCL	p
Intercept	0.60035	0.039084	0.52368	0.67701	<0.0001
Gestational age at delivery	–0.014080	0.0010196	–0.016080	–0.012080	<0.0001
Neonatal birth weight Z-score	–0.036278	0.0026150	–0.041408	–0.031149	<0.0001

LCL = Lower confidence limit; UCL = upper confidence limit.

Table 4. Mean uterine artery PI MoM above the 90th and 95th percentiles in women who subsequently developed PE with or without associated SGA according to gestational age (GA) at delivery

GA, weeks	Total PE					PE with SGA				
	Total, n	Uterine artery PI MoM >90th percentile		Uterine artery PI MoM >95th percentile		Total, n	Uterine artery PI MoM >90th percentile		Uterine artery PI MoM >95th percentile	
		n	% (95% CI)	n	% (95% CI)		n	% (95% CI)	n	% (95% CI)
24–25	11	11	100.0 (74.1–100.0)	11	100.0 (74.1–100.0)	7	7	100.0 (64.6–100.0)	7	100.0 (64.6–100.0)
26–27	17	17	100.0 (81.6–100.0)	15	88.2 (65.7–96.7)	9	9	100.0 (70.1–100.0)	8	88.9 (56.5–98.0)
28–29	44	40	90.9 (78.8–96.4)	38	86.4 (73.3–93.6)	29	27	93.1 (78.0–98.1)	26	89.7 (73.6–96.4)
30–31	46	36	78.3 (64.4–87.7)	27	58.7 (44.3–71.7)	17	16	94.1 (73.0–99.0)	11	64.7 (41.3–82.7)
32–33	87	69	79.3 (69.6–86.5)	58	66.7 (56.2–75.7)	34	29	85.3 (69.9–93.6)	25	73.5 (56.9–85.4)
34–35	118	64	54.2 (45.3–63.0)	47	39.8 (31.5–48.8)	41	31	75.6 (60.7–86.2)	25	61.0 (45.7–74.3)
36–37	261	116	44.4 (38.5–50.5)	90	34.5 (29.0–40.4)	67	47	70.1 (58.3–79.8)	35	52.2 (40.5–63.7)
38–39	508	143	28.1 (24.4–32.2)	84	16.5 (13.6–20.0)	63	40	63.5 (51.1–74.3)	25	39.7 (28.5–52.0)
≥40	350	73	20.9 (16.9–25.4)	44	12.6 (9.5–16.5)	28	13	46.4 (29.5–64.2)	9	32.1 (17.9–50.7)
Total	1,442	569	39.5 (37.0–42.1)	414	28.7 (26.4–31.3)	295	219	74.2 (69.0–78.9)	171	58.0 (52.3–63.5)

gestational age at screening, racial origin (Afro-Caribbean, South Asian and East Asian) and prior history of PE and/or SGA (R^2 = 0.050; table 2), but not maternal weight (p = 0.106), height (p = 0.218), age (p = 0.277), method of conception (p = 0.973), chronic hypertension (p = 0.644), pre-existing diabetes mellitus (p = 0.854) and systemic lupus erythematosus or antiphospholipid syndrome (p = 0.106) due to insignificant p values, and smoking and family history of PE as the ratio of the regression coefficients to the SD of the uterine artery PI $\log_{10}$ MoM was less than 0.1.

In the PE group, there was an inverse significant association between the uterine artery PI $\log_{10}$ MoM and gestational age at delivery (r = −0.458, p < 0.0001; fig. 1) and between the uterine artery PI $\log_{10}$ MoM and neonatal birth weight Z-score (r = −0.473, p < 0.0001; fig. 2). Multiple regression analysis demonstrated significant independent contributions from both gestational age at delivery and neonatal birth weight Z-score in cases of PE (r = –0.550; table 3).

In 295 (20.5%, 95% CI: 18.5–22.6) of the 1,442 cases of PE, there was SGA and the incidence of SGA was inversely related to the gestational age at delivery decreasing from 46.8% (95% CI: 40.1–53.7) before 34 weeks to 28.5% (95% CI: 24.2–33.2) at 34–37 weeks and 10.6% (95% CI: 8.7–12.8) at or after 38 weeks.

The median, 90th and 95th percentiles of uterine artery PI MoM were 0.996, 1.377 and 1.509, respectively. The uterine artery PI MoM was above the 95th percentile in 72.7% (95% CI: 66.2–78.3) of women who developed PE requiring delivery before 34 weeks, in 36.1% (95% CI: 31.5–41.1) of those delivering at 34–37 weeks and in 14.9% (95% CI: 12.7–17.5) of those delivering at or after 38 weeks (table 4; fig. 3). The respective percentages for PE with SGA were 80.2% (95% CI: 71.1–86.9), 55.6% (95% CI: 46.2–64.6) and 37.4% (95% CI: 28.1–47.6), and 66.1% (95% CI: 56.8–74.3), 28.4% (95% CI: 23.4–34.1) and 12.3% (95% CI: 10.1–14.8) for PE without SGA.

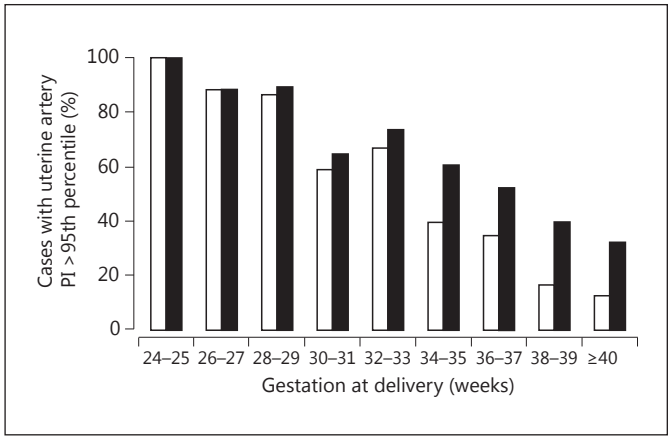

Fig. 3. Percentage of pregnancies that developed PE with mean uterine artery PI MoM above the 95th percentile according to gestational age at delivery. The white histograms are for all cases of PE and the black ones are for PE with delivery of SGA neonates.

Discussion

The findings of this study demonstrate that in normal singleton pregnancies at 20–24 weeks' gestation, uterine artery PI decreases with gestational age, is higher in women of Afro-Caribbean racial origin than in Caucasians, is decreased in South and East Asians, and is increased in multiparous women who developed PE and/or delivered SGA neonates in previous pregnancies. Consequently, adjustments should be made for these maternal characteristics before valid comparisons can be carried out between normal and pathological pregnancies.

In women who develop PE, uterine artery PI at 20–24 weeks' gestation is increased and the increase is particularly marked in those with early PE and in PE with SGA. The uterine artery PI MoM was above the 95th percentile in about 73, 36 and 15% of PE cases requiring delivery at <34, 34–37 and ≥38 weeks, respectively. The percentages for PE with SGA were 80, 56 and 37%, and for PE without SGA the percentages were 66, 28 and 12%. These findings are compatible with the results of previous Doppler studies [7–11, 16] and pathological studies which reported that the prevalence of placental lesions in women with PE is inversely related to the gestational age at delivery [17–19]. The findings are also important in relation to the objectives of screening because there is evolving evidence that the incidence of adverse fetal and maternal short-term and long-term consequences of PE is inversely related to the gestational age at onset of the disease [20–22]. Consequently, the endpoint in screening for PE should not be total disease but rather severe disease, reflected in the need for early delivery and the association with fetal growth restriction.

In our initial studies aiming to capture this gestational age-related severity of disease, we subdivided the condition into early PE and late PE. However, such subdivision could lead to the erroneous conclusion that early PE and late PE are different diseases with different biomarker profiles. As demonstrated by the MoM values of uterine artery PI in pregnancies with PE, the distribution with gestational age is not bimodal. 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. We have therefore proposed a new approach in screening for PE that is based on a survival time model, and the gestation at the time of delivery for PE is treated as a continuous rather than a categorical variable [7, 8]. We are now developing a model in which both the gestation at delivery and the coincidence with SGA are treated as a categorical variable.

The current approach to prenatal care, which involves visits at 16, 24, 28, 30, 32, 34 and 36 weeks' gestation and then weekly until delivery, was established more than 80 years ago [23]. The high concentration of visits in the third trimester implies that (1) most complications occur at this late stage of pregnancy and (2) most adverse outcomes are unpredictable during the first or the second trimester. Extensive research in the last 20 years has shown that many pregnancy complications, including PE, can now be predicted at an integrated first hospital visit at 11–13 weeks combining data from maternal characteristics and history with findings of biophysical and biochemical tests. It is therefore proposed that the traditional pyramid of care should be inverted with the main emphasis placed in the first rather than the third trimester of pregnancy [24]. Early estimation of patient-specific risks for pregnancy complications would improve pregnancy outcome by shifting prenatal 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.

The value of early screening for PE is derived from the evidence that the prophylactic use of low-dose aspirin can result in a major reduction in the prevalence of preterm PE and the associated perinatal mortality, provided the onset of treatment is before rather than after 16 weeks' gestation [25–27]. In the context of the new pyramid of pregnancy care [24], the value of a clinic at 20–24 weeks is to modify the individual patient and disease-specific

estimated risk from the initial assessment at 11–13 weeks and to provide risks for those women who did not have prior screening. In the high-risk group, intensive maternal monitoring for earlier diagnosis of PE and fetal growth restriction could improve outcome by selecting the best time and place for delivery. At present there is no useful pharmacological intervention after 16 weeks that can reduce the prevalence or modify the severity of the disease, but identification of the high-risk group would form the basis of future research that could achieve these objectives.

Acknowledgments

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No. 1037116).

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Study 3

Gallo DM, Wright D, Casanova C, Campanero M, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19-24 weeks' gestation. Am J Obstet Gynecol 2016;214(5):619.e1-619.e17

Impact Factor: 4.704

OBSTETRICS

Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19–24 weeks' gestation

Dahiana M. Gallo, MD; David Wright, PhD; Cristina Casanova, MD; Mercedes Campanero, MD; Kypros H. Nicolaides, MD

BACKGROUND: Preeclampsia (PE) affects 2–3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. The traditional approach to screening for PE is to use a risk-scoring system based on maternal demographic characteristics and medical history (maternal factors), but the performance of such an approach is very poor.

OBJECTIVE: To develop a model for PE based on a combination of maternal factors with second-trimester biomarkers.

STUDY DESIGN: The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending their routine hospital visit at 19–24 weeks' gestation in 3 maternity hospitals in England between January 2006 and July 2014. We had data from maternal factors, uterine artery pulsatility index (UTPI), mean arterial pressure (MAP), serum placental growth factor (PLGF), and serum soluble fms-like tyrosine kinase-1 (SFLT) from 123,406, 67,605, 31,120, 10,828, and 8079 pregnancies, respectively. Bayes' theorem was used to combine the a priori risk from maternal factors with various combinations of biomarker multiple of the median (MoM) values. The modeled performance of screening for PE requiring delivery at <32, <37, and ≥37 weeks' gestation was estimated. The modeled performance was compared to the empirical one, which was derived from 5-fold cross validation. We also examined the performance of screening based on risk factors from the medical history, as recommended by the American Congress of Obstetricians and Gynecologists (ACOG).

RESULTS: In pregnancies that developed PE, the values of MAP, UTPI, and SFLT were increased and PLGF was decreased. For all biomarkers the deviation from normal was greater for early than for late PE, and therefore the performance of screening was inversely related to the gestational age at which delivery became necessary for maternal and/or fetal indications. Screening by maternal factors predicted 52%, 47%, and 37% of PE at <32, <37, and ≥37 weeks' gestation, respectively, at a false-positive rate of 10%. The respective values for combined screening with maternal factors and MAP, UTPI, and PLGF were 99%, 85%, and 46%; the performance was not improved by the addition of SFLT. In our population of 123,406 pregnancies, the DR of PE at <32, <37, and ≥37 weeks with the ACOG recommendations was 91%, 90%, and 91%, respectively, but at a screen positive rate of 67%.

CONCLUSION: The performance of screening for PE by maternal factors and biomarkers in the middle trimester is superior to taking a medical history.

Key words: second-trimester screening, preeclampsia, pyramid of pregnancy care, survival model, Bayes' theorem, uterine artery Doppler, mean arterial pressure, placental growth factor, soluble fms-like tyrosine kinase-1

Preeclampsia (PE) affects 2–3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality.[1,2] The traditional approach to screening for PE is to identify risk factors from maternal demographic characteristics and medical history (maternal factors).[3,4] According to the American Congress of Obstetricians and Gynecologists (ACOG), taking a medical history to evaluate for risk factors is currently the best and only recommended screening approach for PE.[3] In the UK, the National Institute for Health and Clinical Excellence (NICE) has issued guidelines recommending that women should be considered to be at high risk of developing PE if they have any 1 high-risk factor or any 2 moderate-risk factors.[4] However, the performance of such an approach, which essentially treats each risk factor as a separate screening test with additive detection rate (DR) and screen positive rate, is poor, with DR of only 35% of all PE and 40% of preterm PE requiring delivery at <37 weeks' gestation, at a false-positive rate (FPR) of about 10%.[5]

An alternative approach to screening, which allows estimation of individual patient-specific risks of PE requiring delivery before a specified gestation, is to use Bayes' theorem to combine the a priori risk from maternal factors, derived by a multivariable logistic model, with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy.[5-8] We have previously reported that first-trimester screening by a combination of maternal factors with mean arterial pressure (MAP), uterine artery pulsatility index (UTPI), and serum placental growth factor (PLGF) can predict 75% of preterm PE and 47% of term PE, at 10% FPR.[8]

The objective of this study of singleton pregnancies with data on MAP, UTPI, PLGF, and serum soluble fms-like tyrosine kinase-1 (SFLT) at 19–24 weeks' gestation is to examine the potential improvement in performance of

EDITORS' CHOICE

Cite this article as: Gallo DM, Wright D, Casanova C, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19–24 weeks' gestation. Am J Obstet Gynecol 2016;214:619.e1-17.

0002-9378/free

http://dx.doi.org/10.1016/j.ajog.2015.11.016

screening by maternal factors alone with the addition of each biomarker and combinations of biomarkers. We also examined the performance of screening based on risk factors from the medical history, as recommended by ACOG.[3]

Methods

Study design and participants

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for routine pregnancy care at 11^{+0} to 13^{+6} and 19^{+0} to 24^{+6} weeks' gestation in 3 maternity hospitals in the UK (King's College Hospital between January 2006 and July 2014, Medway Maritime Hospital between February 2007 and July 2014, and University College London Hospital between April 2009 and September 2013). Maternal characteristics and medical history were recorded at the visit at 11^{+0} to 13^{+6} weeks (n = 123,406)[5] and measurements of UTPI, MAP, PLGF, and SFLT at 19^{+0} to 24^{+6} weeks. Screening evolved over time in 2 respects. Firstly, there was a change in participating hospitals; although all 3 hospitals were providing routine screening of their local populations, there were differences in the distribution of the racial origin of the study populations, which would affect the prior risk for PE. Secondly, there was a change in the content of the clinics; in the first phase of the study, only UTPI was measured (n = 67,605), then measurement of MAP was added (n = 31,120); and in the final phase serum concentration of PLGF was measured (n = 10,828) and then SFLT was added (n = 8,079). Measurements of all 4 biomarkers were obtained from 7748 pregnancies.

The left and right UTPI were measured by transvaginal color Doppler ultrasound and the mean pulsatility index was calculated.[9] Measurements of MAP were obtained by validated automated devices and a standardized protocol.[10] Measurement of serum concentration of PLGF and SFLT were by an automated biochemical analyzer within 10 minutes of blood sampling (Cobas e411 system; Roche Diagnostics, Penzberg, Germany). The inter-assay coefficients of variation for low and high concentrations were 5.4% and 3.0% for PLGF, and 3.0% and 3.2% for SFLT-1, respectively. Gestational age was determined from measurement of fetal crown-rump length (CRL) at 11–13 weeks or the fetal head circumference at 19–24 weeks.[11,12] The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

The inclusion criteria for this study were singleton pregnancy delivering a nonmalformed live birth or stillbirth at ≥24 weeks' gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities and those ending in termination, miscarriage, or fetal death at <24 weeks.

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE or pregnancy-induced hypertension (PIH), as defined by the International Society for the Study of Hypertension in Pregnancy.[13] Outcome measures were PE delivering at <37 weeks' gestation (preterm PE), PE delivering at ≥37 weeks (term PE), and subgroups of PE delivering at <32, 32^{+0} to 36^{+6}, 37^{+0} to 39^{+6}, and ≥40 weeks. The unaffected group contained all pregnancies without PE or PIH.

Statistical analyses

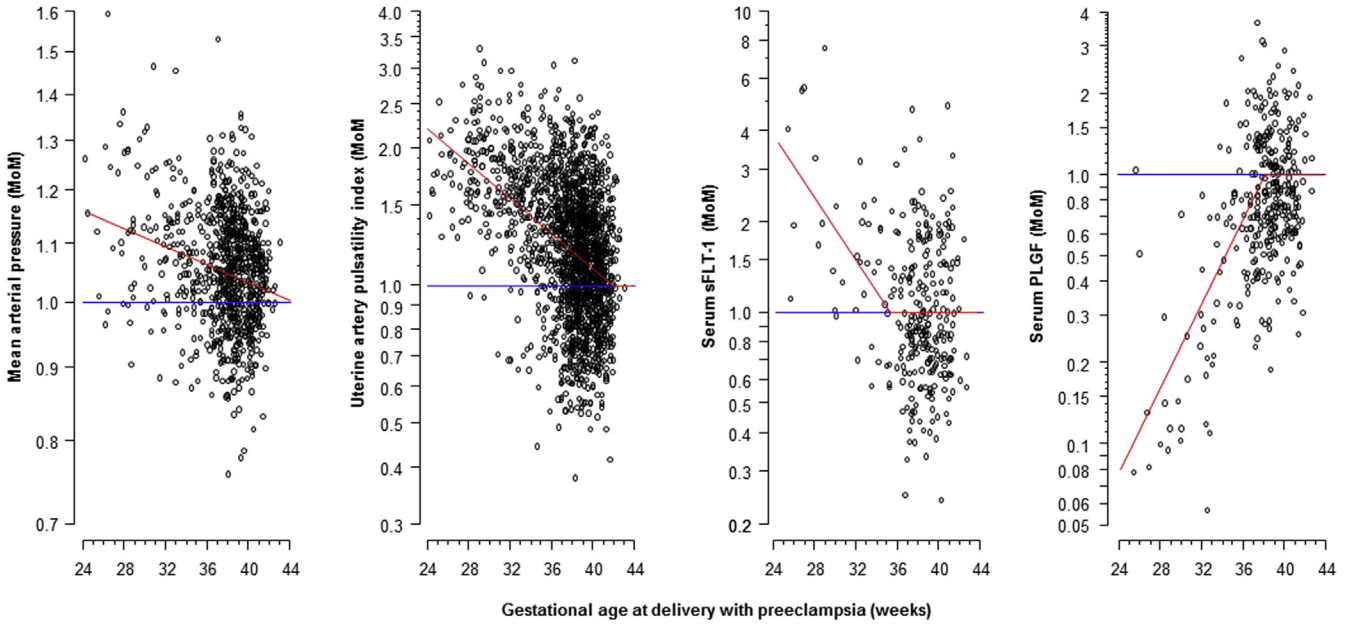
Performance of screening was assessed as follows: firstly, by examining the empirical results in 7748 pregnancies with complete data on UTPI, MAP, PLGF, and SFLT; secondly, by examining the empirical results using all available data for each biomarker; and thirdly, by modeling, whereby values on biomarkers were simulated for all 123,406 cases with available data on maternal factors. In selecting the second option, we wanted to have the maximum possible data for developing the models and examining performance of the various biomarkers; for example, in examining UTPI we could use data from 67,605 pregnancies, rather than just 7748. However, the distribution of maternal factors was not identical in each subset used for assessment of each biomarker or their combinations; consequently, there were differences between the datasets in the maternal factor–related performance of screening and it was therefore difficult to compare meaningfully the additional contribution to performance between biomarkers and their combinations over and above that of maternal factors alone. To overcome this problem we used modeling by imputing values for all biomarkers in the large dataset of 123,406 pregnancies.

Competing risks model

This model assumes that if the pregnancy were to continue indefinitely all women would develop PE, and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE.[6] The effect of maternal factors is to modify the mean of the distribution of gestational age at delivery with PE so that 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 for other reasons before development of PE. In high-risk pregnancies the distribution is shifted to the left; and the smaller the mean gestational age, the higher is the risk for PE. The distribution of biomarkers is specified conditionally on the gestational age at delivery with PE. For any women with specific maternal factors and biomarker multiple of the normal median (MoM) values, the posterior distribution of the time to delivery with PE, assuming there is no other cause of delivery, is obtained from the application of Bayes' theorem.

Gestational age at delivery with PE was defined by 2 components: firstly, the prior distribution based on maternal factors,[5] and secondly, the conditional distribution of MoM biomarker values, given the gestational age, with PE and maternal factors. Values of UTPI, MAP, PLGF, and SFLT were expressed as MoMs adjusting for those characteristics found

FIGURE 1
MoM values and fitted regression relationships with gestational age at delivery



Scatter diagram and regression line for the relationship between (**left**) mean arterial pressure, (**second from left**) uterine artery pulsatility index, (**second from right**) soluble fms-like tyrosine kinase-1, and (**right**) serum placental growth factor multiple of the median (MoM) and gestational age at delivery in pregnancies with preeclampsia.

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to provide a substantive contribution to their values, including the maternal factors in the prior model.[14-17] In the PE group, the mean $\log_{10}$ MoM was assumed to depend linearly with gestational age at delivery and this linear relationship was assumed to continue until the mean $\log_{10}$ MoM of zero, beyond which the mean was taken as zero; this assumption was confirmed by the empirical results shown in Figure 1. Multivariable Gaussian distributions were fitted to the $\log_{10}$ MoM values of the biomarkers and a common covariance matrix was assumed for these distributions. Analysis of residuals was used to check the adequacy of the model and assess the effects of maternal factors on $\log_{10}$-transformed MoM values in pregnancies with PE.

Empirical performance of screening

Empirical performance of screening was carried out for all available data and for the subset of 7748 pregnancies with complete data on UTPI, MAP, PLGF, and SFLT. Five-fold cross validation was used to assess the empirical performance of screening for PE by maternal factors and the combination of maternal factors with biomarkers.[5] The data were divided into 5 equal subgroups; the model was then fitted 5 times to different combinations of 4 of the 5 subgroups and used to predict risk of PE in the remaining fifth of the data. In each case, the maternal factor model, the regression models, and the covariance matrix were fitted to the training dataset comprising four fifths of the data and used to produce risks for the hold-out sample comprising the remaining fifth of the data.

Model-based estimates of screening performance

To provide model-based estimates of screening performance, the following procedure was adopted. First, we obtained the dataset of 123,406 singleton pregnancies, including 2748 (2.2%) with PE, that was previously used to develop a model for PE based on maternal demographic characteristics and medical history.[5] Second, for each case of PE (n = 2748) and pregnancies unaffected by PE or PIH (n = 117,710), the biophysical and biochemical MoM values were simulated from the fitted multivariate Gaussian distribution for log-transformed MoM values. Third, risks were obtained using the competing risk model from the simulated MoM values and the pregnancy characteristics. These 3 steps were applied to the pregnancies within the unaffected group with no restriction on the time of delivery. Fourth, for a given FPR, risks from the unaffected group were used to define a risk cutoff. The proportion of PE risks was then used to obtain an estimate of the associated DR. The area under the receiver operating characteristic curve (AUROC) was also calculated. The simulations were repeated 100 times to reduce variability due to the simulation process and provide suitably precise model-based estimates of performance.

The statistical software package R was used for data analyses.[18] The survival

TABLE 1
Characteristics of the screening population

Variable	Unaffected (n = 117,710)	PE <37 w (n = 790)	PE ≥37 w (n = 1958)	PIH (n = 2948)
Maternal age in years, median (IQR)	31.3 (26.7, 35.1)	31.8 (26.9, 36.5)[a]	31.3 (26.5, 35.8)	31.8 (27.2, 35.5)[a]
Maternal weight in kg, median (IQR)	69.8 (62.4, 79.9)	74.0 (65.0, 88.0)[a]	77.4 (67.8, 91.9)[a]	76.0 (67.0, 88.0)[a]
Maternal height in cm, median (IQR)	164 (160, 169)	163 (158, 167)[a]	164 (160, 168)[a]	165 (160, 169)
Body mass index, median (IQR)	25.8 (23.2, 29.4)	28.4 (24.6, 32.8)[a]	28.8 (25.4, 33.7)[a]	28.1 (25.0, 32.4)[a]
Gestational age in weeks, median (IQR)	22.1 (21.1, 22.7)	22.2 (21.2, 22.8)[a]	22.2 (21.4, 22.7)[a]	22.2 (21.4, 22.7)[a]
Racial origin		[a]	[a]	[a]
White, n (%)	87,373 (74.2)	420 (53.2)	1165 (59.5)	2010 (68.2)
Afro-Caribbean, n (%)	18,313 (15.6)	293 (37.1)	614 (31.4)	668 (22.7)
South Asian, n (%)	6120 (5.2)	51 (6.5)	102 (5.2)	148 (5.0)
East Asian, n (%)	3106 (2.6)	10 (1.3)	37 (1.9)	53 (1.8)
Mixed, n (%)	2798 (2.4)	16 (2.0)	40 (2.0)	69 (2.3)
Medical history				
Chronic hypertension, n (%)	1198 (1.0)	102 (12.9)[a]	186 (9.5)[a]	0 (0.0)[a]
Diabetes mellitus, n (%)	893 (0.8)	30 (3.8)[a]	31 (1.6)[a]	35 (1.2)[a]
SLE/APS, n (%)	207 (0.2)	9 (1.1)[a]	7 (0.4)	9 (0.3)
Conception		[a]	[a]	
Natural, n (%)	113,530 (96.5)	727 (92.0)	1868 (95.4)	2823 (95.8)
In vitro fertilization, n (%)	2632 (2.2)	43 (5.4)	68 (3.5)	83 (2.8)
Ovulation induction drugs, n (%)	1548 (1.3)	20 (2.5)	22 (1.1)	42 (1.4)
Family history of preeclampsia, n (%)	4243 (3.6)	67 (8.5)[a]	134 (6.8)[a]	220 (7.5)[a]
Parity				
Nulliparous, n (%)	57,720 (49.0)	468 (59.2)[a]	1,250 (63.8)[a]	1,888 (64.0)[a]
Parous with no previous PE, n (%)	56,848 (48.3)	196 (24.8)[a]	476 (24.3)[a]	765 (26.0)[a]
Parous with previous PE, n (%)	3142 (2.7)	126 (16.0)[a]	232 (11.9)[a]	295 (10.0)[a]
Inter-pregnancy interval in years, median (IQR)	2.9 (1.9, 4.8)	4.2 (2.4, 7.3)[a]	3.7 (2.3, 6.7)[a]	3.4 (2.0, 5.7)[a]

Comparisons with unaffected group were by χ^2 or Fisher exact test for categorical variables and Mann-Whitney *U* test for continuous variables.

APS, antiphospholipid syndrome; *IQR*, interquartile range; *PE*, preeclampsia; *PIH*, pregnancy-induced hypertension; *SLE*, systemic lupus erythematosus.

[a] Significance value $P < .05$.

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package[19] was used for fitting the maternal factors model and the package pROC[20] was used for the receiver operating characteristic (ROC) curve analysis.

Results

The characteristics of the total population of 123,406 singleton pregnancies are given in Table 1 and those of the subset of 7748 pregnancies with complete data on UTPI, MAP, PLGF, and SFLT are given in Supplemental Table 1 (Appendix).

Distribution of biomarkers

The distributions of $\log_{10}$ MoM values of the biomarkers in unaffected pregnancies and in those that developed PE are shown in Supplemental Tables 2 and 3 (Appendix). In the unaffected group, the median MoM value is 1.0 and on the log scale the distribution of MoM values is very well approximated by a Gaussian distribution with mean zero. The MoM values in the PE group and the fitted regression relationships with gestational age at delivery are shown in Figure 1. All markers showed more separation at earlier than later gestations and this is reflected in their superior performance at detection of early vs late PE.

Performance of screening for preeclampsia

Empirical and model-based performance of screening for PE by maternal factors and combinations of biomarkers are shown in Tables 2 and 3, Supplemental Tables 4-7 (Appendix), and Figures 2 and 3. The empirical

TABLE 2
Empirical detection rate, at false-positive rate of 5% and 10%, in screening for preeclampsia with delivery at <37 and ≥37 weeks' gestation by maternal factors and combinations of biomarkers in the subgroup of 7748 pregnancies with complete data on all biomarkers

	Preeclampsia at <37 weeks				Preeclampsia at ≥37 weeks			
	FPR 5%		FPR 10%		FPR 5%		FPR 10%	
Method of screening	n/N	% (95% CI)[a]	n/N	% (95% CI)[a]	n/N	% (95% CI)[a]	n/N	% (95% CI)[a]
History	21/62	34 (22, 47); 34	29/62	47 (34, 60); 47	55/206	27 (21, 33); 26	75/206	36 (30, 43); 37
MAP	30/62	48 (35, 61); 47	37/62	60 (46, 72); 60	55/206	27 (21, 33); 30	90/206	44 (37, 51); 43
UTPI	37/62	60 (46, 72); 57	47/62	76 (63, 86); 70	52/206	25 (19, 32); 28	78/206	38 (31, 45); 40
PLGF	34/62	55 (42, 68); 64	44/62	71 (58, 82); 73	55/206	27 (21, 33); 27	75/206	36 (30, 43); 37
SFLT	20/62	32 (21, 45); 38	33/62	53 (40, 66); 50	55/206	27 (21, 33); 26	75/206	36 (30, 43); 37
MAP, UTPI	49/62	79 (67, 88); 67	50/62	81 (69, 90); 78	59/206	29 (23, 35); 33	90/206	44 (37, 51); 46
MAP, PLGF	38/62	61 (48, 73); 69	45/62	73 (60, 83); 78	55/206	27 (21, 33); 30	89/206	43 (36, 50); 43
MAP, SFLT	31/62	50 (37, 63); 49	38/62	61 (48, 73); 62	55/206	27 (21, 33); 30	90/206	44 (37, 51); 42
UTPI, PLGF	43/62	69 (56, 80); 72	50/62	81 (69, 90); 81	53/206	26 (20, 32); 28	75/206	36 (30, 43); 40
UTPI, SFLT	41/62	66 (53, 78); 61	45/62	73 (60, 83); 72	54/206	26 (20, 33); 28	78/206	38 (31, 45); 40
PLGF, SFLT	35/62	56 (43, 69); 65	44/62	71 (58, 82); 75	55/206	27 (21, 33); 27	75/206	36 (30, 43); 37
MAP, UTPI, PLGF	45/62	73 (60, 83); 77	52/62	84 (72, 92); 85	58/206	28 (22, 35); 33	90/206	44 (37, 51); 46
MAP, UTPI, SFLT	46/62	74 (62, 84); 69	50/62	81 (69, 90); 79	57/206	28 (22, 35); 33	92/206	45 (38, 52); 46
MAP, PLGF, SFLT	37/62	60 (46, 72); 69	45/62	73 (60, 83); 79	56/206	27 (21, 34); 33	89/206	43 (36, 50); 46
UTPI, PLGF, SFLT	41/62	66 (53, 78); 74	50/62	81 (69, 90); 82	54/206	26 (20, 33); 28	74/206	36 (29, 43); 40
MAP, UTPI, PLGF, SFLT	46/62	74 (62, 84); 78	53/62	85 (74, 93); 86	56/206	27 (21, 34); 33	91/206	44 (37, 51); 46

CI, confidence interval; *FPR*, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

[a] The last numbers in each cell are the values obtained from modeling.

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performance of screening for PE at <37 and ≥37 weeks in the 7748 pregnancies with complete data is shown in Table 2; the DRs at 5% and 10% FPR were compatible with the model-based rates. The AUROC curves for prediction of PE at <32, <37, and ≥37 weeks based on empirical results from all available data are shown in Table 3 and these were compatible with the model-based results. Empirical performance of screening for PE with delivery at <37, ≥37, <32, 32^{+0} to 36^{+6}, 37^{+0} to 39^{+6}, and ≥40 weeks' gestation is shown in Supplemental Tables 4-6 (Appendix); the number of cases for each biomarker and combinations of biomarkers varied, with inevitable differences in composition of the populations and, consequently, differences in performance of screening by maternal factors alone. The model-based performance of screening for PE with delivery at <37, ≥37, <32, 32^{+0} to 36^{+6}, 37^{+0} to 39^{+6}, and ≥40 weeks' gestation is shown in Supplemental Table 7 (Appendix). Figure 2 shows the ROC curves for model-based prediction of PE at <32, <37, and ≥37 weeks' gestation by maternal factors, combination of maternal factors with each biomarker, and combination of maternal factors with MAP, UTPI, and PLGF. Figure 3 shows the empirical performance of screening for PE at <37 and ≥37 weeks, by combination of maternal factors with all available data on MAP, UTPI, and PLGF; the empirical results were compatible with the model-based results.

Empirical performance for early, preterm, and term preeclampsia

On the basis of all available data, the empirical performance of screening for early PE by maternal factors (AUROC, 0.820; 95% CI, 0.791, 0.848) was improved by the addition of MAP (AUROC, 0.902; 95% CI, 0.862, 0.942) or PLGF (AUROC, 0.962; 95% CI, 0.914, 0.999) and the performance of maternal factors and MAP was improved by the addition of PLGF (AUROC, 0.981; 95% CI, 0.957, 0.999), UTPI and PLGF (AUROC, 0.979; 95% CI, 0.949, 0.999), UTPI and SFLT (AUROC, 0.994; 95% CI, 0.989, 0.999), and PLGF and SFLT (AUROC, 0.980; 95% CI, 0.952, 0.999); addition of SFLT to the combination of maternal factors, MAP, UTPI, and PLGF provided a small nonsignificant improvement in performance of screening (AUROC, 0.995; 95% CI, 0.990, 0.999) (Table 3, Figure 2).

The performance of screening for preterm PE by maternal factors (AUROC, 0.789; 95% CI, 0.773, 0.804)

TABLE 3
Areas under the receiver operating characteristic curve in empirical results from all available data and model-based results in screening for preeclampsia by maternal factors and combination of maternal factors and biomarkers

	Areas under the receiver operating characteristic curve					
	PE <32 w		PE <37 w		PE ≥37 w	
Screening	Empirical (95% CI)	Model	Empirical (95% CI)	Model	Empirical (95% CI)	Model
History	0.820 (0.791, 0.848)	0.827	0.789 (0.773, 0.804)	0.796	0.748 (0.737, 0.759)	0.752
MAP	0.902 (0.862, 0.942)	0.906	0.849 (0.824, 0.874)	0.860	0.787 (0.769, 0.805)	0.784
UTPI	0.949 (0.931, 0.968)	0.957	0.898 (0.883, 0.912)	0.895	0.766 (0.753, 0.779)	0.771
PLGF	0.962 (0.914, 0.999)	0.989	0.887 (0.849, 0.926)	0.905	0.732 (0.701, 0.763)	0.752
SFLT	0.906 (0.820, 0.993)	0.875	0.820 (0.771, 0.869)	0.810	0.733 (0.700, 0.766)	0.752
MAP, UTPI	0.969 (0.940, 0.997)	0.975	0.918 (0.895, 0.941)	0.924	0.801 (0.784, 0.819)	0.801
MAP, PLGF	0.981 (0.957, 0.999)	0.992	0.909 (0.875, 0.943)	0.924	0.766 (0.738, 0.795)	0.784
MAP, SFLT	0.941 (0.892, 0.990)	0.924	0.858 (0.811, 0.906)	0.865	0.769 (0.738, 0.801)	0.784
UTPI, PLGF	0.976 (0.947, 0.999)	0.995	0.926 (0.895, 0.956)	0.934	0.736 (0.705, 0.768)	0.771
UTPI, SFLT	0.973 (0.941, 0.999)	0.973	0.909 (0.875, 0.944)	0.903	0.741 (0.707, 0.775)	0.772
PLGF, SFLT	0.957 (0.896, 0.999)	0.993	0.878 (0.836, 0.921)	0.910	0.734 (0.701, 0.768)	0.752
MAP, UTPI, PLGF	0.979 (0.949, 0.999)	0.996	0.932 (0.899, 0.965)	0.948	0.772 (0.742, 0.801)	0.801
MAP, UTPI, SFLT	0.994 (0.989, 0.999)	0.983	0.915 (0.872, 0.958)	0.927	0.780 (0.749, 0.812)	0.801
MAP, PLGF, SFLT	0.980 (0.952, 0.999)	0.983	0.899 (0.859, 0.940)	0.927	0.768 (0.737, 0.800)	0.801
UTPI, PLGF, SFLT	0.984 (0.959, 0.999)	0.998	0.926 (0.894, 0.957)	0.939	0.739 (0.706, 0.773)	0.772
MAP, UTPI, PLGF, SFLT	0.995 (0.990, 0.999)	0.998	0.930 (0.892, 0.968)	0.951	0.773 (0.741, 0.805)	0.801

CI, confidence interval; *FPR*, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.
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was improved by the addition of MAP (AUROC, 0.849; 95% CI, 0.824, 0.874), UTPI (AUROC, 0.898; 95% CI, 0.883, 0.912), or PLGF (AUROC, 0.887; 95% CI, 0.849, 0.926) and the performance of maternal factors and MAP was improved by the addition of either UTPI (AUROC, 0.918; 95% CI, 0.895, 0.941), PLGF (AUROC, 0.909; 95% CI, 0.875, 0.943), or both UTPI and PLGF (AUROC, 0.932; 95% CI, 0.899, 0.965); SFLT did not provide significant improvement to any combination of biomarkers (Table 3, Figure 2).

The performance of screening for term PE by maternal factors (AUROC, 0.748; 95% CI, 0.737, 0.759) was improved by the addition of MAP (AUROC, 0.787; 95% CI, 0.769, 0.805) and both MAP and UTPI (AUROC, 0.801; 95% CI, 0.784, 0.819); serum PLGF and SFLT, either on their own or in combination, did not improve the prediction provided by maternal factors alone (Table 3, Figure 2).

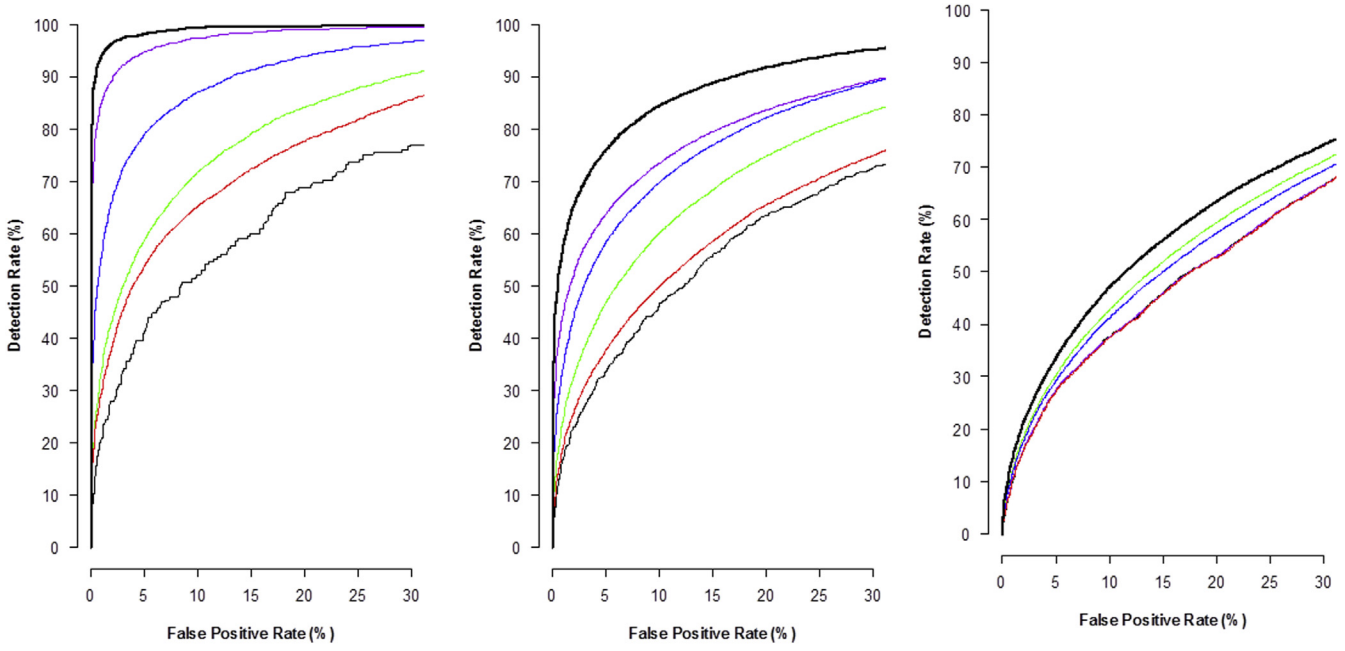
Performance of screening in subgroups of racial origin and obstetric history

In the dataset of 123,406 pregnancies, 61,326 women (49.7%) were nulliparous and 62,080 (50.3%) were parous, including 3795 (6.1%) with history of PE in a previous pregnancy and 58,285 (93.9%) without history of PE. The contribution of parous women to PE was 37.5% (1030/2748), including 34.8% (358/1030) from parous women with PE in a previous pregnancy and 65.2% (672/1030) from parous women without a history of PE.

The model-based performance of screening by a combination of maternal factors, MAP, UTPI, and PLGF in the prediction of preterm PE and term PE for nulliparous and parous women of Afro-Caribbean and white racial origin are given in Table 4. In these calculations a risk cutoff was selected to achieve a screen positive rate of about 10%. At a risk cutoff of 1 in 100 for preterm PE and 1 in 15 for term PE, the FPR and DR were higher in parous women with vs without PE in a previous pregnancy and in those of Afro-Caribbean vs white racial origin. In all groups, the risk of being affected given a screen positive result was considerably higher than the prevalence of the disease, whereas in those with a screen negative result the risk was considerably reduced.

In the lowest-risk group, white parous women with no previous history of PE, the DR for preterm PE was 66% and the FPR was 3.2%; in total, 810 tests would need to be performed for each true positive identified. In the highest-risk group, Afro-Caribbean women with

FIGURE 2
Receiver operating characteristic curves for prediction of preeclampsia



Results are shown at <32 (**left**), <37 (**middle**), and ≥37 weeks' gestation (**right**) by maternal factors (*black*) and combination of maternal factors with uterine artery pulsatility index (*blue*), mean arterial pressure (*green*), serum placental growth factor (*purple*), soluble fms-like tyrosine kinase-1 (*red*), and combination of maternal factors with uterine artery pulsatility index, mean arterial pressure, and serum placental growth factor (*bold black*).

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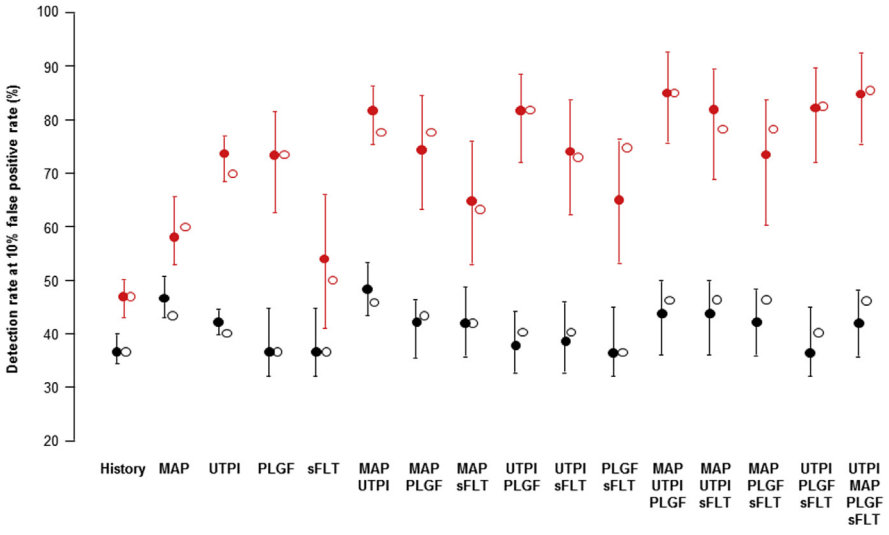
previous history of PE, the DR for preterm PE was 99.6% and the FPR was 57.1%; in total, 15 tests would need to be performed for each true positive identified.

Performance of screening according to ACOG recommendations

The ACOG recommends that screening for PE should be based on taking a medical history to evaluate for risk factors.[3] The risk factors are nulliparity, age >40 years, body mass index ≥30 kg/m^2, conception by in vitro fertilization, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus, systemic lupus erythematosus, or thrombophilia.[21]

In our population of 123,406 singleton pregnancies, the screen positive rate with the ACOG recommendations was 67% and the DR of PE at <32, <37, and ≥37 weeks was 91%, 90%, and 91%, respectively.

FIGURE 3
Empirical performance of screening for preeclampsia



Empirical detection rates, at 10% false-positive rate, of preeclampsia at <37 weeks (*red lines and circles*) and at ≥37 weeks (*black lines and circles*), with 95% confidence interval, in screening by combination of maternal factors with uterine artery pulsatility index, mean arterial pressure, and serum placental growth factor. The open circles represent the model-based detection rates.

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TABLE 4
Model-based performance of screening by an algorithm combining maternal factors, uterine artery pulsatility index, mean arterial pressure, and serum placental growth factor for preeclampsia with delivery at <37 weeks' gestation at a risk cutoff of 1 in 100 and for preeclampsia with delivery at ≥37 weeks at a risk cutoff of 1 in 15

Group	Prevalence (%)	Screen positive (%)	False positive (%)	DR (%)	Risk of being affected given result: Screen positive (%)[a]	Risk of being affected given result: Screen negative (%)[b]
Preeclampsia <37 w						
All pregnancies	0.64	11.4	10.4	85	4.77	0.11
Nulliparous	0.76	14.7	13.7	84	4.37	0.14
Parous	0.52	8.0	7.2	85	5.50	0.08
No previous PE	0.34	5.9	5.4	78	4.45	0.08
Previous PE	3.32	41.6	37.6	97	7.76	0.16
Afro-Caribbean	1.47	23.3	21.1	91	5.78	0.17
Nulliparous	1.64	30.0	27.8	92	5.03	0.20
Parous	1.36	18.8	16.8	91	6.58	0.15
No previous PE	0.93	15.4	14.1	86	5.20	0.15
Previous PE	6.83	62.6	57.1	100	10.87	0.07
White	0.46	8.8	8.2	80	4.20	0.10
Nulliparous	0.62	12.1	11.4	81	4.12	0.13
Parous	0.29	5.2	4.7	78	4.41	0.07
No previous PE	0.19	3.4	3.2	66	3.65	0.07
Previous PE	2.01	34.1	31.5	95	5.61	0.14
Preeclampsia ≥37 w						
All pregnancies	1.59	9.9	9.3	44	7.09	0.98
Nulliparous	2.04	13	12.4	41	6.47	1.38
Parous	1.14	6.9	6.4	50	8.24	0.61
No previous PE	0.82	4	3.8	33	6.66	0.57
Previous PE	6.11	54.8	52.5	85	9.53	1.98
Afro-Caribbean	3.09	28	26.5	70	7.68	1.31
Nulliparous	3.96	41.2	39.8	74	7.07	1.77
Parous	2.51	19.2	18	65	8.52	1.08
No previous PE	1.91	14.6	13.8	52	6.85	1.07
Previous PE	10.13	84	82.4	96	11.58	2.5

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Comment

Principal findings of this study

In pregnancies that developed PE, the second-trimester values of UTPI, MAP, and SFLT were increased and PLGF was decreased. For all biomarkers the deviation from normal was greater for early PE than for late PE, and therefore the performance of screening was inversely related to the gestational age at which delivery became necessary for maternal and/or fetal indications.

Screening for PE by a combination of maternal factors, UTPI, MAP, and PLGF at 19–24 weeks' gestation predicted 99% of early PE, 85% of preterm PE, and 46% of term PE, at an FPR of 10%. Such DRs are superior to the respective values of 52%, 47%, and 37% achieved by screening with maternal factors alone. Serum SFLT-1 improved the performance of screening for early PE but not for PE at ≥32 weeks. We have previously reported that screening by a combination of maternal factors, UTPI, MAP, and PLGF at 11–13 weeks' gestation can predict 89% of early PE, 75% of preterm PE, and 47% of term PE, at an FPR of 10%.[8] Consequently, the performance of screening for early and preterm PE, but not for term PE, is superior at 19–24 vs at 11–13 weeks' gestation.

In the application of Bayes' theorem, the maternal factor–derived prior risk has a strong influence on the posterior risk and, therefore, the performance of screening. The study has highlighted that in screening for PE the FPR and DR are influenced by the characteristics of the study population and for a given risk cutoff they are both higher in nulliparous than in parous women and in those of Afro-Caribbean than in those of white racial origin. Although the risk of PE is higher in nulliparous than parous women, the contribution of the latter group to PE should not be underestimated, because 38% of cases of PE were from parous women, including 13% from parous women with history of PE in a previous pregnancy and 25% from parous women without a history of PE. In all groups, after combined screening, the risk of being affected given a screen positive result was considerably increased and if the screen result was negative the risk was considerably reduced.

Strengths and limitations

The strengths of this second-trimester screening study for PE are, first, examination of a large population of pregnant women attending for routine care in a gestational age range that is widely used for assessment of fetal anatomy and growth; second, recording of data on maternal characteristics and medical

history to identify known risk factors associated with PE and use of multivariable logistic model to define the prior risk; third, use of a specific methodology and appropriately trained doctors to measure UTPI and MAP; fourth, use of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of PLGF and SFLT; fifth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements; and sixth, use of Bayes' theorem to combine the prior risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

A limitation of the study is that some of the findings rely on modeling, which introduces optimistic bias. We have used cross validation on the empirical data, which reduces such bias, and demonstrated that the modeled and empirical performance were similar.

Comparison with previous studies

Several studies have documented that development of PE is associated with second-trimester increase in UTPI, MAP, and SFLT and decrease in serum PLGF.[22-33] In this study we used Bayes' theorem to combine the a priori risk from maternal factors with all 4 biomarkers and conducted 5-fold cross validation to assess performance of screening.

Clinical implications of the study

Screening and diagnosis of PE is traditionally based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second or third trimester of pregnancy. In a proposed new pyramid of pregnancy care,[34] an integrated clinic at 22 weeks' gestation, in which biophysical and biochemical markers are combined with maternal factors, aims to estimate the patient-specific risk of developing PE and, on the basis of such risk, define the subsequent management of pregnancy, including the timing and content of subsequent visits. The objective would be to minimize adverse perinatal events for those that develop PE by determining the appropriate time and place for delivery.

We found that the performance of second-trimester screening for PE is good for preterm PE but poor for term PE. We assume that the performance of screening for term PE would be better if assessment is undertaken at 36, rather than 22, weeks. A previous screening study in the third trimester by a combination of maternal factors, MAP, UTPI, PLGF, and SFLT demonstrated a high performance in the prediction of PE within 6 weeks of screening but poor performance for PE developing beyond this interval.[35] Since the majority of cases of PE occurr at term, it may be necessary that all pregnancies be reassessed at 36 weeks. In this context, the main value of the 22 weeks assessment is to identify, first, the high-risk group for development of early PE that would then require close monitoring for development of high blood pressure and proteinuria at 24–32 weeks; and second, the high-risk group for preterm PE that would require reassessment at around 32 weeks and, on the basis of such assessment, stratification into a high-risk group in need of close monitoring at 32–36 weeks and a low-risk group that would be reassessed at 36 weeks.

Performance of screening for PE by our method is by far superior to those recommended by ACOG[3,21] or NICE.[4] Use of a multivariable logistic model to define the prior risk attributes the appropriate relative importance to each maternal factor and allows estimation of the patient-specific risk of PE requiring delivery before a specified gestation. The prior risk can then be adjusted according to the results of biophysical and biochemical testing. The software for such estimation of prior and adjusted risk is freely available (American Journal of Obstetrics and Gynecology website). Recording maternal history and measurement of blood pressure are universally carried out as part of routine pregnancy care; measurement of MAP requires adherence to a protocol, but it can be undertaken by healthcare assistants after minimal training, with the use of inexpensive equipment, and takes a few minutes to perform. In contrast, measurement of UTPI requires specific training by sonographers and quality assurance of their results; nevertheless, this test can be undertaken within a few minutes by the same sonographers and machines as part of the routine second-trimester scan. Measurement of serum

TABLE 4
Model-based performance of screening by an algorithm combining maternal factors, uterine artery pulsatility index, mean arterial pressure, and serum placental growth factor for preeclampsia with delivery at <37 weeks' gestation at a risk cutoff of 1 in 100 and for preeclampsia with delivery at ≥37 weeks at a risk cutoff of 1 in 15 *(continued)*

Group	Prevalence (%)	Screen positive (%)	False positive (%)	DR (%)	Risk of being affected given result: Screen positive (%)[a]	Risk of being affected given result: Screen negative (%)[b]
White	1.28	6.2	5.8	32	6.62	0.93
Nulliparous	1.74	8.4	8	30	6.16	1.34
Parous	0.79	3.8	3.5	37	7.69	0.51
No previous PE	0.55	1.4	1.3	16	6.53	0.46
Previous PE	4.63	44.8	43.1	76	7.86	2.02

DR, detection rate; *PE*, preeclampsia.

[a] Same as positive predictive value; [b] Same as 1 — negative predictive value.

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PLGF can be undertaken on the same machines as for free ß-human chorionic gonadotropin and pregnancy-associated plasma protein-A, which are widely used in screening for Down syndrome, but there is an inevitable increase in cost. The study provides data on performance of screening for any combinations of the biomarkers. Ultimately, the choice of test for screening will depend not only on the basis of performance, but also on the feasibility of implementation and health economic considerations. ■

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Received Sept. 3, 2015; revised Oct. 3, 2015; accepted Nov. 19, 2015.

The authors report no conflict of interest.

The study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116). The reagents and equipment for the measurement of serum placental growth factor and soluble fms-like tyrosine kinase-1 were provided by Roche Diagnostics Limited. This paper is part of the PhD thesis of Dahiana Gallo for Universidad de Granada, Spain.

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SUPPLEMENTAL TABLE 1
Characteristics of the population with complete data on all biomarkers

Variable	Unaffected (n = 7295)	Preeclampsia (n = 268)	PIH (n = 185)
Maternal age in years, median (IQR)	30.9 (26.4, 34.6)	31.5 (26.5, 35.6)	31.2 (27.1, 35.7)
Maternal weight in kg, median (IQR)	71.0 (63.0, 82.0)	78.0 (68.5, 91.5)[a]	77.0 (69.0, 87.8)[a]
Maternal height in cm, median (IQR)	165 (160, 169)	164 (160, 168)	164 (160, 169)
Body mass index, median (IQR)	26.1 (23.4, 29.9)	28.7 (25.4, 33.2)[a]	28.1 (25.7, 32.6)[a]
Gestational age in weeks, median (IQR)	21.8 (21.2, 22.1)	22.0 (21.1, 22.2)	22.0 (21.2, 22.1)
Racial origin		[a]	[a]
White, n (%)	5596 (76.7)	170 (63.4%)	121 (65.4%)
Afro-Caribbean, n (%)	1127 (15.5)	79 (29.5%)	44 (23.8%)
South Asian, n (%)	299 (4.1)	9 (3.4%)	13 (7.0%)
East Asian, n (%)	134 (1.8)	6 (2.2%)	2 (1.1%)
Mixed, n (%)	139 (1.9)	4 (1.5%)	5 (2.7%)
Medical history			
Chronic hypertension, n (%)	80 (1.1)	30 (11.2)[a]	0 (0.0)
Diabetes mellitus, n (%)	73 (1.0)	8 (3.0)[a]	1 (0.5)
SLE/APS, n (%)	10 (0.1)	0 (0.0)	1 (0.5)
Conception		[a]	[a]
Natural, n (%)	7050 (96.6)	253 (94.4)	174 (94.1)
In vitro fertilization, n (%)	181 (2.5)	9 (3.4)	4 (2.2)
Ovulation induction drugs, n (%)	64 (0.9)	6 (2.2)	7 (3.8)
Family history of preeclampsia, n (%)	215 (3.0)	16 (6.0)[a]	11 (6.0)
Parity		[a]	[a]
Nulliparous, n (%)	3433 (47.1)	169 (63.06%)	123 (66.5)
Parous with no previous PE, n (%)	3623 (49.7)	58 (21.64%)	49 (26.5)
Parous with previous PE, n (%)	239 (3.3)	41 (15.30%)	13 (7.0)
Inter-pregnancy interval in years, median (IQR)	3.1 (2.0, 5.0)	4.3 (2.5, 6.3)[a]	3.3 (2.2, 5.5)
Outcome			
Delivery at <32 weeks, n (%)	41 (0.6)	13 (4.9%)	1 (0.5)
Delivery at <37 weeks, n (%)	377 (5.2)	62 (23.1%)	11 (6.0)

Comparisons with unaffected group were by χ^2 or Fisher exact test for categorical variables and Mann-Whitney *U* test for continuous variables.

APS, antiphospholipid syndrome; *IQR*, interquartile range; *PE*, preeclampsia; *PIH*, pregnancy-induced hypertension; *SLE*, systemic lupus erythematosus.

[a] Significance value $P < .05$.

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SUPPLEMENTAL TABLE 2
Fitted regression models for marker log_{10} multiple of the median (MoM) values on gestation at time of delivery for pregnancies with preeclampsia

Biomarker	Estimate (95% confidence interval)
Uterine artery pulsatility index	
Intercept	0.34798 (0.324785, 0.37117)
Slope	-0.0195256 (-0.021237, -0.01781)
Mean arterial pressure	
Intercept	0.063088 (0.049141, 0.07704)
Slope	-0.002842 (-0.00377, -0.00191)
Placental growth factor	
Intercept	-1.11759 (-1.436384, -0.7988)
Slope	0.078571 (0.048763, 0.10838)
Soluble fms-like tyrosine kinase-1	
Intercept	0.585767 (0.621931, 1.73667)
Slope	-0.052772 (-0.097567, -0.05974)

In the regression models, gestational age was centered at 24 weeks so the intercept represents the mean at 24 weeks.

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SUPPLEMENTAL TABLE 3
Standard deviations and correlations, with 95% confidence limits, for log_{10} multiples of the median biomarker values

	No preeclampsia		Preeclampsia		
	n	Value	n	Value	Pooled
Standard deviation					
MAP	30,261	0.036279 (0.035992, 0.03657)	859	0.040576 (0.038741, 0.042593)	0.036403 (0.036119, 0.036692)
UTPI	65,762	0.113026 (0.112418, 0.113641)	1843	0.137039 (0.13275, 0.141616)	0.113746 (0.113143, 0.114357)
PLGF	9947	0.199612 (0.196865, 0.202438)	335	0.243466 (0.226296, 0.263476)	0.201017 (0.198296, 0.203815)
SFLT	7797	0.212704 (0.209404, 0.216111)	282	0.22947 (0.211936, 0.250191)	0.213306 (0.210053, 0.216661)
Correlations					
MAP and UTPI	28,631	-0.0412 (-0.05246, -0.02993)	817	-0.02828 (-0.09514, 0.03885)	-0.0412 (-0.05246, -0.02993)
MAP and PLGF	9667	-0.05417 (-0.06542, -0.04292)	324	-0.08371 (-0.14991, -0.01675)	-0.05417 (-0.06542, -0.04292)
MAP and SFLT	7621	0.0439 (0.03264, 0.05516)	271	0.04954 (-0.01757, 0.1162)	0.0439 (0.03264, 0.05516)
UTPI and PLGF	9735	-0.07356 (-0.08116, -0.06595)	329	-0.07031 (-0.11566, -0.02467)	-0.07356 (-0.08116, -0.06595)
UTPI and SFLT	7639	-0.16083 (-0.16827, -0.15336)	277	-0.14624 (-0.19069, -0.10119)	-0.16083 (-0.16827, -0.15336)
PLGF and SFLT	7790	0.19361 (0.17454, 0.21253)	282	0.08523 (-0.02262, 0.19112)	0.19361 (0.17454, 0.21253)

Pooled refers to estimates obtained from pooling data for the preeclampsia and no preeclampsia groups.

MAP, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

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SUPPLEMENTAL TABLE 4

Empirical detection rate with 95% confidence interval, at false-positive rate of 5% and 10%, in screening for preeclampsia with delivery at <37 and ≥37 weeks' gestation by maternal factors and combinations of biomarkers

Method of screening	Preeclampsia at <37 weeks					Preeclampsia at ≥37 weeks				
		FPR 5%		FPR 10%			FPR 5%		FPR 10%	
	n	History	Combined	History	Combined	n	History	Combined	History	Combined
History	790	34 (30, 37)	34 (30, 37)	47 (43, 50)	47 (43, 50)	1958	27 (25, 29)	27 (25, 29)	37 (35, 40)	37 (35, 40)
MAP	223	37 (30, 43)	44 (38, 51)	48 (41, 55)	59 (52, 66)	636	30 (26, 34)	32 (29, 36)	41 (37, 45)	47 (43, 51)
UTPI	520	37 (33, 41)	63 (58, 67)	49 (45, 53)	73 (69, 77)	1323	28 (25, 30)	30 (27, 32)	38 (36, 41)	42 (40, 45)
PLGF	81	35 (24, 46)	56 (44, 67)	52 (40, 63)	70 (59, 80)	254	28 (22, 33)	28 (22, 33)	37 (31, 44)	37 (31, 44)
SFLT	69	30 (20, 43)	32 (21, 44)	46 (34, 59)	54 (41, 66)	213	28 (22, 34)	28 (22, 34)	37 (31, 44)	37 (31, 44)
MAP, UTPI	211	37 (30, 44)	74 (67, 80)	48 (41, 55)	82 (76, 87)	606	30 (26, 33)	34 (30, 38)	40 (36, 44)	49 (44, 53)
MAP, PLGF	75	37 (26, 49)	67 (55, 77)	52 (40, 64)	75 (63, 84)	249	27 (22, 33)	28 (22, 34)	37 (31, 43)	41 (35, 47)
MAP, SFLT	63	33 (22, 46)	51 (38, 64)	46 (33, 59)	65 (52, 77)	208	27 (21, 33)	28 (22, 35)	37 (30, 43)	42 (35, 49)
UTPI, PLGF	79	35 (25, 47)	67 (56, 77)	52 (40, 63)	81 (71, 89)	250	27 (21, 33)	26 (21, 32)	37 (31, 43)	38 (32, 44)
UTPI, SFLT	67	31 (21, 44)	64 (52, 76)	46 (34, 59)	73 (61, 83)	210	27 (21, 34)	27 (21, 34)	37 (30, 44)	39 (32, 46)
PLGF, SFLT	69	30 (20, 43)	54 (41, 66)	46 (34, 59)	65 (53, 76)	213	28 (22, 34)	28 (22, 34)	37 (31, 44)	37 (31, 44)
MAP, UTPI, PLGF	74	38 (27, 50)	72 (60, 81)	53 (41, 64)	85 (75, 92)	246	26 (21, 32)	28 (22, 34)	37 (31, 43)	43 (37, 50)
MAP, UTPI, SFLT	62	34 (22, 47)	73 (60, 83)	47 (34, 60)	81 (69, 90)	206	27 (21, 33)	30 (23, 36)	36 (30, 43)	43 (36, 50)
MAP, PLGF, SFLT	63	33 (22, 46)	57 (44, 70)	46 (33, 59)	73 (60, 83)	208	27 (21, 33)	27 (21, 34)	37 (30, 43)	42 (36, 49)
UTPI, PLGF, SFLT	67	31 (21, 44)	67 (55, 78)	46 (34, 59)	82 (71, 90)	210	27 (21, 34)	26 (20, 32)	37 (30, 44)	38 (31, 45)
MAP, UTPI, PLGF, SFLT	62	34 (22, 47)	73 (60, 83)	47 (34, 60)	85 (74, 93)	206	27 (21, 33)	27 (21, 33)	36 (30, 43)	42 (35, 49)

The performance of screening with history varies with each biomarker or their combination because of differences in composition of the studied populations.

FPR, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

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SUPPLEMENTAL TABLE 5

Empirical detection rate with 95% confidence interval, at false-positive rate of 5% and 10%, in screening for preeclampsia with delivery at <32 and 32^{+0} to 36^{+6} weeks' gestation by maternal factors and combinations of biomarkers

Method of screening	Preeclampsia at <32 weeks					Preeclampsia at 32^{+0} to 36^{+6} weeks				
		FPR 5%		FPR 10%			FPR 5%		FPR 10%	
	n	History	Combined	History	Combined	n	History	Combined	History	Combined
History	205	41 (35, 49)	41 (35, 49)	52 (45, 59)	52 (45, 59)	585	31 (27, 35)	31 (27, 35)	45 (41, 49)	45 (41, 49)
MAP	60	50 (37, 63)	57 (43, 69)	65 (52, 77)	72 (59, 83)	163	32 (25, 40)	39 (32, 43)	42 (34, 50)	55 (47, 62)
UTPI	148	46 (38, 54)	82 (75, 88)	56 (48, 64)	87 (81, 92)	372	33 (29, 38)	57 (52, 75)	46 (41, 51)	68 (63, 72)
PLGF	19	42 (20, 67)	89 (67, 99)	68 (43, 87)	89 (67, 99)	62	32 (21, 45)	45 (32, 67)	47 (34, 60)	68 (55, 79)
SFLT	15	40 (16, 68)	60 (32, 84)	67 (38, 88)	73 (45, 92)	54	28 (16, 42)	26 (15, 32)	41 (28, 55)	48 (34, 62)
MAP, UTPI	57	49 (36, 63)	95 (85, 99)	65 (51, 77)	96 (88, 100)	154	32 (25, 40)	66 (58, 85)	42 (34, 50)	76 (68, 82)
MAP, PLGF	17	47 (23, 72)	88 (64, 99)	71 (44, 90)	94 (71, 100)	58	34 (22, 48)	60 (47, 64)	47 (33, 60)	69 (55, 80)
MAP, SFLT	13	46 (19, 75)	69 (39, 91)	69 (39, 91)	77 (46, 95)	50	30 (18, 45)	46 (32, 39)	40 (26, 55)	62 (47, 75)
UTPI, PLGF	18	44 (22, 69)	89 (65, 99)	67 (41, 87)	89 (65, 99)	61	33 (21, 46)	61 (47, 65)	48 (35, 61)	79 (66, 88)
UTPI, SFLT	14	43 (18, 71)	86 (57, 98)	64 (35, 87)	93 (66, 100)	53	28 (17, 42)	58 (44, 57)	42 (28, 56)	68 (54, 80)
PLGF, SFLT	15	40 (16, 68)	87 (60, 98)	67 (38, 88)	87 (60, 98)	54	28 (16, 42)	44 (31, 60)	41 (28, 55)	59 (45, 72)
MAP, UTPI, PLGF	17	47 (23, 72)	94 (71, 100)	71 (44, 90)	94 (71, 100)	57	35 (23, 49)	65 (51, 71)	47 (34, 61)	82 (70, 91)
MAP, UTPI, SFLT	13	46 (19, 75)	100 (75, 100)	69 (39, 91)	100 (75, 100)	49	31 (18, 45)	65 (50, 75)	41 (27, 56)	76 (61, 87)
MAP, PLGF, SFLT	13	46 (19, 75)	85 (55, 98)	69 (39, 91)	92 (64, 100)	50	30 (18, 45)	50 (36, 55)	40 (26, 55)	68 (53, 80)
UTPI, PLGF, SFLT	14	43 (18, 71)	93 (66, 100)	64 (35, 87)	93 (66, 100)	53	28 (17, 42)	60 (46, 66)	42 (28, 56)	79 (66, 89)
MAP, UTPI, PLGF, SFLT	13	46 (19, 75)	100 (75, 100)	69 (39, 91)	100 (75, 100)	49	31 (18, 45)	65 (50, 75)	41 (27, 56)	82 (68, 91)

The performance of screening with history varies with each biomarker or their combination because of differences in composition of the studied populations.

FPR, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

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SUPPLEMENTAL TABLE 6

Empirical detection rate with 95% confidence interval, at false-positive rate of 5% and 10%, in screening for preeclampsia with delivery at 37^{+0} to 39^{+6} and at ≥40 weeks' gestation by maternal factors and combinations of biomarkers

Method of screening	Preeclampsia at 37^{+0} to 39^{+6} weeks					Preeclampsia at ≥40 weeks				
	n	FPR 5%		FPR 10%		n	FPR 5%		FPR 10%	
		History	Combined	History	Combined		History	Combined	History	Combined
History	1315	31 (29, 34)	31 (29, 34)	41 (38, 44)	41 (38, 44)	643	19 (16, 22)	20 (17, 23)	30 (27, 34)	30 (27, 34)
MAP	435	35 (31, 40)	39 (34, 44)	47 (42, 51)	52 (47, 57)	201	18 (13, 24)	18 (13, 24)	29 (23, 36)	35 (28, 42)
UTPI	881	32 (29, 35)	34 (31, 37)	42 (39, 46)	46 (43, 49)	442	19 (15, 22)	22 (19, 27)	29 (25, 34)	35 (31, 40)
PLGF	172	32 (25, 40)	32 (25, 40)	42 (35, 50)	42 (34, 50)	82	17 (10, 27)	18 (11, 28)	24 (16, 35)	28 (19, 39)
SFLT	146	32 (25, 40)	32 (25, 40)	42 (34, 50)	42 (34, 50)	67	16 (8, 27)	18 (10, 29)	22 (13, 34)	27 (17, 39)
MAP, UTPI	410	34 (30, 39)	41 (37, 46)	45 (40, 50)	55 (50, 60)	196	18 (13, 25)	18 (13, 25)	30 (23, 37)	34 (28, 41)
MAP, PLGF	168	31 (24, 39)	33 (26, 40)	42 (34, 50)	48 (40, 55)	81	17 (10, 27)	17 (10, 27)	25 (16, 36)	27 (18, 38)
MAP, SFLT	142	31 (24, 39)	32 (25, 41)	41 (33, 49)	47 (39, 56)	66	17 (9, 28)	20 (11, 31)	24 (15, 36)	30 (20, 43)
UTPI, PLGF	168	31 (24, 39)	32 (25, 40)	42 (34, 50)	42 (35, 50)	82	17 (10, 27)	15 (8, 24)	24 (16, 35)	28 (19, 39)
UTPI, SFLT	143	31 (24, 40)	33 (25, 41)	41 (33, 50)	45 (36, 53)	67	16 (8, 27)	15 (7, 26)	24 (14, 36)	7 (17, 39)
PLGF, SFLT	146	32 (25, 40)	32 (25, 40)	42 (34, 50)	41 (33, 50)	67	16 (8, 27)	18 (10, 29)	22 (13, 34)	28 (18, 41)
MAP, UTPI, PLGF	165	30 (23, 38)	33 (26, 40)	41 (34, 49)	50 (42, 58)	81	17 (10, 27)	17 (10, 27)	25 (16, 36)	30 (20, 41)
MAP, UTPI, SFLT	140	31 (23, 39)	36 (28, 44)	41 (32, 49)	49 (40, 57)	66	17 (9, 28)	17 (9, 28)	24 (15, 36)	30 (20, 43)
MAP, PLGF, SFLT	142	31 (24, 39)	31 (24, 39)	41 (33, 49)	48 (39, 56)	66	17 (9, 28)	20 (11, 31)	24 (15, 36)	30 (20, 43)
UTPI, PLGF, SFLT	143	31 (24, 40)	31 (23, 39)	41 (33, 50)	42 (34, 50)	67	16 (8, 27)	15 (7, 26)	22 (13, 34)	28 (18, 41)
MAP, UTPI, PLGF, SFLT	140	31 (23, 39)	32 (25, 41)	41 (32, 49)	49 (41, 58)	66	17 (9, 28)	15 (8, 26)	24 (15, 36)	27 (17, 40)

The performance of screening with history varies with each biomarker or their combination because of differences in composition of the studied populations.

FPR, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

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SUPPLEMENTAL TABLE 7

Model-based detection rate of preeclampsia, at false-positive rates of 5% and 10%, in screening by maternal factors and combination of maternal factors and biomarkers

Method of screening	Gestational age at delivery with preeclampsia (w)											
	<32		32^{+0} to 36^{+6}		37^{+0} to 39^{+6}		≧40		<37		≧37	
	FPR 5%	FPR 10%	FPR 5%	FPR 10%	FPR 5%	FPR 10%	FPR 5%	FPR 10%	FPR 5%	FPR 10%	FPR 5%	FPR 10%
History	41	52	31	45	30	40	19	30	34	47	26	37
MAP	60	72	43	56	34	47	22	35	47	60	30	43
UTPI	79	88	50	63	33	46	19	31	57	70	28	40
PLGF	95	97	53	65	30	40	19	30	64	73	27	37
SFLT	54	65	32	44	30	40	19	30	38	50	26	37
MAP, UTPI	88	94	59	72	39	53	23	36	67	78	33	46
MAP, PLGF	96	98	59	71	34	47	22	35	69	78	30	43
MAP, SFLT	67	78	43	56	34	47	22	35	49	62	30	42
UTPI, PLGF	98	99	63	74	33	46	19	31	72	81	28	40
UTPI, SFLT	87	93	51	65	33	46	19	31	61	72	28	40
PLGF, SFLT	97	98	54	66	30	40	19	30	65	75	27	37
MAP, UTPI, PLGF	98	99	69	80	39	53	23	35	77	85	33	46
MAP, UTPI, SFLT	92	96	60	73	39	53	23	36	69	79	33	46
MAP, PLGF, SFLT	92	96	60	73	39	53	23	36	69	79	33	46
UTPI, PLGF, SFLT	99	100	65	76	34	46	19	31	74	82	28	40
MAP, UTPI, PLGF, SFLT	99	100	70	81	39	53	23	36	78	86	33	46

FPR, false-positive rate; *MAP*, mean arterial pressure; *PLGF*, placental growth factor; *SFLT*, soluble fms-like tyrosine kinase-1; *UTPI*, uterine artery pulsatility index.

Gallo et al. Second-trimester screening for preeclampsia. Am J Obstet Gynecol 2016.

Study 4

Wright D, Gallo DM, Gil Pugliese, Casanova C, Nicolaides KH. Contingent screening for preterm preeclampsia. Ultrasound Obstet Gynecol 2016;47(5):554-9.

Impact Factor: 4.254

Ultrasound Obstet Gynecol 2016; **47**: 554–559
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15807

Contingent screening for preterm pre-eclampsia

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KEYWORDS: Bayes' theorem; contingent screening; mean arterial pressure; placental growth factor; pre-eclampsia; pyramid of pregnancy care; survival model; uterine artery Doppler

ABSTRACT

Objective *Effective screening for pre-eclampsia resulting in delivery < 37 weeks' gestation (preterm PE) is provided by assessment of a combination of maternal factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) at 11–13 or 19–24 weeks' gestation. This study explores the possibility of carrying out routine screening for preterm PE by maternal factors and MAP in all pregnancies and reserving measurements of UtA-PI and PlGF for a subgroup of the population, selected on the basis of the risk derived from screening by maternal factors and MAP alone.*

Methods *Study data were derived from prospective screening for adverse obstetric outcomes in women attending their routine hospital visit at 11–13 and/or 19–24 weeks' gestation. Bayes' theorem was used to derive the* a-priori *risk for preterm PE from maternal factors and MAP. The posterior risk was obtained by the addition of UtA-PI and PlGF. We estimated the detection rate (DR) of preterm PE, at an overall false-positive rate (FPR) of 10%, from a policy in which first-stage screening by a combination of maternal factors and MAP defines screen-positive, screen-negative and intermediate-risk groups, with the latter undergoing second-stage screening by UtA-PI and PlGF.*

Results *At 11–13 weeks' gestation, the model-based DR of preterm PE, at a 10% FPR, when screening the whole population by maternal factors, MAP, UtA-PI and PlGF was 74%. A similar DR was achieved by two-stage screening, with screening by maternal factors and MAP in the first stage and reserving measurement of UtA-PI and PlGF for the second stage and for only 50% of the population. If second-stage screening was offered to 30% of the population, there would be only a small reduction in DR from 74% to 71%. At 19–24 weeks, the model-based DR of preterm PE, at a 10% FPR, when screening the whole population by maternal factors, MAP, UtA-PI and PlGF was 84%. A similar DR was achieved by two-stage screening with measurements of UtA-PI and PlGF in only 70% of the population; if second-stage screening was offered to 40% of the population, the DR would be reduced from 84% to 81%.*

Conclusions *High DR of preterm PE can be achieved by two-stage screening in the first and second trimesters with maternal factors and MAP in the whole population and measurements of UtA-PI and PlGF in only some of the pregnancies.* Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

In screening for pre-eclampsia (PE), we advocate use of Bayes' theorem to combine the *a-priori* risk from maternal demographic characteristics and medical history (maternal factors) with the results of various combinations of biophysical and biochemical measurements[1–4]. This approach of screening, which allows estimation of individual patient-specific risks of PE requiring delivery before any specified gestational age, has a performance which is by far superior to that of risk-scoring systems based on maternal factors alone[3–6].

We reported recently that screening for PE at 11–13 or 19–24 weeks' gestation by a combination of maternal factors and mean arterial pressure (MAP) can predict about 60% of preterm PE, requiring delivery < 37 weeks, but only about 45% of PE delivering ≥ 37 weeks, at a false-positive rate (FPR) of 10%[3,4]. Addition of uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) improved the detection rate (DR) of preterm PE to about 75% at 11–13 weeks and 85% at 19–24 weeks[3,4]. Recording of maternal history and measurement of blood pressure are universally carried out as part of routine pregnancy care. In contrast, measurement of UtA-PI requires specific training by sonographers and quality assurance of their results; nevertheless this test can be undertaken within a few minutes by the same sonographers and machines as part of the routine second-trimester scan. Measurement of serum PlGF can be

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Accepted: 27 October 2015

undertaken on the same machines as for free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A, which are widely used in screening for Down syndrome, but there is an inevitable increase in cost.

The objective of this study was to explore the possibility of carrying out routine screening by maternal factors and MAP in all pregnancies and reserving measurements of UtA-PI and PlGF for only a subgroup of the population, selected on the basis of the risk derived from screening by maternal factors and MAP.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending their routine first- and/or second-trimester hospital visits at King's College Hospital, University College London Hospital and Medway Maritime Hospital, UK. These visits, which were held at 11 + 0 to 13 + 6 and 19 + 0 to 24 + 6 weeks' gestation, included first, recording of maternal characteristics and medical history, second, measurement of the left and right UtA-PI by color Doppler ultrasound and calculation of the mean PI by transabdominal ultrasound in the first trimester and by transvaginal ultrasound in the second trimester[7,8], third, measurement of MAP by validated automated devices and standardized protocol[9] and fourth, measurement of serum concentration of PlGF by an automated biochemical analyzer within 10 min of blood sampling (Cobas e411 system, Roche Diagnostics, Penzberg, Germany).

Gestational age was determined from measurement of fetal crown–rump length at 11–13 weeks or the fetal head circumference at 19–24 weeks[10,11]. The women were screened between March 2006 and July 2014 and gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

The inclusion criteria for this study were singleton pregnancy delivering a morphologically normal live birth or stillbirth at or after 24 weeks' gestation. Pregnancies with aneuploidy or major fetal abnormality and those ending in termination, miscarriage or fetal death < 24 weeks were excluded.

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[12]. The outcome measure for this study was preterm PE.

Statistical analysis

Our competing-risks model for gestational age at delivery with PE is defined by two components: first, the prior distribution based on maternal factors[1] and second, the conditional distribution of multiples of the median (MoM) values of UtA-PI, MAP and PlGF[13–15] given the gestational age with PE and maternal factors[4]. Model-based estimates of screening performance were obtained as follows. Samples of 500 000 records with preterm PE and 500 000 without PE or pregnancy-induced hypertension were sampled with replacement from a population of 123 406 pregnancies with available data on maternal factors. For each record, the prior distribution of time to delivery was obtained from a competing-risks model[1]. MAP, UtA-PI and PlGF MoM values were simulated from the fitted multivariate Gaussian distribution for log-transformed MoM values[3,4]. Posterior distributions of time to delivery with PE were obtained by combining the prior risk[1] and the likelihoods of the biomarkers using Bayes' theorem. Risks of PE were obtained by calculating the area under the posterior distribution. The different contingent screening policies were then applied to the risks to provide model-based estimates of screening performance.

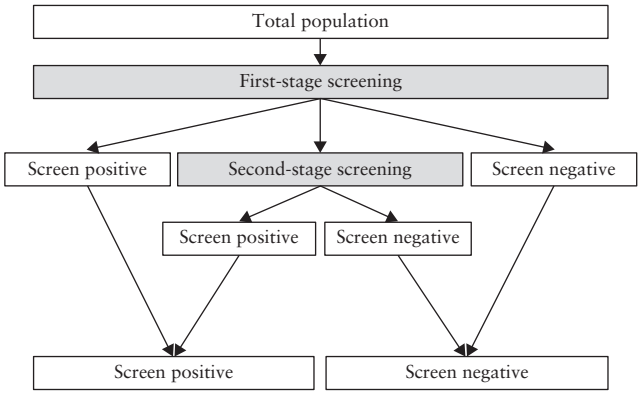

Figure 1 Two-stage screening strategy for preterm pre-eclampsia in which the whole population undergoes first-stage screening by maternal factors and mean arterial pressure and a selected proportion of those considered to be at intermediate risk undergo second-stage screening by uterine artery pulsatility index and placental growth factor.

We examined the performance of screening for preterm PE by a two-stage strategy (Figure 1). In the first stage, which is applied to the whole population, the risk of preterm PE was derived from maternal factors and MAP. On the basis of the results of first-stage screening, the population was divided into a low-risk group considered to be screen negative, a high-risk group considered to be screen positive and an intermediate-risk group in need of further testing with UtA-PI and PlGF; after such testing, the patients were again classified as screen negative or screen positive. The screen-positive rate in the normal-outcome group (same as FPR for the whole population) is the sum of the screen-positive groups from first- and second-stage screening and was fixed at 10%.

The statistical software package R was used for data analyses[16].

RESULTS

The characteristics of the total population of 123 406 singleton pregnancies are given in Table 1. In the first

Table 1 Characteristics of study population of pregnant women screened at 11–13 weeks or 19–24 weeks' gestation for preterm pre-eclampsia (PE) (delivery < 37 weeks) according to pregnancy outcome

Characteristic	*Unaffected* (n = *117 710*)	*Preterm PE* (n = *790*)	*Term PE* (n = *1958*)	*PIH* (n = *2948*)
Maternal age (years)	31.3 (26.7–35.1)	31.8 (26.9–36.5)	31.3 (26.5–35.8)	31.8 (27.2–35.5)
Maternal weight (kg)	69.8 (62.4–79.9)	74.0 (65.0–88.0)	77.4 (67.8–91.9)	76.0 (67.0–88.0)
Maternal height (cm)	164 (160–169)	163 (158–167)	164 (160–168)	165 (160–169)
Body mass index (kg/m^2)	25.8 (23.2–29.4)	28.4 (24.6–32.8)	28.8 (25.4–33.7)	28.1 (25.0–32.4)
Gestational age (weeks)	22.1 (21.1–22.7)	22.2 (21.2–22.8)	22.2 (21.4–22.7)	22.2 (21.4–22.7)
Racial origin				
Caucasian	87 373 (74.2)	420 (53.2)	1165 (59.5)	2010 (68.2)
Afro-Caribbean	18 313 (15.6)	293 (37.1)	614 (31.4)	668 (22.7)
South Asian	6120 (5.2)	51 (6.5)	102 (5.2)	148 (5.0)
East Asian	3106 (2.6)	10 (1.3)	37 (1.9)	53 (1.8)
Mixed	2798 (2.4)	16 (2.0)	40 (2.0)	69 (2.3)
Medical history				
Chronic hypertension	1198 (1.0)	102 (12.9)	186 (9.5)	0 (0.0)
Diabetes mellitus	893 (0.8)	30 (3.8)	31 (1.6)	35 (1.2)
SLE/APS	207 (0.2)	9 (1.1)	7 (0.4)	9 (0.3)
Mode of conception				
Spontaneous	113 530 (96.5)	727 (92.0)	1868 (95.4)	2823 (95.8)
In-vitro fertilization	2632 (2.2)	43 (5.4)	68 (3.5)	83 (2.8)
Ovulation induction drugs	1548 (1.3)	20 (2.5)	22 (1.1)	42 (1.4)
Family history of PE	4243 (3.6)	67 (8.5)	134 (6.8)	220 (7.5)
Parity				
Nulliparous	57 720 (49.0)	468 (59.2)	1250 (63.8)	1888 (64.0)
Parous				
No previous PE	56 848 (48.3)	196 (24.8)	476 (24.3)	765 (26.0)
Previous PE	3142 (2.7)	126 (16.0)	232 (11.9)	295 (10.0)
Interpregnancy interval (years)	2.9 (1.9–4.8)	4.2 (2.4–7.3)	3.7 (2.3–6.7)	3.4 (2.0–5.7)

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; PIH, pregnancy induced hypertension; SLE, systemic lupus erythematosus.

trimester, MAP was measured in 77 343 cases, UtA-PI in 92 712 and PlGF in 40 212, and the respective values in the second trimester were 31 120, 67 605 and 10 282.

Screening at 11–13 weeks' gestation

The model-based DR of preterm PE, at a FPR of 10%, when screening the whole population by maternal factors, MAP, UtA-PI and PlGF in the first trimester was 74% (Table 2 and Figure 2). A similar DR was achieved by two-stage screening; by maternal factors and MAP in the first stage and reserving measurement of UtA-PI and PlGF for the second stage and for only 50% of the population (Table 2 and Figure 2). Similarly, if second-stage screening was offered to 30% of the population, the DR with the addition of UtA-PI or PlGF would be reduced only mildly from 74% to 71%.

The results of a policy in which the population is divided after first-stage screening into screen-positive, screen-negative and intermediate-risk groups, with the latter undergoing second-stage screening, is shown in Figure 2 and Table S1. If the selected population for second-stage screening is > 30%, the maximum DR is achieved without the need for identifying a screen-positive group in first-stage screening. In contrast, if the selected population for second-stage screening is ≤ 30%, the DR is higher if a screen-positive group is introduced. For example, if after first-stage screening 0% of the population is classified as screen-positive and 20% is selected for second-stage screening, the DR, at a 10% FPR, would be 66.7%; if after first-stage screening 5% of the population is classified as screen positive and 20% is selected for second-stage screening, the DR, at a 10% FPR, would be 68.7%.

The DR for preterm PE, at a 10% FPR, of two-stage screening at 11–13 weeks' gestation in the population, subdivided according to racial origin and obstetric history is shown in Table S2. In these calculations, a policy was selected whereby, after first-stage screening, 2% of the population was classified as screen positive, 68% as screen negative and 30% were selected for second-stage screening. The FPR was lower and DR higher in parous than in nulliparous women, in parous women with PE in a previous pregnancy than in parous women without PE in a previous pregnancy and in those of Afro-Caribbean racial origin than in those of Caucasian racial origin.

Screening at 19–24 weeks' gestation

The model-based DR of preterm PE, at a FPR of 10%, when screening the whole population by maternal factors, MAP, UtA-PI and PlGF in the second trimester was 84% (Table 2 and Figure 2). A similar DR was achieved by two-stage screening; by maternal factors and MAP in the first stage and reserving measurements of UtA-PI and PlGF for the second stage and for only 70% of the population (Table 2 and Figure 2). Similarly, if second-stage screening was offered to 40% of the population, the DR with the addition of UtA-PI or PlGF would be reduced only mildly from 84% to 81%.

The results of a policy in which the population is divided after first-stage screening into screen-positive, screen-negative and intermediate-risk groups, with the

Table 2 Model-based detection rate (DR) of preterm pre-eclampsia (PE), at overall false-positive rate of 10%, by two-stage screening with maternal factors and mean arterial pressure at the first stage and uterine artery pulsatility index and serum placental growth factor at the second stage, at 11–13 or 19–24 weeks' gestation

Screening at 11–13 weeks				*Screening at 19–24 weeks*			
Proportion undergoing second-stage screening (%)				*Proportion undergoing second-stage screening (%)*			
All	*Unaffected*	*Preterm PE*	*DR (%)*	*All*	*Unaffected*	*Preterm PE*	*DR (%)*
15	13.8	64.3	62.8	15	13.7	66.7	66.1
20	18.7	71.1	66.7	20	18.6	73.3	71.2
25	23.6	76.6	69.3	25	23.5	78.4	74.7
30	28.6	81.0	70.9	30	28.5	82.4	77.2
35	33.6	84.6	72.0	35	33.5	85.8	79.1
40	38.6	87.6	72.7	40	38.5	88.6	80.5
45	43.6	90.2	73.3	45	43.5	90.9	81.5
50	48.6	92.3	73.6	50	48.6	92.8	82.3
55	53.7	94.0	73.8	55	53.7	94.4	82.9
60	58.8	95.5	74.0	60	58.7	95.8	83.4
65	63.9	96.7	74.0	65	63.8	96.9	83.8
70	69.0	97.6	74.1	70	69.0	97.8	84.0
75	74.1	98.4	74.1	75	74.1	98.5	84.2
80	79.3	99.0	74.1	80	79.2	99.0	84.3
85	84.4	99.4	74.1	85	84.4	99.4	84.4
90	89.6	99.7	74.1	90	89.6	99.6	84.4
95	94.8	99.9	74.1	95	94.8	99.8	84.4
100	100	100	74.1	100	100	100	84.4

In the first stage, applied to the whole population, risk of preterm PE is assessed by maternal factors and mean arterial pressure, defining a higher risk group that continues to the second stage. In the second stage, uterine artery pulsatility index and serum placental growth factor are measured and the combined risk is used to identify a screen-positive group. The risk cut-off in the first stage is determined to achieve the proportion continuing to the second stage. The second-stage risk cut-off is determined so that the two stages combined have a false-positive rate of 10%.

latter undergoing second-stage screening, is shown in Figure 2 and Table S3. If the selected population for second-stage screening is > 40%, the maximum DR is achieved without the need for identifying a screen-positive group in first-stage screening. In contrast, if the selected population for second-stage screening is ≤ 40%, the DR is higher if a screen-positive group is introduced. For example, if after first-stage screening 0% of the population is classified as screen positive and 20% is selected for second-stage screening, the DR, at a 10% FPR, would be 71.6%; if after first-stage screening 7% of the population is classified as screen positive and 20% is selected for second-stage screening, the DR, at a 10% FPR, would be 74.9%.

The DR for preterm PE, at a 10% FPR, of two-stage screening at 19–24 weeks' gestation in the population subdivided according to racial origin and obstetric history is shown in Table S4. In these calculations, a policy was selected whereby, after first-stage screening, 2% of the population was classified as screen positive, 58% as screen negative and 40% were selected for second-stage screening. The FPR and DR were higher in nulliparous than in parous women, in parous women with PE in a previous pregnancy than in women without PE in a previous pregnancy and in those of Afro-Caribbean racial origin than in those of Caucasian racial origin.

DISCUSSION

Principal findings of the study

The findings of this study demonstrate that, in screening the whole population for preterm PE at 11–13 or 19–24 weeks' gestation by a combination of maternal factors, MAP, UtA-PI and PlGF, the DR, at a 10% FPR, is about 75% and 85%, respectively. A similar performance can be achieved by a two-stage strategy with screening by maternal factors and MAP in the whole population in the first stage and reserving measurement of UtA-PI and PlGF for the second stage and for only some of the population; 50% of the population when screening at 11–13 weeks and 70% of the population when screening at 19–24 weeks. Further reduction in the proportion of the population undergoing second-stage screening to 30% at 11–13 weeks and 40% at 19–24 weeks would result in only a small decrease in DR.

We propose a methodology and provide data on the estimated overall DR based on the proportion of the population selected for second-stage screening, and this could form the basis for heath economic evaluations that would define the most appropriate strategy for different healthcare systems.

In the application of Bayes' theorem, the maternal-factor derived prior risk has a strong influence on the posterior risk and therefore the performance of screening. This is well recognized in the case of screening for Down syndrome for which the maternal-age derived prior risk is combined with the measurement of first- and or second-trimester biomarkers to derive the posterior risk; at a fixed risk cut-off, both the DR and FPR increase with maternal age and therefore the overall performance of screening depends on the maternal age distribution of a given study population. In screening for PE, important contributors to the prior risk are racial origin, maternal weight and height, method of conception as well as

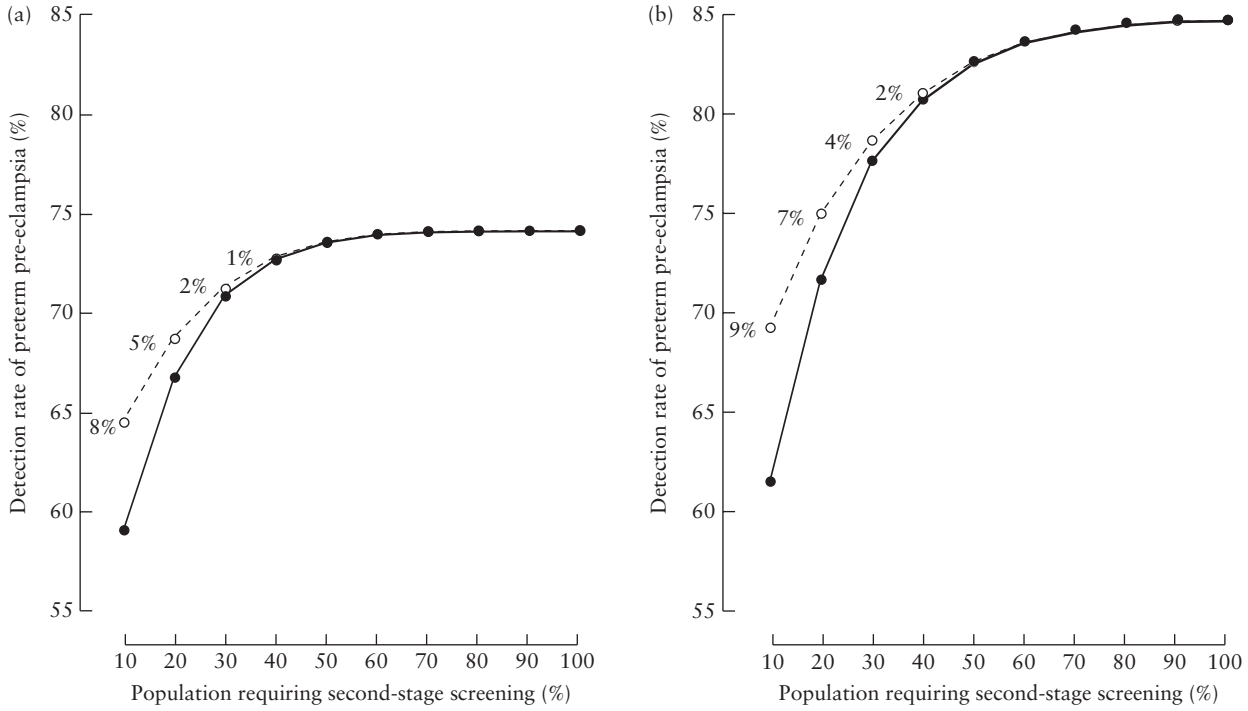

Figure 2 Relationship between the detection rate of preterm pre-eclampsia and the proportion of the population requiring second-stage screening by uterine artery pulsatility index and placental growth factor after first-stage screening by maternal factors and mean arterial pressure at: (a) 11–13 weeks; or (b) 19–24 weeks' gestation. Filled circles and solid lines represent the performance of screening if the population is divided after the first stage into a screen-negative group and a group in need of second-stage screening. Open circles and dashed lines represent the performance of screening if the population is divided after the first stage into a screen-positive group, a screen-negative group and an intermediate-risk group in need of second-stage screening. Values adjacent to the open circles are the proportion of the population classified as screen positive after the first stage.

components of family, obstetric and medical history; consequently, at a fixed FPR, the risk cut-off and DR are inevitably dependent on the distribution of maternal factors of a given study population.

Strengths and limitations

The strength of this study relies on the basic principle that first-stage screening identifies a group that is at such high risk and another that is at such low risk that further testing with additional biomarkers is unlikely to change their classification from screen positive and screen negative, respectively. Second-stage testing is restricted to an intermediate-risk group for which additional measurements are likely to make a difference to their final screening result. The first stage uses maternal factors and MAP; taking a medical history and recording blood pressure are an integral part of routine antenatal care. The second stage uses UtA-PI and PlGF; measures that incur additional costs or require specialist expertise or equipment.

Previous studies have demonstrated that contingent strategies provide a cost-effective way of screening for Down syndrome; the performance of screening by a combination of first-trimester fetal nuchal translucency thickness and first- and second-trimester serum biochemistry in all pregnancies, as in the integrated test, is similar to contingent screening in which second-trimester testing is carried out in only about 25% of the population, who were identified as being at intermediate risk by first-trimester screening[17,18].

A limitation of the study is that although a large dataset of prospectively examined patients undergoing routine pregnancy care in the first and/or second trimesters was used, the performance of screening was estimated from modeling. Prospective evaluation is required to confirm the results, after appropriate adjustments for the distribution of maternal factors in the study populations.

Comparison to alternative strategies of screening for preterm PE

In the USA, the American College of Obstetricians and Gynecologists (ACOG) recommends that the best and only approach to screening for PE should be by taking a medical history to evaluate for the following risk factors: nulliparity, age > 40 years, body mass index ≥ 30 kg/m^2, conception by *in-vitro* fertilization, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus and systemic lupus erythematosus or thrombophilia[5]. However, the performance of such a strategy in screening for preterm PE is very poor, with a DR of 90% but a FPR of 67%[4]. In the UK, the National Institute for Health and Clinical Excellence (NICE) recommends that women should be considered to be at high risk of developing PE if they have any one high-risk factor (hypertensive

disease in previous pregnancy, chronic hypertension, chronic renal disease, diabetes mellitus or autoimmune disease) or any two moderate-risk factors (nulliparity, age ≥ 40 years, body mass index ≥ 35 kg/m^2, interpregnancy interval > 10 years or family history of PE)[6]. However, the performance of such a strategy in screening for preterm PE is also very poor with a DR of 40% and a FPR of 11%[1].

In our approach to screening, maternal factors are not treated as independent screening tests, as advocated by ACOG[4] and NICE[6], but rather they are combined into a multivariable logistic model which attributes the appropriate value to each factor and takes into consideration their interrelations to derive the individual patient-specific *a-priori* risk. Bayes' theorem is then used to combine the information on maternal factors with that from biomarkers to estimate the patient-specific posterior risk. We have shown that useful biomarkers in both the first and second trimesters are MAP, UtA-PI and PlGF and, when these are measured in all pregnancies, the DR of preterm PE, at a 10% FPR, is about 75% and 85%, respectively[3,4]. In this study, we have shown that a similarly high DR can be achieved by a two-stage screening strategy, at substantially lower costs than carrying out screening with all biomarkers in the whole population. The software for implementation of this approach is freely available (https://fetalmedicine.org/calculator/preeclampsia).

ACKNOWLEDGMENTS

The study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116). The reagents and equipment for the measurement of serum placental growth factor were provided by Roche Diagnostics Limited. This paper is part of the PhD thesis of Dahiana M. Gallo for Universidad de Granada, Spain.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Model-based detection rate (DR) of preterm pre-eclampsia (PE) by two-stage screening at 11–13 weeks' gestation at a fixed overall false-positive rate of 10%

Table S2 Effects of two-stage screening for preterm pre-eclampsia (PE) (delivery < 37 weeks) at 11–13 weeks' gestation in the total population, subdivided according to racial origin and obstetric history

Table S3 Model-based detection rate (DR) of preterm pre-eclampsia (PE) by two-stage screening at 19–24 weeks' gestation at a fixed overall false-positive rate of 10%

Table S4 Effects of two-stage screening for preterm pre-eclampsia (PE) (delivery < 37 weeks) at 19–24 weeks' gestation in the total population, subdivided according to racial origin and obstetric history

Ultrasound Obstet Gynecol 2016; **47**: 554–559
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15807

RESUMEN

Objetivo Proporcionar un cribado eficaz de la preeclampsia que causa el parto antes de la semana 37 de gestación (PE pretérmino), mediante la evaluación de una combinación de factores maternos, la presión arterial media (PAM), el índice de pulsatilidad de la arteria uterina (IP artUt) y el factor de crecimiento placentario (PlGF, por sus siglas en inglés) en las semanas de gestación 11–13 o 19–24. Este estudio explora la posibilidad de realizar cribados rutinarios para la PE pretérmino mediante factores maternos y la PAM en todos los embarazos y tan solo medir el UtA-PI y el PlGF en un subgrupo de la población, seleccionado en función del riesgo derivado del cribado empleando solo factores maternos y la PAM.

Métodos Los datos del estudio se obtuvieron del cribado prospectivo de resultados obstétricos adversos en mujeres que realizaron su visita rutinaria en el hospital en las semanas de gestación 11–13 y/o 19–24. Se empleó el teorema de Bayes para calcular el riesgo a priori de PE pretérmino a partir de factores maternos y la PAM. El riesgo a posteriori se obtuvo al añadir el UtA-PI y el PlGF. Se estimó la tasa de detección (TD) de PE pretérmino, con una tasa de falsos positivos (TFP) en general del 10%, a partir de una política en la que la primera etapa de cribado mediante una combinación de factores maternos y PAM define los grupos de cribado en resultados positivos, negativos y de riesgo intermedio, siendo este último al que se le aplicaría una segunda fase de cribado por UtA-PI y PlGF.

Resultados En las semanas de gestación 11–13, la TD de PE pretérmino, basada en un modelo con TFP de 10% en el que se cribó la totalidad de la población mediante factores maternos, la PAM, el UtA-PI y el PlGF, fue del 74%. Mediante el cribado de dos etapas se logró una TD similar, empleando factores maternos y PAM en la primera etapa y tan solo midiendo el UtA-PI y el PlGF durante la segunda etapa y tan sólo para el 50% de la población. Si la segunda etapa de cribado se hiciera al 30% de la población, solo habría una ligera reducción en la TD del 74% al 71%. En las semanas de gestación 19–24, la TD de PE pretérmino, basada en un modelo con TFP de 10% en el que se cribó la totalidad de la población mediante factores maternos, la PAM, el UtA-PI y el PlGF, fue del 84%. Mediante el cribado de dos etapas se logró una TD similar, midiendo el UtA-PI y el PlGF en tan sólo el 70% de la población; si la segunda etapa de cribado se hiciera al 40% de la población, la TD se reduciría del 84% al 81%.

Conclusiones Es posible lograr una elevada TD de la PE pretérmino mediante un cribado en dos etapas en el primer y el segundo trimestre, a partir de factores maternos y la PAM en toda la población y la medición del UtA-PI y el PlGF en tan sólo algunos de los embarazos.

目的：通过综合评估孕11~13周或孕19~24周时的母体因素、平均动脉压（mean arterial pressure，MAP）、子宫动脉搏动指数（uterine artery pulsatility index，UtA-PI）和血清胎盘生长因子 (PlGF)，有效筛查导致孕37周前分娩的先兆子痫。本研究探讨了对所有孕妇通过母体因素和MAP进行早产PE常规筛查，并对通过母体因素和MAP进行筛查后所确定的高风险亚组进行UtA-PI和PlGF检测的可能性。

方法：研究资料来自在孕11~13周和/或孕19~24周时，进行常规检查孕妇的不良产科结局的前瞻性筛查。应用贝叶斯法则，根据母体因素和MAP得到早产 PE的验前风险，通过增加UtA-PI和PlGF检测得到验后风险。采用母体因素和MAP进行第一阶段筛查，分为筛查阳性组、筛查阴性组和中度风险组，后者采用UtA-PI和PlGF进行第二阶段筛查，根据这一策略，我们评估在总的假阳性率（false-positive rate，FPR）为10%时早产PE的检出率（detection rate，DR）。

结果：孕11~13周时，当根据母体因素、MAP、UtA-PI和PlGF对整个人群进行筛查时，在10% FPR时，基于模型的早产PE的DR为74%。两阶段筛查所得的DR相似，第一阶段采用母体因素和MAP进行筛查，第二阶段仅对50%的人群进行UtA-PI和PlGF检测。如果对30%的人群进行第二阶段筛查，则DR从74%降至71%，下降很小。孕19~24周时，当根据母体因素、MAP、UtA-PI和PlGF对整个人群进行筛查时，在10% FPR时，基于模型的早产PE的DR为84%。仅对70%的人群进行UtA-PI 和PlGF检测的两阶段筛查所得DR相似；如果对40%的人群进行第二阶段筛查，DR将从84%降至81%。

结论：在妊娠早期和妊娠中期，通过检测整个人群的母体因素和MAP以及仅检测某些孕妇的UtA-PI和PlGF进行两阶段筛查，早产PE的DR较高。

CHAPTER 3 SUMMARY

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3.1 ENGLISH

STUDY 1

The objectives of this screening study in singleton pregnancies examined at both 11-13 and 20-24 weeks, were firstly, to examine the maternal characteristics that affect mean arterial pressure (MAP) in normal pregnancies and secondly, to compare the performance of screening for preeclampsia (PE) by MAP in the first and second trimesters of pregnancy.

MAP was measured at 11-13 and 20–24 weeks in 17,383 singleton pregnancies, including 70 with early-PE, requiring delivery <34 weeks' gestation, 143 with preterm-PE, delivering <37 weeks and 537 with total-PE. MAP was expressed as multiple of the median (MoM) after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes. The performance of screening for PE by maternal characteristics and MAP MoM at 11-13 weeks (MAP-1), MAP MoM at 20-24 weeks (MAP-2) and their combination was evaluated.

In normal pregnancies, MAP was affected by maternal characteristics and medical history. At both 11-13 and 20-24 weeks' gestation, MAP decreased with gestational age and height, increased with maternal weight, it was higher in women with chronic hypertension and in those with a personal or family history of PE and lower in women of Afro-Caribbean racial origin, in smokers and in parous women with no previous PE. In the PE group, compared to the normal group, the median MAP-1 and MAP-2 were significantly increased and there was a significant inverse association between gestational age at delivery with both MAP-1 and MAP-2. In screening by maternal characteristics and MAP-1, at false positive rate (FPR) of 10%, the detection rates (DR) of early-

PE, preterm-PE and total-PE were 74.3%, 62.9% and 49.3%, respectively; the DR at FPR of 5% were 52.9%, 42.7% and 35.8%. In screening by MAP-1 and MAP-2 the DR, at FPR of 10%, were 84.3%, 65.7% and 52.5%; the DR at FPR of 5% were 60.0%, 49.7% and 37.6%.

It was concluded that the measured MAP must be adjusted for maternal characteristics and medical history and expressed as a MoM before valid comparisons can be carried out between normal and pathological pregnancies. The performance of screening for PE by MAP MoM is best when measurements are taken at both 11-13 and 20-24 weeks' gestation than at only one of these gestational ranges.

STUDY 2

The objectives of this screening study are firstly, to determine the maternal characteristics that affect uterine artery pulsatility index (UTPI) in normal pregnancies at 20-24 weeks' gestation, and secondly, to examine in pregnancies with PE the relation between UTPI multiple of the median (MoM) and the severity of the disease, defined by the gestational age at delivery and the presence of fetal growth restriction.

The study population consisted of singleton pregnancies undergoing a routine ultrasound examination at 20-24 weeks' gestation, which was preceded by combined screening for aneuploidies at 11-13 weeks' gestation between 2006 and 2013 at three hospitals in and around London. UTPI was measured at 20–24 weeks in 50,490 singleton pregnancies, including 1,442 (2.9%) that developed PE. UTPI was expressed as MoM after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes. In PE, the correlation between Ut-PI MoM with gestational age at delivery and neonatal birth weight Z-score was determined.

In the normal group, there were significant independent contributions to UTPI from gestational age at screening, racial origin and prior history of PE and/or small for gestational age (SGA). In the PE group, there was an inverse

significant association between UTPI MoM and both gestational age at delivery and neonatal birth weight Z-score (P<0.0001). In 295 (20.5%) of the 1,442 cases of PE there was SGA and the incidence of SGA was inversely related to the gestational age at delivery decreasing from 46.8% <34 weeks to 28.5% at 34-37 weeks and 10.6% at ≥38 weeks. UTPI was above the 95th percentile (1.509 MoM) in 72.7%, 36.1% and 14.9% of cases of PE requiring delivery at <34, 34-37 and ≥38 weeks, respectively, and the percentages for PE with SGA were 80.2%, 55.6% and 37.4%.

It was concluded that in normal pregnancy UTPI is affected by maternal characteristics. In women that develop PE, UTPI at 20-24 weeks' gestation is increased and the increase is particularly marked in those with early-PE and in PE wth SGA.

STUDY 3

The objective of this study was to develop a model for PE based on a combination of maternal factors with second-trimester biomarkers.

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at 19-24 weeks' gestation in three maternity hospitals in England between January 2006 and July 2014. We had data from maternal factors, uterine artery pulsatility index (UTPI), mean arterial pressure (MAP), serum placental growth factor (PLGF) and serum soluble fms-like tyrosine kinase-1 (SFLT) from 123,406, 67,605, 31,120, 10,828 and 8,079 pregnancies, respectively. Bayes theorem was used to combine the *a priori* risk from maternal factors with various combinations of biomarker multiple of the median (MoM) values. The modeled performance of screening for PE requiring delivery at <32, <37 and ≥37 weeks' gestation was estimated. The modeled performance was compared to the empirical one which was derived from five-fold cross validation. We also examined the performance of screening based on risk factors from the medical history, as recommended by ACOG.

In pregnancies that developed PE, the values of MAP, UTPI and SFLT were increased and PLGF was decreased. For all biomarkers the deviation from normal was greater for early than late PE and therefore the performance of screening was inversely related to the gestational age at which delivery become necessary for maternal and or fetal indications. Screening by maternal factors predicted 52%, 47% and 37% of PE at <32, <37 and ≥37 weeks' gestation, respectively, at false positive rate of 10%. The respective values for combined screening with maternal factors, MAP, UTPI and PLGF were 99%, 85% and 46%; the performance was not improved by the addition of SFLT. In our population of 123,406 pregnancies, the DR of PE at <32, <37 and ≥37 weeks with the ACOG recommendations was 91%, 90% and 91%, respectively, but at a screen positive rate of 67%.

It was concluded that the performance of screening for PE by maternal factors and biomarkers in the mid-trimester is superior to taking a medical history.

STUDY 4

The objective of this study is to explore the possibility of carrying out routine screening for preterm-PE by maternal factors and MAP in all pregnancies and reserving UTPI and PlGF measurements only for a subgroup of the population selected on the basis of the risk derived from screening by maternal factors and MAP alone at 11-13 or 19-24 weeks' gestation.

The data for the study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at 11-13 and / or 19-24 weeks' gestation. Bayes theorem was used to derive the *a priori* risk for preterm-PE from maternal factors and MAP. The posterior risk was obtained by the addition of UTPI and PLGF. We estimated the detection rate (DR) of preterm-PE, at an overall false positive rate (FPR) of 10%, from a policy in which first-stage screening by a combination of maternal factors and MAP defines a screen-positive, screen-negative and intermediate-risk group, which then undergoes second-stage screening with UTPI and PLGF. We estimated the detection rate (DR) of preterm-PE, at an overall false positive rate (FPR) of

10%, from a policy in which first-stage screening by a combination of maternal factors and MAP defines a screen-positive selecting a combination of maternal factors and MAP defines a screen-positive, screen-negative and intermediate risk group, which then undergoes second stage screening with UTPI and PLGF.

At 11-13 weeks' gestation, the model-based DR of preterm-PE, at 10% FPR, in screening the whole population by maternal factors, MAP, UTPI and PLGF was 74%. A similar DR was achieved by two-stage screening with maternal factors and MAP in the first-stage and reserving measurements of UTPI and PLGF for the second-stage to only 50% of the population. If second-stage screening was offered to 30% of the population, there would be only a small reduction in DR from 74% to 71%. At 19-24 weeks, the model-based DR of preterm-PE, at 10% FPR, in screening the whole population by maternal factors, MAP, UTPI and PLGF was 84%. A similar DR was achieved by two-stage screening with measurements of UTPI and PLGF in only 80% of the population; if second-stage screening was offered to 40% of the population, the DR would be reduced from 84% to 81%.

It was concluded that high DR of preterm-PE can be achieved by two-stage screening in the first- and second-trimester with maternal factors and MAP in the whole population and measurements of UTPI and PLGF in only some of the pregnancies.

3.2 ESPAŃOL

ESTUDIO 1

Es un estudio de cribado de preeclampsia (PE) en embarazos únicos entre las 11-13 y las 20-24 semanas de gestación cuyos objetivos fueron: primero, evaluar las características maternas que afectan la presión arterial media en embarazos normales y segundo, comparar el desempeño del cribado de preeclampsia usando presión arterial media (PAM) en el primer y segundo trimestre del embarazo.

La presión arterial media (PAM) fue medida entre las 11-13 y las 20-24 semanas de gestación en 17.383 embarazos únicos, incluyendo 70 pacientes con preeclampsia temprana requiriendo parto antes de las 34 semanas de gestación, 143 con parto prétermino requiriendo parto antes de las 37 semanas de gestación y 537, el total de preeclampsia. La PAM fue expresada como múltiplo de la mediana (MoM) después de ajustarse por características maternas y corregirse por los resultados adversos del embarazo. Se evaluó el rendimiento del cribado de PE por características maternas y la PAM expresada en MoM a las 11-13 semanas (PAM-1), PAM expresada en MoM a las 20-24 semanas (PAM-2) y su combinación.

En embarazos normales, la PAM fue afectada por características maternas e historia médica. En ambos, a las 11-13 semanas y a las 20-24 semanas la PAM disminuyó con la edad gestacional y la altura e incrementó con el peso materno. La PAM fue más alta en pacientes con hipertensión arterial crónica y en aquellas con historia personal o familiar de preeclampsia y más baja en mujeres afro caribeñas, fumadoras y multíparas sin historia previa de preeclampsia.

En el grupo de preeclampsia, comparado con el grupo normal, la mediana de la PAM-1 y la PAM-2 estaban significativamente aumentadas y hubo una asociación inversa significativa entre la edad gestacional al parto con la PAM-1 y la PAM-2. En cribado por características maternas y PAM-1, a una tasa de falsos positivos (TFP) del 10%, la tasa de detección (TD) para preeclampsia

temprana, PE pretérmino y el total de PE fue 74,3%, 62,9% y 49,3% respectivamente; la tasa de detección con una tasa de falsos positivos (TFP) del 5% fue 52,9%, 42,7% y 35,8%. En cribado por PAM-1 y PAM-2 la tasa de detección (TD), con TFP del 10%, fue 84,3%, 65,7% y 52,5%; la TD con tasa de falsos positivos del 5% fue 60,0%, 49,7% y 37,6%, respectivamente.

Se concluyó que la medida de PAM tiene que ser ajustada por características maternas e historia médica y debe ser expresada en MoM antes de hacer comparaciones válidas entre embarazos normales y patológicos. El rendimiento del cribado para PE por PAM expresado en MoM es mejor cuando se toman ambas medidas, a las 11-13 y 20-24 semanas que cuando se toma uno de los dos rangos de edad gestacional.

ESTUDIO 2

Los objetivos de este estudio de cribado son primero, determinar las características maternas que afectan el índice de pulsatilidad de la arteria uterina (IPAU) en embarazos normales a las 20-24 semanas de gestación y segundo, examinar en los embarazos con preeclampsia (PE) la relación entre los múltiplos de la mediana (MoM) de la arteria uterina y la severidad de la enfermedad, definida por la edad gestacional al parto y la presencia de restricción del crecimiento fetal.

La población de estudio son mujeres con embarazos únicos que fueron a ultrasonido de rutina entre las 20-24 semanas, las cuales fueron precedidas por un cribado combinado de aneuploidías entre las 11-13 semanas de gestación entre 2006 y 2013 en tres hospitales de Londres y sus alrededores. El índice de pulsatilidad de la arterias uterinas fue medido entre las 20-24 semanas de gestación en 50.490 embarazos únicos, incluyendo 1442 (2.9%) que desarrollaron preeclampsia. El índice de pulsatilidad de la arteria uterina fue expresado como MoM después de ajustarse por características maternas y corregirse por los resultados adversos del embarazo. En preeclampsia, la correlación entre el índice de pulsatilidad de la arteria uterina en MoM con la

edad gestacional al parto y el peso al nacimiento expresado en z score fue determinado.

En el grupo normal, hubo contribuciones independientes significativas al índice de pulsatilidad de la arteria uterina al momento del cribado, el origen racial, la historia previa del PE y/o pequeños para la edad gestacional (PEG). En el grupo de PE, hubo una asociación significativa inversa entre el índice de pulsatilidad de la arteria uterina MoM y la edad gestacional al momento del parto y el peso al nacer expresado en Z-score (P<0,0001). En 295 (20.5%) de 1442 casos de PE se encontró PEG y la incidencia de PEG fue inversamente relacionada a la edad gestacional al momento del parto disminuyendo desde 46.8% antes de las 34 semanas hasta 28.5% a las 34-37 semanas y 10.6% ≥ 38 semanas. El IP de la arteria uterina estuvo por encima del percentil 95 (1.509 MoM) en el 72.7%, 36,1% y 14,9% de los casos de PE requiriendo parto antes de las 34, 34-37 y ≥ 38 semanas respectivamente. Los porcentajes de PE con PEG fueron 80.2%, 55.6% y 37,4%.

Se concluyó que en el embarazo normal, el IP de la arteria uterina se ve afectado por las características maternas. En mujeres que desarrollaron PE, el IP de la arteria uterina entre las 20-24 semanas de gestación está incrementado y el incremento es particularmente marcado en aquellos con PE temprana y en PE con PEG.

ESTUDIO 3

El objetivo de este estudio fue desarrollar un modelo para PE basado en la combinación de factores maternos con biomarcadores en el segundo trimestre.

Los datos de este estudio se obtuvieron a partir de un cribado prospectivo de resultados obstétricos adversos en mujeres que acuden a su visita de rutina al hospital entre 19 y 24 semanas de gestación en tres hospitales de maternidad en Inglaterra entre enero del 2006 y julio del 2014. Teníamos datos de factores maternos, índice de pulsatilidad de la arteria uterina (IPAU), presión arterial media (PAM), factor de crecimiento placentario sérico y tirosina quinasa-1 sérica en 123.406, 67.605, 31.120, 10.828 y 8.079 embarazos

respectivamente. El teorema de Bayes se utilizó para combinar el riesgo a priori de los factores maternos con varias combinaciones de los múltiplos de la mediana (MoM) de biomarcadores. Se estimó el rendimiento del modelo de detección de PE requiriendo parto antes de las 32, 37 y mayor a las 37 semanas de gestación. El modelo de cribado de preeclampsia se comparó con el modelo empírico el cual se deriva de una validación cruzada. También se examinó el rendimiento del cribado basado en los factores de riesgo a partir de la historia clínica, según lo recomendado por el ACOG.

En los embarazos que desarrollaron PE, los valores de PAM, IPAU y Factor de tirosin quinasa 1 estaban incrementados y el factor de crecimiento placentario disminuido. Para todos los biomarcadores la desviación de lo normal fue mayor para preeclampsia temprana que para PE tardía y por lo tanto el rendimiento del cribado fue inversamente relacionado a la edad gestacional a la cual el parto se hizo necesario por indicaciones maternas y/o fetales. El cribado por factores maternos tuvo una predicción del 52%, 47% y 37% para PE <32, <37 y > 37 semanas de gestación respectivamente, a una tasa de falsos positivos del 10%. Los valores respectivos para el cribado combinado con factores maternos, PAM, IPAU y factor de crecimiento placentario fueron 99%, 85% y 46%; el rendimiento no se mejoró con la adición del factor de tirosinquinasa 1. En nuestra población de 123,406 embarazos, la tasa de detección de PE <32, <37 y >37 semanas con las recomendaciones de la ACOG fue del 91%, 90% y 91%, respectivamente, pero a una tasa positiva de pantalla del 67%.

Se concluyó que el rendimiento del cribado de PE por factores maternos y biomarcadores en la mitad del trimestre es superior que la historia medica.

ESTUDIO 4

El objetivo de este estudio es explorar la posibilidad de realizar un cribado de rutina para preeclampsia temprana por factores maternos y PAM en todos los embarazos y reservar medidas del índice de pulsatilidad de la arteria uterina y factor de crecimiento placentario solo para un subgrupo de población

seleccionado con base en el riesgo derivado del cribado por factores maternos y PAM ente las 11-13 o 19-24 semanas de gestación.
Los datos de este estudio se obtuvieron a partir de un cribado prospectivo de resultados obstétricos adversos en mujeres que acuden a su visita de rutina al hospital entre las 11 y 13 semanas y/o 19 y 24 semanas de gestación. El teorema de Bayes se utilizó para calcular el riesgo a priori de preeclampsia temprana a partir de factores maternos y PAM. El riesgo posterior se obtuvo adicionando el índice de pulsatilidad de la arteria uterina y el factor de crecimiento placentario. Nosotros estimamos la tasa de detección de preeclampsia temprana con una tasa de falsos positivos del 10% a partir de un protocolo en el cual la primera fase del cribado se realiza con una combinación de factores maternos y PAM y define un cribado positivo, cribado negativo y un grupo de riesgo intermedio, el cual posteriormente va a una segunda fase de cribado con índice de pulsatilidad de la arteria uterina y el factor de crecimiento placentario.

Entre las 11 y 13 semanas de gestación, el modelo estimó la tasa de detección de preeclampsia temprana con una tasa de falsos positivos del 10%. En el cribado de toda la población por factores maternos, PAM, IPAU y factor de crecimiento placentario la tasa de detección fue de un 74%. Una tasa de detección similar se obtuvo con una segunda etapa de cribado incluyendo factores maternos y PAM en la primera etapa y reservando el IPAU y el factor de crecimiento placentario para solo el 50% de la población. Si la segunda etapa de cribado fuera ofrecida al 30% de la población habria solo una pequeña reducción en la tasa de detección del 74% al 71%. Entre las 19 y 24 semanas de gestación, el modelo estimó la tasa de detección de preeclampsia temprana con una tasa de falsos positivos del 10%. En el cribado de toda la población por factores maternos, PAM, IPAU y factor de crecimiento placentario la tasa de detección fue de un 84%. Una tasa de detección similar se obtuvo con una segunda etapa de cribado incluyendo IPAU y el factor de crecimiento placentario en solo el 80% de la población. Si la segunda etapa de cribado fuera ofrecida al 40% de la población habría solo una pequeña reducción en la tasa de detección del 84% al 81%.

Se concluyó que la alta tasa de detección de preeclampsia temprana se puede lograr mediante un cribado de dos etapas en el primer y segundo trimestre con factores maternos y PAM en toda la población y mediciones de IPAU y factor de crecimiento placentario sólo en algunos de los embarazos.

CHAPTER 4 DISCUSSION

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The studies in this thesis have to a large extent fulfilled the aims as outlined in chapter 1.5.

Prediction of preeclampsia by mean arterial pressure at 11-13 and 20-24 weeks' gestation

This prospective study in 17,383 singleton pregnancies having screening for PE in their routine first and second ultrasound scan by measurement of MAP, provided data on the maternal characteristics that affect MAP in normal pregnancies and compared the performance of screening for PE by MAP in the first and second trimesters of pregnancy.

In normal singleton pregnancies, MAP is affected by maternal characteristics and medical history. At both 11-13 and 20-24 weeks' gestation, MAP decreases with gestational age and height, increases with maternal weight, it is higher in women with chronic hypertension and in those with a personal or family history of PE and lower in women of Afro-Caribbean racial origin, in smokers and in parous women with no previous PE. Consequently, the measured MAP must be adjusted for these variables and expressed as a MoM before valid comparisons can be carried out between normal and pathological pregnancies.

In pregnancies that develop PE, MAP MoM at 11-13 and 20-24 weeks' gestation is higher than in normal pregnancies and the increase is inversely related to the gestational age at delivery. We used a survival time model in screening for PE by a combination of maternal characteristics and history with MAP. In this model the gestation at the time of delivery for PE, for maternal and or fetal indications, is treated as a continuous rather than a categorical variable.

The study has also highlighted that in screening by maternal characteristics and MAP-1, at FPR of 10%, the DR of early-PE, preterm-PE and total-PE were

74%, 63% and 49%, respectively, whereas in screening by MAP-1 and MAP-2 the DR, at FPR of 10%, were 84%, 66% and 53%.

A potential limitation of the study is that in previous studies we combined data from maternal characteristics and history with the measurements of MAP, UTPI and maternal serum PLGF and PAPP-A at 11-13 weeks' gestation to establish an algorithm for effective screening for PE. This study was limited to defining the factors affecting MAP, describing, the relation of MAP MoM with gestation at birth in pregnancies complicated by PE, and examining the performance of screening by maternal characteristics and history with MAP at 11-13 and 20-24 weeks. The development of an algorithm combining MAP with other biomarkers will be the subject of future studies.

Our findings that the performance of screening for PE by MAP at 11-13 and 20-24 weeks was similar, is compatible with the results of previous longitudinal studies which reported that in pregnancies developing PE the MAP was increased from the first trimester and the deviation from normal increased only after 31 weeks (Moutquin *et al.,* 1985; Villar *et al.,* 1989). The performance of screening is contradictory with our results with DRs of 8-93% and FPRs of 2-55%, as a consequence of the varied methods in selection of the screened population, measurement of blood pressure, cut-offs used in defining the screen positive group and definitions of PE. The sample size ranged from 80 to 2,582, the incidence of PE was 3-53% and MAP was measured by either mercury sphygmomanometers or different types of automated devices at a wide range of gestations between 5 and 40 weeks (Oney *et al*., 1983; Villar *et al*., 1989; Conde-Agudelo *et al*., 1993; Higgins *et al*., 1997; Iwasaki *et al*., 2002)

In our study we used a standardized approach for measurement of MAP in a large number of pregnant women during two hospital visits at which an ultrasound examination is carried out routinely, adjusted the measured MAP to correct for maternal characteristics, used the definition of PE proposed by the international society for the study of hypertension in pregnancy (ISSHP) and reported the relation between MAP MoM and gestational age at delivery for PE, rather than erroneously considering the disease as being homogeneous across all gestational ages.

An important clinical implication of the data is that screening for PE at 11-13 weeks defines the high-risk group which could benefit from prophylactic treatment with low-dose aspirin and identifies the group that needs closer surveillance of the maternal and fetal condition and thereby defines the best time for delivery.

Prediction of preeclampsia by uterine artery Doppler at 20-24 weeks' gestation

This study involved the measurement of UTPI at 20–24 weeks' gestation in 50,490 singleton pregnancies, including 1,442 (2.9%) that developed PE. The objectives of the study were to determine maternal characteristics affecting UTPI in normal pregnancies and examine in pregnancies with PE the relation between UTPI MoM and severity of disease.

The finding of the study demonstrates that in normal singleton pregnancies at 20-24 weeks' gestation, uterine artery PI decreases with gestational age, is higher in women of Afro-Caribbean racial origin than in Caucasians, it is decreased in South and East Asians and it is increased in multiparous women who developed PE and / or delivered SGA neonates in previous pregnancies. Consequently, adjustments should be made for these maternal characteristics before valid comparisons can be carried out between normal and pathological pregnancies.

In women that developed PE, UTPI was increased and the increase was particularly marked in those with early-PE and in PE wth SGA. The uterine artery PI MoM was above the 95th percentile in about 73%, 36% and 15% of cases of PE requiring delivery at <34, 34-37 and $\geq$38 weeks, respectively. The percentages for PE with SGA were 80%, 56% and 37% and for PE without SGA were 66%, 28% and 12%. These findings are compatible with the results of previous Doppler studies (Wright *et al*., 2012; Akolekar *et al*., 2013; Yu *et al*., 2008 Lai *et al.*, 2013; Pedrosa *et al.*, 2011; Papageorghiou *et al*., 2002) and pathological studies which reported that the prevalence of placental lesions in women with PE is inversely related to the gestational age at delivery (Moldenhauer *et al*., 2003; Sebire *et al* 2005; Egbor *et al*.,2006). The findings

are also important in relation to the objectives of screening because there is evolving evidence that the incidence of adverse fetal and maternal short-term and long-term consequences of PE is inversely related to the gestational age at onset of the disease (Witlin *et al*., 2000; Irgens *et al*., 2001; Von Dadelszen *et al*., 2000). Consequently, the end-point in screening for PE should not be total disease but rather severe disease, reflected in the need for early delivery and the association with fetal growth restriction.

In our initial studies aiming to capture this gestational age related severity of disease we subdivided the condition into early-PE and late-PE. However, such subdivision could lead to the erroneous conclusion that early-PE and late-PE are different diseases with different biomarker profiles. As demonstrated by the MoM values of uterine artery PI in pregnancies with PE the distribution with gestational age is not bimodal. 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. We have therefore proposed a new approach in screening for PE, which is based on a survival time model and the gestation at the time of delivery for PE is treated as a continuous rather than a categorical variable (Wright *et al*., 2012; Akolekar *et al*., 2013). We are now developing a model in which both the gestation at delivery and the coincidence with SGA are treated as a categorical variable.

The current approach to prenatal care, which involves visits at 16, 24, 28, 30, 32, 34, 36 weeks' gestation and then weekly until delivery, was established more than 80 years ago. The high concentration of visits in the third-trimester implies that firstly, most complications occur at this late stage of pregnancy and secondly, most adverse outcomes are unpredictable during the first- or the second-trimester. Extensive research in the last 20 years has shown that many pregnancy complications, including PE, can now be predicted at an integrated first hospital visit at 11-13 weeks combining data from maternal characteristics and history with findings of biophysical and biochemical tests. It is therefore proposed that the traditional pyramid of care should be inverted with the main emphasis placed in the first- rather than the third-trimester of pregnancy (Nicolaides et al., 2011). Early estimation of patient-specific risks for pregnancy

complications would improve pregnancy outcome by shifting prenatal 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.

The value of early screening for PE is derived from the evidence that the prophylactic use of low-dose aspirin can result in a major reduction in the prevalence of preterm PE and the associated perinatal mortality, provided the onset of treatment is before rather than after 16 weeks' gestation (Bujold *et al.,* 2010 Roberge *et al.*, 2012 Roberge *et al*., 2013).

In the context of the new pyramid of pregnancy care (Nicolaides et al., 2011), the value of a clinic at 20-24 weeks is to modify the individual patient and disease-specific estimated risk from the initial assessment at 11-13 weeks and to provide risks for those women who did not have prior screening. In the high-risk group, intensive maternal monitoring for earlier diagnosis of PE and fetal growth restriction could improve outcome by selecting the best time and place for delivery. At present there is no useful pharmacological intervention after 16 weeks that can reduce the prevalence or modify the severity of the disease, but identification of the high-risk group would form the basis of future research that could achieve these objectives.

Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19-24 weeks' gestation

The objective of this study was to develop a model for PE based on a combination of maternal factors with second-trimester biomarkers. The data for the study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at 19-24 weeks' gestation in three maternity hospitals in England. We had data from maternal factors, UTPI, MAP, serum PLGF and serum SFLT from 123,406, 67,605, 31,120, 10,828 and 8,079 pregnancies, respectively. Bayes theorem was used to combine the *a priori* risk from maternal factors with various combinations of biomarker MoM values. The modeled performance of screening for PE requiring delivery at <32, <37 and $\geq$37 weeks' gestation was estimated. The modeled performance was compared to the empirical one which was derived from five-fold cross validation. We also examined the performance of screening

based on risk factors from the medical history, as recommended by ACOG.

In pregnancies that developed PE, the values of MAP, UTPI and SFLT were increased and PLGF was decreased. For all biomarkers the deviation from normal was greater for early than late PE and therefore the performance of screening was inversely related to the gestational age at which delivery become necessary for maternal and or fetal indications. Screening by maternal factors predicted 52%, 47% and 37% of PE at <32, <37 and ≥37 weeks' gestation, respectively, at FPR of 10%. The respective values for combined screening with maternal factors, MAP, UTPI and PLGF were 99%, 85% and 46%; the performance was not improved by the addition of SFLT. In our population of 123,406 pregnancies, the DR of PE at <32, <37 and ≥37 weeks with the ACOG recommendations was 91%, 90% and 91%, respectively, but at a screen positive rate of 67%.

In the application of Bayes theorem, the maternal factor derived *prior* risk has a strong influence on the posterior risk and therefore the performance of screening. The study has highlighted that in screening for PE the FPR and DR 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. Although the risk of PE is higher in nulliparous than parous women, the contribution of the latter group to PE should not be underestimated because 38% of cases of PE were from parous women, including 13% from parous women with history of PE in a previous pregnancy and 25% from parous women without a history of PE. In all groups, after combined screening, the risk of being affected given a screen positive result was considerably increased and if the screen result was negative the risk was considerably reduced.

The strengths of the study are first, examination of a large population of pregnant women attending for routine care in a gestational age range which is widely used for assessment of fetal anatomy and growth, second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE and use of multivariable logistic model to define the *prior* risk, third, use of a specific methodology and appropriately trained doctors

to measure UTPI and MAP, fourth, use of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of PLGF and SFLT, fifth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements, and sixth, use of Bayes theorem to combine the *prior* risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

A limitation of the study is that some of the findings rely on modeling which introduces optimistic bias. We have used cross validation on the empirical data which reduces such bias and demonstrated that the modeled and empirical performance were similar.

Screening and diagnosis of PE is traditionally based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second- or third-trimester of pregnancy. In a proposed new pyramid of pregnancy care, an integrated clinic at 22 weeks' gestation, in which biophysical and biochemical markers are combined with maternal factors, aims to estimate the patient-specific risk of developing PE and on the basis of such risk define the subsequent management of pregnancy, including the timing and content of subsequent visits. The objective would be to minimize adverse perinatal events for those that develop PE by determining the appropriate time and place for delivery.

We found that the performance of second-trimester screening for PE is good for preterm-PE but poor for term-PE. We assume that the performance of screening for term-PE would be better if assessment is undertaken at 36, rather than 22 weeks. A previous screening study in the third-trimester by a combination of maternal factors, MAP, UTPI, PLGF and SFLT demonstrated a high performance in the prediction of PE within six weeks of screening but poor performance for PE developing beyond this interval (Garcia et al., 2015). Since the majority of cases of PE occurr at term it may be necessary that all pregnancies are reassessed at 36 weeks. In this context, the main value of the 22 weeks assessment is to identify first, the high-risk group for development of early-PE which would then require close monitoring for development of high

blood pressure and proteinuria at 24-32 weeks and second, the high-risk group for preterm-PE which would require reassessment at around 32 weeks and on the basis of such assessment stratified into a high-risk group in need of close monitoring at 32-36 weeks and a low-risk group that would be reassessed at 36 weeks.

Performance of screening for PE by our method is by far superior to those recommended by ACOG or NICE. Use of a multivariable logistic model to define the *prior* risk attributes the appropriate relative importance to each maternal factor and allows estimation of the patient-specific risk of PE requiring delivery before a specified gestation. The *prior* risk can then be adjusted according to the results of biophysical and biochemical testing. The software for such estimation of *prior* and adjusted risk is freely available (Fetal Medicine Foundation website).

Recording maternal history and measurement of blood pressure are universally carried out as part of routine pregnancy care; measurement of MAP requires adherence to a protocol, but can be undertaken by healthcare assistants after minimal training, with the use of inexpensive equipment and takes a few minutes to perform. In contrast, measurement of UTPI requires specific training by sonographers and quality assurance of their results; nevertheless, this test can be undertaken within a few minutes by the same sonographers and machines as part of the routine second-trimester scan. Measurement of serum PLGF can be undertaken on the same machines as for free ß-hCG and PAPP-A, which are widely used in screening for Down syndrome, but there is an inevitable increase in cost. The study provides data on performance of screening for any combinations of the biomarkers. Ultimately, the choice of test for screening will depend not only on the basis of performance, but also the feasibility of implementation and health economic considerations.

Contingent screening for preterm preeclampsia

Effective screening for preeclampsia resulting in delivery at <37 weeks' gestation (preterm-PE) is provided by a combination of maternal factors, MAP, UTPI and serum PLGF at 11-13 or 19-24 weeks' gestation. This study explores

the possibility of carrying out routine screening for preterm-PE by maternal factors and MAP in all pregnancies and reserving measurements of UTPI and PLGF only for a subgroup of the population selected on the basis of the risk derived from screening by maternal factors and MAP alone.

The data for the study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at 11-13 and / or 19-24 weeks' gestation. Bayes theorem was used to derive the *a priori* risk for preterm-PE from maternal factors and MAP. The posterior risk was obtained by the addition of UTPI and PLGF. We estimated the DR of preterm-PE, at an overall FPR of 10%, from a policy in which first-stage screening by a combination of maternal factors and MAP defines a screen-positive, screen-negative and intermediate-risk group, which then undergoes second-stage screening with UTPI and PLGF.

The findings of this study demonstrate that in screening the whole population for preterm-PE at 11-13 or 19-24 weeks' gestation, by a combination of maternal factors, MAP, UTPI and PLGF, the DR at 10% FPR, is about 75% and 85%, respectively. A similar performance can be achieved by a two-stage strategy with maternal factors and MAP for the whole population in the first-stage and reserving measurements of UTPI and PLGF for the second-stage to only some of the population; 50% at 11-13 weeks and 80% at 19-24 weeks. Further reductions in the proportion of the population undergoing second-stage screening to 30% at 11-13 weeks and 40% at 19-24 weeks would result in only a small decrease in DR.

We propose a methodology and provide data on the estimated overall DR based on the proportion of the population selected for second-stage screening and this can form the basis for heath economic evaluations that would define the most appropriate strategy for different healthcare systems.

The strength of this study relies on the basic principle that first-stage screening identifies a group that is at such high-risk and another that is at such low-risk that further testing with additional biomarkers is unlikely to change their classification from screen-positive and screen-negative, respectively. Second-

stage testing is restricted to an intermediate-risk group for which additional measurements are likely to make a difference into their final screening result. The first-stage uses maternal factors and MAP; taking a medical history and recording blood pressure are an integral part of routine antenatal care. The second-stage uses UTPI and PLGF; measures that incur additional costs or require specialist expertise or equipment.

Previous studies have demonstrated that contingent strategies provide a cost effective way of screening for Down syndrome; the performance of screening by a combination of first-trimester fetal nuchal translucency (NT) and first- and second-trimester serum biochemistry in all pregnancies, as in the integrated test, is similar to contingent screening in which second-trimester testing is carried out in only about 25% of the population, identified by first-trimester screening as being at intermediate-risk (Wright *et al.,* 2004; Cuckle *et al.,* 2008)

A limitation of the study is that although large datasets of prospectively examined patients undergoing routine pregnancy care in the first- and / or second-trimester were used, the performance of screening was estimated from modeling. Prospective evaluation is required to confirm the results, after appropriate adjustments for the distribution of maternal factors in the study populations.

In the USA, the ACOG recommends that the best and only approach to screening for PE should be taking a medical history to evaluate for the following risk factors: nulliparity, age >40 years, body mass index $\geq$30 kg/m^2, conception by *in vitro* fertilization, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus and systemic lupus erythematosus or thrombophilia. However, the performance of such strategy in screening for preterm-PE is very poor, with DR of 90% but FPR of 67%. In the UK, the National Institute for Health and Clinical Excellence (NICE) recommends that women should be considered to be at high-risk of developing PE if they have any one high-risk factor (hypertensive disease in previous pregnancy, chronic hypertension, chronic renal disease, diabetes mellitus or autoimmune disease) or any two moderate-risk factors (nulliparity, age $\geq$40 years, body mass index $\geq$35 kg/m^2, inter-pregnancy interval >10 years

and family history of PE). However, the performance of such strategy in screening for preterm-PE is very poor with DR of 40% and FPR of 11%.

In our approach to screening, maternal factors are not treated as independent screening tests as advocated by ACOG and NICE, but rather they are combined into a multivariable logistic model, which attributes the appropriate value to each factor and takes into consideration their interrelations, to derive the individual patient-specific *a priori* risk. Bayes theorem is then used to combine the information on maternal factors with that from biomarkers to estimate the patient-specific *posterior* risk. We have shown that useful biomarkers in both the first and second-trimesters are MAP, UTPI and PLGF and when these are measured in all pregnancies the DR of preterm-PE, at 10% FPR, is about 75% and 85%, respectably. In this study we have shown that a similar high DR can be achieved with a two-stage strategy of screening, at substantially lower costs than with carrying out screening with all biomarkers in the whole population.

The software for implementation of this approach is freely available (https://fetalmedicine.org/calculator/preeclampsia).

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