Assessment and stratification of women with hypertension in the second half of pregnancy: a clinical, biochemical, economical and outcome evaluation

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Assessment and stratification of women with hypertension in the second half of pregnancy: a clinical, biochemical, economical and outcome evaluation

The PELICAN Study

Pre-EcLampsia: Clinical Application of PIGF

Dr Susan Duckworth

Student number: 0400515

A thesis submitted to the University of London for the degree of Medical Doctorate
**Dedication**

Throughout this project I learnt more about pregnancy and the hypertensive diseases associated with it; the physiology, the pathology, the potential. I had the privilege of working with clinical specialists and scientists, as well as many women experiencing pregnancy but I also became a mother myself.

This thesis is dedicated to my own mother; my role model, my strength and my best friend.

‘Motherhood: all love begins and ends there’ Robert Browning
Abstract

Pre-eclampsia is a disease unique to pregnancy. Prevalence in the UK is between 5-8% of pregnancies yet diagnosis remains challenging. The PELICAN study was a multi-centre, observational cohort study. The primary aim was to evaluate the diagnostic accuracy of plasma placental growth factor (PlGF) in the second half of pregnancy, in predicting the need for delivery for pre-eclampsia within 14 days of testing. 649 women presenting with suspected pre-eclampsia were recruited between January 2011 and February 2012, across seven consultant-led units within England and Ireland. Blood samples were taken at enrolment; PlGF measurements were performed but results blinded until the study was complete and diagnoses and pregnancy outcome known. A further 47 biomarkers were measured (using 57 assays) to evaluate whether the diagnostic potential of PlGF could be improved further.

Using a pre-specified cut off of <5th centile, a low (>12pg/ml < 5th centile) or very low (<12pg/ml) PlGF concentration was shown to have high sensitivity (0.95 CI (0.89-0.99) in women <35 weeks’ gestation) to determine need for delivery within 14 days. When compared with other biologically plausible biomarkers, the area under the ROC curve for low or very low PlGF (0.87, standard error 0.03), was greater than all other commonly utilised tests either singly or in combination (range 0.58–0.76; p<0.001 for all comparisons). Data from 100 women were then used to perform a budget impact analysis. A hypothetical decision analytical model using data extracted from case note review and reference cost tariffs, suggested a mean cost saving associated with the PlGF test (in the PlGF plus management arm) of £35,087
(95% CI -£33,181 to -£36,992) per 1,000 women, equating to a saving of £582 (95% CI -£552 to -£613) per woman tested.

PIGF testing could be used to risk-stratify women with suspected pre-eclampsia with the aim of improving pregnancy outcome.
Acknowledgements

I would like to acknowledge the help and support of the doctors, midwives, laboratory and administrative staff at all seven hospital trusts involved in the PELICAN study. I would also like to express gratitude to all the women who participated in the study and helped reveal some promising data, as well as the staff at The Division of Women’s Health for the invaluable help and guidance they have provided over the last five years.

I also need to extend enormous thanks to my supervisors, Professor Andrew Shennan and Professor Lucy Chappell, for their support, patience and direction; they have been an inspiration throughout my time at the unit and I am so grateful for the opportunities and experiences they have offered me. I am indebted to Paul Seed, Rachael Hunter, Paul Sheard, Melanie Griffin, Annette Briley, Jenny Carter, Gulsena Eivazova and Professor Lucilla Poston for their expertise, teaching and time. I have learnt so much but also made some firm friendships and collected some happy memories.

Funding for this project was provided from Tommy’s Baby Charity and Alere. Without this investment, the project would not have taken place, so I would like to further thank the people involved with these organisations and the public who support them.
Declaration

Unless specifically stated in the text, all work described in this thesis is my own.

No part of this work has been previously accepted for, or is currently being submitted, in candidature for another degree.

Suzy Duckworth

July 2016
Trusts involved in the study:

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The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Department of Obstetrics and Gynaecology, University College Cork, Ireland
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CHAPTER 1

Introduction
1.1 Introduction

1.1.1 Synopsis

Hypertensive disorders of pregnancy affect around 15% of pregnant women (≈120,000 women per year in UK). (World Health Organization, 2013) Pre-eclampsia is characterised by hypertension and other features of multi-organ disease, including proteinuria, liver derangement or renal damage and complicates around 5% of all pregnancies. (Levine et al., 2004) Pre-eclampsia is associated with increased serious maternal morbidity and is the leading cause of iatrogenic preterm birth, responsible for substantial neonatal mortality and morbidity.

Current diagnosis and risk stratification of women presenting with suspected disease is subject to considerable test and observer error (Conde-Agudelo et al., 2004a). Recent improvements in understanding of the biological roles of placentally-derived angiogenic/anti-angiogenic factors in pre-eclampsia and fetal growth restriction has led to their development as potential diagnostic biomarkers, including at point of care, in suspected disease. The PELICAN study was designed to test the hypothesis that abnormally low PlGF concentrations are associated with pre-eclampsia requiring delivery within 14 days and the primary findings of that study are discussed in this thesis.

In chapter one, the need for improved clinical assessment tools is demonstrated by showing the scale of the problem and the impact of pre-eclampsia in both developed and low resource settings. Current definitions are set out and the underlying
pathophysiological theories are explored, to highlight the need for a point of care test based on disease aetiology. PlGF is compared with current surveillance tools, as well as other relevant biomarker targets, identified following an extensive literature review.

The PELICAN study further provided the opportunity to supplement data from the literature with actual resource use to calculate the cost of current practice and to model the savings if PLGF were used in management decisions. A proportion of these women also completed a patient questionnaire-style survey, with the aim of producing a Patient Reported Outcome Measure, to assist clinician awareness of the emotional and practical difficulties that the monitoring and uncertainty of this disease places on women and their families.

1.1.2 Pre-eclampsia: the scale of the problem

Pre-eclampsia complicates 3-8% of all pregnancies (Redman et al., 2014) and is a serious complication of the second half of pregnancy, labour and the early postnatal period. In high-income settings, it accounts for the majority of iatrogenic pre-term delivery (Steegers et al., 2010b) and between 2006 and 2009, 19 women died as a direct consequence of the disease in the UK. (Knight et al., 2014) Up to 15% of pregnant women present with gestational hypertension (Hall et al., 1980) with around a third of those going on to develop pre-eclampsia (Saudan et al., 1998). Once in the antenatal day unit for assessment, most women undergo a series of blood tests (creatinine, liver transaminases, uric acid and platelet count) along with fetal monitoring, to assess wellbeing and activity.
Pre-eclampsia remains a global health problem. Worldwide, pre-eclampsia is the second commonest cause of maternal mortality (Kassebaum et al., 2014) and an important cause of fetal growth restriction, accounting for a quarter of very low birth weight infants (Shennan et al., 2001). It has been estimated that hypertensive diseases of pregnancy are associated with about 20% of intrapartum and 10% of antepartum stillbirths and 6% of neonatal deaths. (Lawn et al., 2011) The sequelae of pre-eclampsia lead to approximately 60,000 maternal deaths per year (Stokowski, 2005) worldwide. In high-income countries, the risk of death is between 0% and 1.8% whereas, in low resource settings, that figure rises to 15%. (Staff et al., 2013a) In addition, more than three million neonatal deaths occur as a result. (Friberg et al., 2010). There is an obvious need for an accurate predictive test aligned with effective preventative treatments.

In the UK, diagnostic uncertainty and imperfect risk stratification leads to treatment delays or over-management and high costs for an already over-stretched health service. In addition to adverse outcomes, our current imperfect tests for evaluating women with suspected pre-eclampsia lead to repeated antenatal monitoring and in-patient admissions. This can lead to substantial emotional stress for the women involved, as well as placing a financial burden on pregnant women and their families because of hospital attendance. (Hadker et al., 2010) Pre-eclampsia is estimated to account for one-fifth of antenatal admissions and two-thirds of referrals to day assessment units. (Rosenberg and Twaddle, 1990) Improvements in confirmation of diagnosis have the
potential to improve clinical outcomes and significantly reduce costs, to women as well as the health service, by directing resources to women most at need.

1.2 Associated morbidity and mortality

1.2.1 Short-term fetal complications

Pre-eclampsia has the potential to cause life-limiting complications for mother, baby, or both. (Redman and Sargent, 2009) Hypertensive disorders of pregnancy are associated with poor outcomes. (Sibai, 2006) Early, intrauterine risks for the baby include poor growth and prematurity (Sibai, 2006), leading to infants being born with complications of prematurity or small for gestational age and contributing to an increased risk of perinatal death. As a result, in low and middle income countries, limited access to neonatal intensive care means mortality and morbidity is considerably higher than in settings where such facilities are available. (Duley, 2009)

With gestational age and weight at delivery being the most important predictors (Withagen et al., 2005) of adverse outcome for the baby, preterm delivery and growth restriction following pre-eclampsia are important causes of perinatal complications.

Hypertension (with/ without additional features of pre-eclampsia) is the leading single identifiable risk factor in pregnancy associated with stillbirth (one in five stillbirths in otherwise viable babies) (Centre for Maternal and Child Enquiries (CMACE), 2011). A multinational study exploring the impact of being born premature in low and middle income countries reported that the relative risk of neonatal mortality was 6.82 (CI 1.32 to 2.5). If the baby was also small for gestational age, the risk rose to 15.42 (CI
9.11 to 16.12). (Katz et al., 2013) Worldwide, pre-eclampsia is associated with a 10% perinatal and neonatal death rate, (Altman et al., 2002) most commonly due to premature delivery, carried out to preserve the life of the mother.

1.2.2 Short term maternal complications

Over half a million women die each year from pregnancy related causes, 99% of whom are in low and middle income countries. (Duley, 2009) The Millennium Development Goals (www.un.org) have prioritised maternal health within the struggle against poverty and inequality, yet 10% to 15% of direct maternal deaths are associated with preeclampsia and eclampsia. (Duley, 2009) Most deaths are attributable to eclampsia, a rare but important complication associated with approximately 1 in 2000 deliveries in Europe. (von Dadelszen et al., 2011a) Pre-eclampsia can affect the brain, kidneys, clotting system and liver, which can lead to haemolysis, elevated liver enzymes, low platelets (HELLP syndrome). Approximately 1% of women with HELLP syndrome will die in the perinatal period, even in high income settings (Sibai et al., 1993) and a greater proportion will experience life threatening sequelae, such as disseminated intravascular coagulopathy, acute renal failure, retinal detachment or pulmonary oedema.

1.2.3 Long term baby complications

Placental insufficiency (associated with pre-eclampsia), places the infant at increased risk of being born growth restricted, which has implications for long-term health. Not only has it been suggested to be the highest attributable cause of
stillbirth in high income countries (Lawn et al., 2011) but it is also associated with increased incidence of respiratory distress syndrome, intraventricular haemorrhage, necrotising enterocolitis (McIntire et al., 1999) and seizures (Bukowski et al., 2003) within the perinatal period. Disadvantage also extends into childhood and later life: a reported increased risk of cerebral palsy, by up to seven-fold, (Jacobsson et al., 2008) has been linked to placental disease, as well as lower intelligence, behavioural problems and poor social skills. (Gray et al., 1996) Several studies have also suggested a higher incidence of coronary heart disease and hypertension in adults who experienced early in utero placental deficiency or growth restriction. (Bonamy et al., 2011) (Huxley et al., 2000)

### 1.2.4 Long term maternal complications

Pre-eclampsia usually ends when the placenta is delivered but risk of long-term cardiovascular disease, perhaps due to a shared pathophysiology, persists in mothers. Approximately 20% of women with pre-eclampsia develop hypertension within seven years of their pregnancy, compared with only 2% of women following a normotensive pregnancy. (Nisell et al., 1995) A large, Norwegian study, following over 600,000 parents for a median of 13 years after delivery, found reduced survival in women who had been diagnosed with pre-eclampsia during their pregnancy (with no significant increase in mortality of fathers). Women who had pre-term deliveries with pre-eclampsia, had a 2.71 fold higher risk of death and this rose to 8.12 fold in those with a cardiovascular cause of death (CI 4.31 to 15.33). An increased risk of early mortality persisted in women who had a term pre-eclamptic delivery but was marginal in women with a healthy pre-term delivery. (Irgens et al., 2001) Studies in
other populations corroborate these findings that pre-eclampsia infers an increased risk of future cardiovascular disease and death in women, despite post-natal normotension. (Funai et al., 2005) Pre-eclampsia also predisposes women to develop end-stage renal disease in later life. (Vikse et al., 2006)

In summary, women with previous pre-eclampsia are more likely to develop cardiovascular disease later in life (Mosca et al., 2011) and this association is more profound in women who develop the pre-eclampsia at earlier gestations (Melchiorre et al., 2011). However, pre-eclampsia does not only increase the risk of pervasive physical ill health: the combination of experiencing a serious illness, possible emergency intervention or unplanned delivery of a premature or disabled child has obvious psychological consequences for mother, including depression, tocophobia and post-traumatic stress disorder. (van Pampus et al., 2004) A Dutch study, investigating women following pre-eclampsia and/or HELLP syndrome, found that psychological therapy (psycho-education, eye movement desensitisation, support techniques) improved coping strategies in this cohort. (Poel et al., 2009) The researchers affirm the importance of timely diagnosis and suggest that it can shorten duration of required therapeutic support.

1.2.5 Pre-eclampsia: historical context

The following account of pre-eclampsia at 24 weeks’ gestation, documented in 1914, suggests little progress in diagnosis and management over the last century. It is notable that the diagnosis of pre-eclampsia was made on the findings of hypertension and proteinuria in 1914 and that these remain the NICE criteria for
diagnosis in 2015, despite the considerable amount of research into the pathophysiology of the disorder.

Dr. Pocock, of Beaconsfield, who was called in, examined a specimen of her urine and found it to contain a very large quantity of albumin, together with a certain amount of blood. It is important to note that Dr. Beauchamp had examined the urine ten days previously and it was then normal.

The urine contained a good deal of blood and enough albumin to solidify on boiling, and the quantity passed per diem was much reduced.

On examination I found the uterus the size of a twenty-four weeks’ pregnancy, the cervix long and the os closed. We decided that in so acute a case of pregnancy intoxication evacuation of the uterus was the only course. Induction of premature labour either by bougies and Champetier’s bag, or by dilatation of the cervix with Hegar’s dilators followed by Champetier’s bag, was considered, but all of us agreed that it was fraught with the risk of an eclamptic seizure taking place before the child could be delivered. The alternative of immediate emptying of the uterus by operative means was all, therefore, that remained. Of the two methods of performing this, abdominal and vaginal Cæsarean section, the former was decided on as being cleaner, quicker, and having the least risk.

The uterus retracted well and the wounds in it and the parietes were closed in the usual way. An extraordinarily rapid improvement in the patient’s condition followed. By next day the jaundice and œdema had disappeared, the vomiting had ceased, 25½ oz. of urine were passed containing a much reduced amount of albumin and no blood, and the eyesight was in large measure restored.

Taken from Bonney MS, ‘A case of pre-eclampsia at the twenty-fourth week’

C section Proc Roy Soc Med 1914
1.3 Defining hypertensive disorders of pregnancy

Pre-eclampsia was first described over 2000 years ago; Hippocrates warned of the combination of headaches, heaviness and convulsions in pregnant women associated with a dry uterus. At that time, treatment strategies focussed on releasing excess ‘humor’ through a variety of mechanisms including purging and blood-letting. (Bell, 2010) Throughout the 19th century, more theories emerged as to the underlying pathophysiology, with physicians and scientists acknowledging the prodromal syndrome as distinct from the seizure activity of eclampsia. In the 1500s, Fallopius first described the female pelvic anatomy and identified the placenta was only found in the gravid uterus, (Bell, 2010) yet it took another century until eclampsia was described in the literature and primigravid women suggested to be at increased risk. Today, the exact mechanisms of disease remain elusive, yet the disease claims more than 60,000 maternal lives worldwide annually. (World Health Organization, 2013)

Classically, pre-eclampsia has been defined as new hypertension of over 140/90 mmHg and proteinuria >0.3g/24 hours after 20 weeks’ gestation. (National Institute for Health and Clinical Excellence, 2010) In recent years, concerns have been raised that this relatively narrow definition (in which only proteinuria is the additional feature required to make the diagnosis of pre-eclampsia) could lead to inadequacies in diagnosis and risk delayed intervention. (Brown et al., 2001) For the purposes of this thesis, we adopted the extended definition of pre-eclampsia (American College of Obstetricians and Gynaecologists (ACOG)/ International Society for the Study of Hypertension in Pregnancy (ISSHP)) in which other multi-organ features of the
disease (see below) may be utilised to make a pre-eclampsia diagnosis. (Brown et al., 2001) The international consensus has been to adopt these definitions, as they appear to reflect the clinical situation more closely:

**American College of Obstetricians and Gynaecologists' (ACOG) definition**

*Pre-eclampsia:* Raised BP (>140 systolic or 90 diastolic) after 20 weeks' gestation with proteinuria (>300mg over 24 hours) or any of the following features of severe pre-eclampsia:

- Severe hypertension (>160/>110)
- Thrombocytopenia
- Impaired liver function or persistent right upper quadrant or epigastric pain
- New renal insufficiency or doubling of creatinine in the absence of renal disease
- Pulmonary oedema
- New cerebral or visual disturbances

*Gestational hypertension:* Raised BP after 20 weeks' gestation in the absence of proteinuria or any features of severe pre-eclampsia

*Chronic hypertension:* Raised BP of any cause that pre-dates pregnancy
Chronic hypertension with superimposed pre-eclampsia: Chronic BP in association with pre-eclampsia

International Society for the Study of Hypertension in Pregnancy (ISSHP) definition

Pre-eclampsia: de novo hypertension in the presence of proteinuria (>30mg/mmol, >300mg/day, >2+ on dipstick) and/or maternal organ dysfunction (renal insufficiency with creatinine >90umol/l, liver derangement, neurological symptoms or haematological complications such as thrombocytopenia or haemolysis) and/or fetal growth restriction.

Gestational hypertension: de novo hypertension (>140/90mmHg) after 20 weeks’ gestation without proteinuria, other features of pre-eclampsia or evidence of utero-placental dysfunction (fetal growth restriction)

Chronic hypertension: hypertension that pre-dates pregnancy. Practically, this may include hypertension recorded during the first trimester.

Chronic hypertension with superimposed pre-eclampsia: Essential hypertension with one of the above features of pre-eclampsia

National Institute of Clinical Excellence
**Pre-eclampsia:** new hypertension presenting after 20 weeks’ gestation with significant proteinuria.

**Severe pre-eclampsia:** pre-eclampsia with severe hypertension and/or with symptoms, and/or biochemical and/or haematological impairment

**Gestational hypertension:** new hypertension presenting after 20 weeks without significant proteinuria

**Chronic hypertension:** hypertension present at the booking visit or before 20 weeks or if the woman is already taking anti-hypertensive medication when referred to maternity services. It can be primary or secondary.

### 1.3.1 Epidemiology of pre-eclampsia

Previous studies have reported that approximately half of multiparous women diagnosed with pre-eclampsia have a history of the disease. (Seed et al., 2011) Their risk doubles if the previous pre-eclampsia was early onset or required delivery before 32 weeks’ gestation. (Bramham et al., 2011) Nulliparity, maternal age over 40 years and obesity (>80kg in first trimester) (Duckitt and Harrington, 2005) are recognised risk factors, which may contribute to the secular increases in chronic hypertension, gestational hypertension and severe pre-eclampsia in recent years. (Ananth et al., 2013) Despite an increasing research focus on hypertensive disorders in pregnancy
over the last decade (including preventative strategies), pre-eclampsia rates have remained stable.

An overall decline in eclampsia in the western world in recent years (Hutcheon et al., 2011) likely results from improved antenatal awareness and use of prophylactic treatments (such as magnesium sulphate), highlighting the value of ongoing research in this area. A retrospective study of over 1200 women between 1931 and 1990 suggests a 90% decline in the incidence of eclampsia (particularly antenatal and intra-partum) over this period, which has also resulted in a drop in associated mortality: perinatal death has dropped and there were no eclampsia-related maternal deaths after 1964. (Leitch et al., 1997) A large prospective study of over 210,000 women diagnosed with either severe pre-eclampsia or eclampsia between 1999 and 2003 found only 82 women had eclamptic seizures, with no maternal deaths. Over half of the seizures occurred antenatally, prior to hospital presentation. (Tuffnell et al., 2005) This study supports the use of regional clinical management guidelines to prevent serious adverse outcome.

The figure below shows the rate of pre-eclampsia in the United States between 1980 and 2010; total cases and number of pregnancies with mild pre-eclampsia have remained stable over this period, but cases of severe pre-eclampsia have risen from 0.3% in 1980 to 1.4% in 2010. The prevalence has risen mainly in the youngest and oldest mothers.
1.3.2 Current management

1.3.2.1 Primary prevention

Low dose aspirin has been shown to reduce pre-eclampsia, stillbirth and serious adverse outcome. A meta-analysis of over 30,000 women recruited to 31 studies found that women taking anti-platelet therapy had a lower relative risk (RR 0.90 (95% CI 0.84–0.97)) of developing pre-eclampsia and of delivering prior to 34 weeks’ gestation (RR 0.90; 0.83–0.98). (Askie et al., 2007) This review also reported a 14% reduction in fetal or neonatal deaths (RR 0.86; 0.76 to 0.98).
In a contradictory manner, exercise and rest have both been associated with hypertensive disease outside of pregnancy. (van Duijnhoven et al., 2010) However, data relating to this association within pregnancy are sparse. A Cochrane review suggests that rest, for up to four hours a day, can reduce pre-eclampsia developing in normotensive, high risk women. (Duley et al., 2006) The same review concluded there was no clear direction as to appropriate dietary advice to protect against pre-eclampsia, including reduced salt intake or low fat diets.

1.3.2.2 Secondary prevention

The most recent Cochrane meta-analysis to assess the effects of antihypertensive drug treatments for women with mild to moderate hypertension during pregnancy concluded that ‘it remains unclear whether antihypertensive drug therapy for mild to moderate hypertension during pregnancy is worthwhile’, (Abalos et al., 2014) although there is consensus that antihypertensive medication should be given to women with severe hypertension to prevent ongoing high blood pressures. (Brown et al., 2000). It is unclear whether antihypertensive treatment reduces adverse outcome or makes a woman less likely to develop pre-eclampsia. The recent Control of Hypertension in Pregnancy Study (CHIPS) trial confirmed that ‘tight’ blood pressure control reduces episodes of severe hypertension without any adverse effects on fetal growth. (Magee et al., 2015).

Anticonvulsants are used with the aim of preventing eclamptic seizures. The MAGPIE Trial included data from over 10,000 women, with blood pressure >140/90
and >1+ proteinuria, who were randomised to either receive magnesium sulphate or placebo. Women who received magnesium had a 58% lower risk of suffering eclampsia. (Altman et al., 2002) Maternal morbidity was also lower in this cohort. Overall, the trial identified a halving in the risk of developing eclampsia, with improved maternal outcomes, without proven risk to the baby, leading to widespread use of magnesium sulphate in high risk women across the United Kingdom (and worldwide). These findings are summarised in the forest plot below.

**Figure 1.2: Interventions in the prevention of pre-eclampsia, taken from (Duley et al., 2006)**

![Forest plot showing interventions in the prevention of pre-eclampsia](image-url)
1.4 Pathophysiology of pre-eclampsia

Pre-eclampsia is a multi-factorial, multi-stage (Chaiworapongsa et al., 2013c) condition and its exact pathophysiology has been the subject of decades of research. We now have a better understanding of the disease syndrome and how this process can be used to develop improved diagnostic adjuncts.

Most theories now focus on the ischaemic placenta, which is considered to release bioactive factors into the maternal circulation in response to hypoxia. In turn this is followed by multi-organ clinical manifestations of the disease. The only curative action remains delivery of the placenta (irrespective of the fetus). In the early 1900s, animal studies demonstrated seizures and liver damage in guinea pigs injected with placental tissue from women who had died of eclampsia. (Young, 1914) These studies were later supported by evidence that reducing aortic blood flow to the uterus of pregnant dogs produced signs of pre-eclampsia, which resolved with removal of the aortic clamp. Interestingly, this phenomenon was not reproduced in non-pregnant dogs or dogs post-hysterectomy. (Chaiworapongsa et al., 2014a) This phenomenon is depicted in the below figure, taken from the paper by Chaiworapongsa and colleagues.
Figure 1.3: Schematic of effects of reducing uterine blood flow in pregnant and non-pregnant dogs: Clamping of the renal artery led to hypertension in dogs, whereas clamping of the aorta only led to hypertension in pregnant dogs: no hypertension was seen in dogs without a gravid uterus or those who had undergone hysterectomy. Taken from (Chaiworapongsa et al., 2014a)

1.4.1 Normal placental development

Effective placentation necessitates fetal villous invasion into the maternal uterine decidua. This process is supported by the trophoblast, which consists of two layers: the inner single nucleated cytotrophoblast and the outer multinucleated continuous syncytiotrophoblast that forms the interface between the developing fetal vessels and the maternal circulation. This takes place between 8 and 18 weeks’ gestation, resulting in cytotrophoblast remodelling of the maternal spiral arteries. (Ashton et al., 2005) This remodelling is aided by uterine natural killer cells and results in loss of maternal artery smooth muscle and collagen matrix and the development of new fibrinoid material, to allow sufficient maternal blood flow to the placenta and support
a developing fetus. (Redman and Sargent, 2009). Pressure and pulsatility are reduced to optimise maternal-fetal exchange. (Burton et al., 2009)

Haemodynamic changes occur in normal pregnancy: sodium and water retention increases plasma volume by nearly 50%, (Faupel-Badger et al., 2007) raising cardiac output and stroke volume. Despite this, in a healthy pregnancy, reduced peripheral vascular resistance means blood pressure drops by the first trimester and often remains lowered throughout (relative to the non-pregnant state). This process is reversed in pre-eclampsia; a generally vasoconstricted state leads to increased arterial blood pressure and low cardiac output. (Maynard and Karumanchi, 2011) The plasma-extended state is dependent on the normal functioning of maternal endothelium and immune interactions. Measuring blood pressure is a non-invasive screening test, used antenatally and during labour, to alert clinicians of the possibility of pre-eclampsia. However, Doppler studies suggest (Noori et al., 2010) that uterine vascular resistance is significantly raised before a measureable increase in blood pressure can be detected by the clinician.

### 1.4.2 Poor placentation

In pre-eclampsia, inadequate trophoblast invasion leads to diminished dilatation of spiral arteries and an inadequately perfused placenta. Smooth muscle cells persist within the spiral arteries (Lim et al., 1997) causing impaired blood flow and reduced maternal acceptance of the fetal trophoblast. (Redman et al., 2014) These poorly adapted spiral arteries may develop atherosclerosis, causing impaired blood flow
and thrombus formation (Staff et al., 2014) which further compromises placental blood supply and, therefore, impedes fetal growth. The figure below shows a healthy, dilated spiral artery, with trophoblasts extending into the myometrium, in comparison with a narrowed, poorly developed artery of pre-eclampsia, with inadequate trophoblast invasion.

Figure 1.4: Figure showing altered modelling of spiral arteries in pre-eclampsia
Taken from (Chaiworapongsas et al., 2014a)

Such disruption of flow leads to oxidative stress (Burton and Jauniaux, 2011) and a generalised systemic inflammatory response, causing abnormal cellular
development (Staff et al., 2013c) consistent with hypoxic injury. Reactive oxygen species can overwhelm mammalian natural defences. Resultant oxidative stress occurs due to increased production of superoxides in the presence of reduced antioxidants. (Chappell et al., 1999) Additional evidence of inadequate antioxidant defence in women with pre-eclampsia includes reduced blood mRNA encoding haem oxygenases, catalase and superoxide dismutase (antioxidant enzymes) in women with pre-eclampsia compared with healthy controls. (Nakamura et al., 2009)

An overview of the mechanism underlying the placental contribution to pre-eclampsia is shown in the figure below.

Figure 1.5: Overview of pathophysiology of pre-eclampsia (Whitley and Cartwright, 2010)
In summary, the placenta plays a vital role in the development of pre-eclampsia but the process is complex. The underlying pathophysiology takes place before the outward clinical features develop. In the first trimester, inadequate fetal villous invasion sets up compromised maternal-fetal blood flow and inadequate spiral artery remodelling. This leads to laminar flow, artery atherosclerosis and hypoxia/reperfusion injury. The combined effect is a poorly functioning placenta, an inflammatory oxidative stress response and systemic effects of pre-eclampsia. (Walker, 2000) Poor placental invasion can, however, exist in the absence of any features of pre-eclampsia, (Brosens, 2011) meaning additional factors must contribute to the development of the maternal syndrome. Indeed, inappropriate placentation and maladapted spiral arteries can be seen in women with placental abruption (Tikkanen, 2011) and fetal growth restriction. (Redline, 2008) This two stage process is demonstrated below:
1.4.3 Angiogenesis

As discussed above, the development of a healthy pregnancy requires adequate invasion of fetal vasculature into the spiral arteries, to set up an effective low resistance circulation. Angiogenesis is the term used to describe the development of new vessels and is an active and important part of this process. Pregnancy has been reported in a PIGF knock-out mouse experiment, implying pregnancy can continue in the absence of PIGF in the mouse model (Carmeliet et al., 2001) but its role in activating pro-angiogenic genes when it binds to the tyrosine kinase receptor VEGF-R1 (also known as soluble Flt-1) (Maynard and Karumanchi, 2011, Chaiworapongsa et al., 2011b) has been demonstrated to promote vasodilatation and vascular
permeability. This process is depicted below and the angiogenic factors are discussed in more detail later.

![Diagram of angiogenesis and lymphangiogenesis](image)

**Figure 1.7**: PIGF binds to VEGFR-1 to promote angiogenesis, Taken from (Tammela et al., 2005)

The pre-eclamptic placenta is now thought to secrete (although not exclusively) bioactive factors, such as PIGF, vascular endothelial growth factors, tyrosine kinases and other biological debris into the maternal circulation. The resulting imbalance of angiogenic and anti-angiogenic factors accounts for the widespread clinical manifestation of the disease. (Redman and Sargent, 2005) A growing body of
research has now targeted these markers as a possible aid to the risk stratification and classification of pre-eclampsia. A later table demonstrates the role of a variety of implicated biomarkers and their maternal blood concentrations in pre-eclampsia.

1.4.4 Inflammatory response to pre-eclampsia

In pre-eclampsia, the inflammatory changes associated with normal pregnancy, are exaggerated; pre-eclampsia is less distinct from normal pregnancy than from the non-pregnant state. (Johansen et al., 1999) In a healthy pregnancy, there is an immune reaction between maternal natural killer cells and the fetal cytotrophoblast, such that, by the second half of pregnancy, it is the main immune interface between mother and fetus. (Germain and Nelson-Piercy, 2006) The precise stimulus for this exaggerated inflammatory response remains unclear but theories include alloimmune interaction (Tan et al., 2008) or innate systemic inflammatory activation. Research in this area implies specific cytokine targets in the excessive inflammatory pathways linked to pre-eclampsia: TNF-α, IL-12, IL-18 and IFN-γ (Redman and Sargant, 2003) and suggests this inflammatory response increases with advancing gestation, (Brewster et al., 2008) possibly accounting for the incidence late onset pre-eclampsia without placental disease.

Successful pregnancy requires the maternal immune system to accept the trophoblast; trophoblastic HLA-C molecules, regulatory T cells and maternal natural killer cells have been implicated in this complex interaction. (Sargant et al., 2007) The uterine natural killer cells release cytokines that promote appropriate
placentation when they bind with HLA-C1 molecules. It has been proposed that inappropriate binding with HLA-C2 could influence a woman’s likelihood of developing pre-eclampsia. (Moffett and Hiby, 2007) The high resistance system and subsequent hypoxic conditions of the utero-placental bed of pre-eclamptic women places the cellular endoplasmic reticulum under stress and suspends protein folding (the ‘unfolded protein response’) (Chaiworapongsa et al., 2014a). If severe, this leads to trophoblast apoptosis and can cause a generalised intravascular inflammatory response.

1.4.5 Oxidative stress

Oxidative stress occurs when the reactive oxygen species overwhelm the body’s innate anti-oxidant defences. (Chaiworapongsa et al., 2014a) This process is relevant to the pathogenesis of pre-eclampsia because it generates an exaggerated release of pro-inflammatory mediators. Once in the maternal circulation, they trigger widespread cellular activation, which alters the concentrations of acute phase proteins seen in the pre-eclamptic mother. The outcome is maternal endothelial dysfunction. (Conway et al., 2009) In healthy humans, bilirubin and biliverdin (breakdown products converted from red blood cells by haem oxidases) provide antioxidant protection. Mice deficient in a haem oxidase isoform develop hypertension, small placentas and elevated sFlt-1. (Zhao et al., 2009)

1.4.6 Endothelial dysfunction
Maternal endothelial activation has been shown to be induced by syncytiotrophoblast microvesicles (Cockell and Poston, 1997) and other microparticles including neutrophils, (Mellembakken et al., 2001) angiotensin II receptor agonist antibodies (Herse et al., 2009) and angiogenic factors. (Levine et al., 2004) Endothelial dysfunction has been assessed by measuring flow in the brachial artery, which is impaired in women with pre-eclampsia compared with healthy controls, even before the onset of clinical features (Savvidou et al., 2003) especially in women with early-onset disease. (Noori et al., 2010)

1.5 Biomarkers of placental disease

Despite pre-eclampsia being well known as a ‘disease of the placenta’, evidence is emerging to suggest most of its damaging effects are due to maternal endothelial insult (Maynard and Karumanchi, 2011) and downstream organ damage of associated ischaemia. This has led to suggestions that circulating markers in the maternal blood could be responsible for the systemic effects of the disease. The International Programme on Chemical Safety, led by the World Health Organisation defined a biomarker as “any substance, structure or process that can be measured in bio specimens and may be associated with health-related outcomes.” (Strimbu and Tavel, 2010) By definition, they are an objective measure of a medical state that can be identified, recorded accurately and therefore, reproduced. Their use has now become commonplace in medical practice and they are an accepted endpoint in clinical trials, for a range of medical conditions.
Biomarkers relating to specific cellular and molecular events can inform of early biological mechanisms often prior to clinical manifestation of disease. Careful selection of appropriate biomarker, or panel of biomarkers, is required. Selection is usually based upon disease aetiology: complex diseases may require multiple biomarkers. Evidence has been emerging over the last decade as to clinically appropriate biomarkers for the diagnosis and prediction of adverse outcome in pre-eclampsia. This next section will discuss some of the main placentally-derived biomarkers that contribute to the studies later described in this thesis.

1.5.1 Placental Growth Factor (PIGF)

PIGF was first identified in 1991 and has four isoforms (PIGF 1-4) made up of amino acids. PIGF-2 and -4 have heparin binding domains, whereas PIGF-1 and -3 do not. (Hauser and Weich, 1993). PIGF is a potent angiogenic glycoprotein, secreted by the placenta and increases in healthy pregnancy from eight weeks’ gestation, with peak concentrations at 29 to 32 weeks being up to ten-fold higher than those of the first trimester, followed by a fall towards delivery. (Benton et al., 2012b) It has a role in endothelial cell activation and proliferation. In vitro studies have shown that trophoblastic expression of PIGF reduces in hypoxic conditions, leading to the hypothesis that PIGF would be low in women with pre-eclampsia.

This theory was explored via a case-control study by Torry and colleagues. (Torry et al., 1998) Serum PIGF concentrations in pre-eclamptic women were compared with age matched normotensive participants. Results showed that PIGF concentrations


fluctuate according to gestation, even in healthy pregnancy, rising in the second trimester and dropping at term. Despite a small sample size (30 women with pre-eclampsia versus 30 controls), PIGF concentrations were consistently low in women with diagnosed pre-eclampsia and significantly higher in women with healthy pregnancies. Moreover, concentrations were noted to be lowest in women with the most severe phenotypes, despite there being no difference in placental mass. (Torry et al., 1998)

A subsequent paper reported a prospective case-control study of women at risk of pre-eclampsia, designed to explore the behaviour of biochemical variables in the serum of women from 20 weeks' gestation to delivery. The researchers suggested PIGF to be predictive of pre-eclampsia. The figure below shows that PIGF was lowest in women who developed pre-eclampsia (triangles).
Figure 1.8: Graph showing change in PIGF concentrations, according to gestational age and disease status. (Chappell et al., 2002) The closed squares show results from low risk women. The closed triangles show concentrations in high risk women. The results represented by closed circles were from women who did not develop pre-eclampsia but went on to develop small for gestational age babies.

Other case control studies also describe PIGF concentrations to be lower in women with severe early onset pre-eclampsia compared with severe late onset disease (Levine et al., 2004) and lower in women with severe pre-eclampsia compared with mild pre-eclampsia. (Robinson et al., 2006) Eclampsia is associated with serum PIGF concentrations comparable to women with severe pre-eclampsia, suggesting a common underlying disease pathway. (Vaisbuch et al., 2011) Despite this, there is evidence to suggest that PIGF is a marker of placental pathology but not specific to
pre-eclampsia: low concentrations of PIGF have also been shown to be associated with small for gestational age babies, even in mothers without pre-eclampsia. (Benton et al., 2012a) It is important to identify a biomarker capable of predicting pre-eclampsia at earlier gestations, as this is when the disease poses the greatest management challenge and potential harm. PIGF is a placentally derived biomarker, present in normal pregnancy, but which has markedly different concentrations in pre-eclamptic pregnancies, with the difference greatest prior to 37 weeks’ gestation.

1.5.2 Vascular endothelial growth factors (VEGF)

Vascular endothelial growth factors play an important role in angiogenesis, by contributing to endothelial relaxation via the nitric oxide pathway. VEGF-A was initially discovered in 1989 when it was known as vascular permeability factor. (Keck et al., 1989) Since then, many more have been identified and are seen in high quantities in highly angiogenic organs such as the placenta.

1.5.3 Soluble fms-like tyrosine kinase 1 (sFlt-1)

Soluble Flt-1, (also known as vascular endothelial growth factor R1 or VEGF-R1), binds to PIGF and stimulates angiogenesis. PIGF is up-regulated in many pathological conditions and displaces VEGF-A from sFlt-1, activating an amplified VEGFA-driven angiogenesis. Concentrations of sFlt-1 are high in the serum and placenta of women with pre-eclampsia. (Maynard et al., 2003).
Figure 1.9: sFlt and its ligands: direct stimulation of angiogenesis through sFlt-1 and indirect stimulation of pathological angiogenesis via enhancement of VEGF-A (Fischer et al., 2008)

Administration of exogenous sFlt-1 in rats produced hypertension and proteinuria. (Maynard et al., 2003) Similarly, reduced VEGF concentration in a murine model has been associated with significant proteinuria. (Levine et al., 2004) These findings suggest that excess sFlt-1 could be linked to the pathogenesis of pre-eclampsia, by binding to circulating VEGF and PIGF molecules. Increased sFlt-1 concentrations are seen in some clinical scenarios associated with an increased risk of pre-
eclampsia: twin pregnancy has been shown to yield sFlt-1 concentrations double that of singleton pregnancy (Bdolah et al., 2008) and in pregnancies with a trisomy 13 (the gene location of sFlt-1) fetus. (Steinberg et al., 2009) It has been proposed that the low sFlt-1 concentrations in pregnant smokers may partly explain the protective effects of smoking against pre-eclampsia.(Jeyabalan et al., 2008)

1.5.4 Soluble Endoglin (S Eng)

Endoglin is a co-receptor responsible for transforming growth factor β1 and β3. It is found on endothelial cell membranes and syncytiotrophoblasts (Levine et al., 2006b) but in pre-eclampsia is upregulated, leading to release of soluble endoglin into the maternal circulation. (Venkatesha et al., 2006) Venkatesha and colleagues went on to show that over expression of endoglin caused hypertension in rodents. When sFlt-1 was included in the adenoviral mediated over-expression, this was associated with severe hypertension with proteinuria and features of HELLP syndrome, supporting the theory that soluble endoglin behaves as a powerful anti-angiogenic agent and plays a part in the pathogenesis of pre-eclampsia.

The Calcium for the Prevention of Pre-eclampsia trial (CPEP) was a randomised double blind trial, (Levine et al., 2006) investigating the effect of calcium supplementation on over 3500 women. A random selection of participants, with and without hypertensive disease, was tested in a nested case-control analysis. The study identified significant elevations in soluble endoglin (sEng) concentrations in
women with pre-eclampsia, particularly those with pre-term disease, but calcium supplementation did not impact on circulating markers of pro-angiogenesis.

The graph below shows sEng across cohorts of pregnant women: concentrations were highest in women who developed preterm pre-eclampsia and lowest in healthy controls.

![Graph showing soluble endoglin concentrations across cohorts of pregnant women.](image)

**Figure 1.10:** Soluble endoglin concentrations are higher in women with pre-eclampsia, taken from (Levine et al., 2006a)

### 1.5.5 Other biomarkers

A far-reaching range of other potential biomarker targets were also included in the secondary analysis of the PELICAN study, selected on biological plausibility and their availability on a commercial platform. The table at the end of this section lists
the biomarkers tested (the results of which are discussed in chapter 4). The biomarkers have been grouped under biologically relevant headings below.

1.5.5.1 Podocyte proteins

Glomerular podocytes are highly specialised cells that function to prevent renal protein loss. Cellular damage following glomerular disease can lead to shedding into the urine. Evidence suggests that the quantities of four podocyte markers (podocin, podocalyxin, synaptopodin, and nephrin) found in the urine of proteinuric pre-eclamptics were not found in the urine of non-proteinuric normotensive controls. (Karumanchi and Lindheimer, 2007) However, podocyturia is not pathognomonic of pre-eclampsia and glomerular endotheliosis has been demonstrated in up to 40% of normotensive pregnant women. (Strevens et al., 2003)

1.5.5.2 Endothelial markers

Several biomarkers released by the endothelium have been proposed as potential diagnostic tools for pre-eclampsia, including endothelin and Neutrophil gelatinase-associated lipocalin (NGAL). Endothelin is a potent vasoconstrictor, which may contribute to the hypertension observed in pre-eclampsia. (Chaiworapongsa et al., 2014a) NGAL is released in response to ischaemic damage and elevated plasma concentrations have been linked with the presence and severity of pre-eclampsia. (Kim et al., 2013) Arginase, also released by the endothelium, reduces nitric oxide causing increased superoxide formation (Sankaralingam et al., 2009).
1.5.5.3 Metabolic markers

Pregnancy associated plasma protein A (PAPP-A) is a metalloprotease produced by the syncytiotrophoblast and promotes fetal growth. Multiple studies have investigated the role of PAPP-A, mainly when measured in the first trimester to determine pregnancies at risk of placental dysfunction. Reduced maternal concentrations have been reported in pre-eclampsia (Smith et al., 2007) but large-scale meta-analysis has found low predictive accuracy. (Morris et al., 2008)

1.5.5.4 Natriuretic peptides

The association between placental and cardiovascular disease has already been discussed and provides the basis for suggested promise of cardiovascular biomarkers holding diagnostic potential in pre-eclampsia. Brain Natriuretic Peptide (BNP) has been seen in high concentrations in pre-eclamptic women (Szabo et al., 2011) although concentrations usually rise in response to volume expansion. For this reason, BNP is more commonly used in the diagnosis of cardiac failure.
Table 1.1: Angiogenic biomarkers and their biological action* in placental disease

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biomarker full name</th>
<th>Mechanism of action</th>
<th>↑ or ↓ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlGF</td>
<td>Placental Growth Factor</td>
<td>Angiogenic marker produced by trophoblastic tissues</td>
<td>↓</td>
</tr>
<tr>
<td>VEGF-C</td>
<td>Vascular endothelial growth factor C</td>
<td>Angiogenic marker produced by trophoblastic tissues</td>
<td>↑</td>
</tr>
<tr>
<td>sFlt-1 (VEGFR1)</td>
<td>Soluble fms-like tyrosine kinase-1</td>
<td>Binds to VEGF causing reducing plasma concentrations</td>
<td>↑</td>
</tr>
<tr>
<td>Endoglin</td>
<td>Endoglin</td>
<td>Anti-angiogenic cell surface glycoprotein</td>
<td>↑</td>
</tr>
<tr>
<td>Angiogenin</td>
<td>Angiogenin</td>
<td>Potent angiogenic factor which interacts with endothelial cells</td>
<td>↑</td>
</tr>
<tr>
<td>C-Met</td>
<td>Tyrosine kinase</td>
<td>Proto-oncogene which promotes angiogenesis</td>
<td>↓</td>
</tr>
</tbody>
</table>

Table 1.2: Endothelial biomarkers and their biological action

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Biomarker full name</th>
<th>Mechanism of action</th>
<th>↑ or ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase-1</td>
<td>Arginase 1</td>
<td>Enzyme which compete with nitric oxide synthase (NOS)</td>
<td>↑</td>
</tr>
<tr>
<td>Endothelin</td>
<td>Endothelin</td>
<td>Potent vasoconstricting peptide produced by the endothelium</td>
<td>↑</td>
</tr>
<tr>
<td>NGAL</td>
<td>Neutrophil gelatinase-associated lipocalin</td>
<td>Protein released post ischaemic damage or sepsis</td>
<td>↑</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia inducible factor 1-alpha inhibitor</td>
<td>Inhibits transcription factor HIF-1alpha, which mediates cellular responses to hypoxia, preventing tissue repair</td>
<td>↑</td>
</tr>
<tr>
<td>PODXL</td>
<td>Podocalyxin</td>
<td>Renal marker expressed in glomerular podocytes and vascular endothelium, correlates to eGFR</td>
<td>↑</td>
</tr>
</tbody>
</table>
Table 1.3: Cell adhesion biomarkers and their biological action

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Biomarker full name</th>
<th>Mechanism of action</th>
<th>↑ or ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM 9</td>
<td>Disintegrin and metalloproteinase domain-</td>
<td>Modulates cell-cell interactions possibly affecting trophoblast invasion and</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>containing protein 9</td>
<td>spiral artery formation. Role in angiogenesis. Marker in renal and prostate cancers</td>
<td></td>
</tr>
<tr>
<td>CPA-4</td>
<td>Carboxypeptidase A4</td>
<td>Cleaves angiotensin-1, a potent vasoconstrictor. Low concentrations in normal tissue</td>
<td>↓</td>
</tr>
<tr>
<td>ESAM-1</td>
<td>Endothelial Cell-selective adhesion molecule</td>
<td>Cellular adhesion molecule expressed by vascular endothelium</td>
<td>↓</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
<td>Signalling protein involved in immune activation</td>
<td>↑</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule</td>
<td>Signalling protein involved in immune activation.</td>
<td>↑</td>
</tr>
<tr>
<td>Kunitz-2 (HAI-2)</td>
<td>Kunitz-type protease inhibitor 2</td>
<td>Inhibits clotting factors</td>
<td>↓</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Matrix metalloproteinase-9</td>
<td>Expressed by cytotrophoblast, to aid trophoblast invasion and remodeling of spiral arteries</td>
<td>↑</td>
</tr>
</tbody>
</table>
### Table 1.4: Inflammatory biomarkers and their biological action

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Biomarker full name</th>
<th>Mechanism of action</th>
<th>↑ or ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>C reactive protein</td>
<td>Non-specific inflammatory marker raised in the acute phase response</td>
<td>↑</td>
</tr>
<tr>
<td>CXCL10</td>
<td>CXC motif chemokine 10</td>
<td>Immune activator released by endothelial cells</td>
<td>↑</td>
</tr>
<tr>
<td>Elafin</td>
<td>Elafin</td>
<td>Protease inhibitor involved in inflammation</td>
<td>↑</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Interleukin 1 receptor antagonist</td>
<td>Activates inflammatory response with release of prostaglandins</td>
<td>↓</td>
</tr>
<tr>
<td>MIF</td>
<td>Macrophage migration inhibitory factor</td>
<td>Pro-inflammatory cytokine</td>
<td>↓</td>
</tr>
<tr>
<td>PCT</td>
<td>Procalcitonin</td>
<td>Involved in calcium homeostasis (↓ plasma [calcium]) and raised in inflammation</td>
<td>↑</td>
</tr>
<tr>
<td>ST2</td>
<td>Interleukin-1 receptor-like 1</td>
<td>Detected in liver, kidney, pancreas, prostate, spleen, small intestine and placenta. Activation produces modulatory cytokines.</td>
<td>↑</td>
</tr>
<tr>
<td>TGFβ-R2</td>
<td>Transforming growth factor beta- receptor 2</td>
<td>Receptor for TGFβ, a multifunctional protein controlling proliferation</td>
<td>↓</td>
</tr>
</tbody>
</table>
### Table 1.5: Coagulation/metabolic biomarkers and their biological action

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Biomarker full name</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 and -2</td>
<td>Plasminogen activator inhibitor 1 and 2</td>
<td>Produced by trophoblasts, inhibits fibrinolysis</td>
</tr>
<tr>
<td>Pentraxin-3</td>
<td>Pentraxin-related protein PTX3</td>
<td>Involved in the activation of the complement system</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>Pregnancy specific plasma protein A</td>
<td>Produced by the syncytiotrophoblast, promotes fetal and placental growth</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin growth factor 1</td>
<td>IGF-1 suppresses catabolism in fetal tissues</td>
</tr>
<tr>
<td>Leptin</td>
<td>Leptin</td>
<td>Released by the placenta, stimulates growth and inhibits apoptosis. Produced in response to hypoxia</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Biomarker full name</td>
<td>Mechanism of action</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>ANP</td>
<td>Natriuretic peptide A</td>
<td>Cardiac hormone causing vasodilatation. Released by the atria in response to stretch</td>
</tr>
<tr>
<td>BNP</td>
<td>Natriuretic peptide B</td>
<td>Cardiac hormone causing vasodilatation, and inhibition of renin and aldosterone. Synthesised in ventricle in response to volume expansion and pressure overload.</td>
</tr>
<tr>
<td>Nephrin</td>
<td>Nephrin</td>
<td>Renal marker essential for normal glomerular function and cardiovascular development.</td>
</tr>
<tr>
<td>Cystatin</td>
<td>Cystatin</td>
<td>Renal marker. Inhibits cysteine proteases possibly reducing trophoblast invasion.</td>
</tr>
</tbody>
</table>
1.6 **Classification of pre-eclampsia**

The range of pre-eclamptic phenotypes (including gestational age at onset and disease severity) is likely to be influenced by maternal individual differences, including co-morbidities, physical characteristics, obstetric history, recreational behaviours and genetic factors, and the resultant impact these may have on a developing placenta.

With improved understanding of pre-eclampsia pathophysiology, two phenotypes have been described, but with overlapping clinical features:

- **Placental pre-eclampsia**: driven by inadequate placental development, and associated with fetal growth restriction, usually requiring early pre-term delivery
- **Maternal pre-eclampsia**: associated with pre-existing maternal disease with vascular dysfunction which is exaggerated by the inflammatory burden of pregnancy

1.6.1 **Maternal pre-disposition**

Maternal chronic conditions, such as essential hypertension, renal disease, diabetes mellitus, systemic lupus erythematosus, obesity and antiphospholipid syndrome are associated with an increased risk of developing pre-eclampsia, mainly due to impaired placental function. (Yoge et al., 2004) (Duckitt and Harrington, 2005) (Steegers et al., 2010b) Increasing age is a risk factor for developing pre-eclampsia, with women over the age of 40 years being at twice the risk of those who are
younger at conception (Duckitt and Harrington, 2005); data from the United States suggest a 30% increased risk for every year past the age of 34. (Saftlas et al., 1990) A body mass index (BMI) over 35 doubled the risk of developing pre-eclampsia (Sibai et al., 1997) and a BMI less than 20 reduced the risk. (Sebire et al., 2001).

1.6.2 Placental contribution to pre-eclampsia

Pre-eclampsia is a multifactorial condition, but placental dysfunction, exacerbated by maternal disease is the underlying pathological process in the majority of cases. Improvements in the understanding of the pathophysiological processes underlying placental disease has aided the identification of upstream markers altered early in the pathological process allowing earlier detection and targeted intervention. These processes are depicted by the schematic below:
Recent research compares placental invasion in humans with other primates. Some primates, including lemurs and lorises, do not exhibit pre-eclamptic phenotypes; perhaps due to a more shallow trophoblastic invasion in these species. (Carter et al., 2015) (Pijnenborg et al., 2011) There is an apparent tendency towards deeper invasion during primate evolution, such that gorillas, great apes and humans get pre-eclampsia, suggesting that pre-eclampsia represents a failure in deep endovascular trophoblast invasion. In the second half of pregnancy, it is the cytotrophoblast that accounts for this process and is responsible for remodelling the spiral arteries.

Figure 1.11: Diagrammatic representation of pre-eclamptic phenotypes, taken from (Staff et al., 2010)
Deeper invasion into the endometrium and inner myometrium is seen in gorillas, chimps and humans.

It is thought defective deep placentation is associated with a range of adverse syndromes of pregnancy: placental abruption, preterm labour, fetal growth restriction and spontaneous miscarriage. It is hypothesised that poor placentation restricts transformation of the spiral arteries and that this defective process is of a greater severity at the peripheries of the placental bed, as shown in the diagram below:

Figure 1.12: A-normal pregnancy, B-Defective deep placentation, characterized by non-transformation of the myometrial spiral arteries reducing the central area with deep placentation. Taken from (Brosens et al., 2011)
1.7 The need for a new test

As discussed, pre-eclampsia is a complex, multi-factorial disease that is challenging to detect and diagnose, particularly in its early stages. Assessment is usually initiated by routine detection of signs that could indicate evolving disease (such as proteinuria or raised blood pressure) during antenatal monitoring in the community. (North et al., 2011) With one in 10 pregnant women developing symptoms suggestive of pre-eclampsia (headache, abdominal pain) but only 20% of these reaching a diagnosis of pre-eclampsia, there is a clear need for improved testing at time of presentation with suspected disease. (Chaiworapongsa et al., 2013c) NICE recommends evaluation of risk factors, such as first pregnancy, BMI, previous pre-eclampsia, more than 10 years between pregnancies, age over 40 years and physical co-morbidity at the time of booking (National Institute for Health and Clinical Excellence, 2010) but, as yet, a robust means of accurately attributing risk does not exist. It is possible to test prediction, in the first trimester of pregnancy or test to diagnose disease at the time of presentation with symptoms.

1.7.1 First trimester prediction

The SCOPE (Screening for Pregnancy Endpoints) study aimed to develop and evaluate screening tests to predict pre-eclampsia in the first half of pregnancy. The study ran over four years, across five international units (UK, Ireland, Australia and New Zealand) and recruited over 3500 women. An extensive range of variables were explored including dietary intake, lifestyle, mental health questionnaires, physical measurements, blood pressure, family history, early pregnancy complications such
as hyperemesis and vaginal bleeding and blood sampling. Participants were then followed up throughout pregnancy and delivery. (North et al., 2011) Results suggested promise for the development of an individual risk algorithm to which biomarker analysis could be added. Guidance exists to promote early detection and timely specialist input (Milne et al., 2005) for women at greatest risk. It is suggested that appropriate surveillance and intervention has the potential to improve adverse perinatal outcomes associated with pre-eclampsia and superimposed pre-eclampsia. (Chappell et al., 2008)

Screening risk in the first trimester is useful but the management options of proven value in terms of outcome for mother and baby are largely limited to aspirin (Duley et al., 2007) and an increased surveillance strategy (currently of uncertain value). Using this information to better predict the women that will go on to experience complications associated with their disease could better define risk and direct resources to reduce morbidity (Duckitt and Harrington, 2005), at a time when medication or iatrogenic delivery can alleviate disease. In a study designed to develop a predictive tool, based on data from over 3500 nulliparous women, based on individual clinical characteristics, only modest predictive potential was reported. Assessment of both risk factors and protective factors included personal characteristics (smoking, maternal birth weight, body mass index and obstetric history) as well as family history (cardiovascular disease, pre-eclampsia). The researchers conclude that risk profiling in this way could gain improved validity if added to biomarker analysis. No improvement was found by adding uterine artery Doppler (area under curve remained 0.71). The figure below shows how a risk
prediction model of this kind could be used to identify the high risk cohort of women who would require specialist care. (North et al., 2011)

Figure 1.13: Framework of care pathway according to estimated risk, showing specialist referral if presumed risk of pre-eclampsia >15%. Taken from (North et al., 2011)

1.7.2 Diagnostic tests

Studies have explored the role of PIGF in both making the diagnosis of pre-eclampsia, usually in the third trimester and in determining disease prognosis in women already diagnosed, or of adverse outcome at a fixed point in the second half of pregnancy. For the purposes of this thesis, a predictive test is defined as one capable of detecting disease in asymptomatic women (e.g. in the first trimester) and
In women with a viable fetus, decisions around delivery are challenging and often made to save the mother, resulting in complications of prematurity in the fetus. The identification of bioactive proteins in the maternal circulation, which hold diagnostic and prognostic potential in pre-eclampsia, hold potential to allow intervention prior to the onset of fulminant disease. Fulminant pre-eclampsia is a multi-organ disorder, causing derangement of renal, liver, clotting and cerebral functioning and contributing to sudden infant death, still birth and haemorrhage. Delivery of the fetus, and crucially, its poorly functioning placenta, remains the only means of ameliorating the clinical manifestation of the disease. While a variety of biomarkers and imaging techniques have been evaluated for improving detection, to date none has adequate sensitivity, specificity, and convenience for the diagnosis or prediction of pre-eclampsia. Development of a test using a biomarker implicated in its pathophysiology, such as PIGF, has attraction over the traditional measurement of blood pressure and urinary protein which are consequences of established disease.

In a retrospective study of over 500 women, Anumba and colleagues explored the validity of frequently used serum tests in identifying adverse outcome. (Anumba et al., 2010) Their work suggested the use of laboratory ‘cut-offs’ and subsequent interpretation of blood results varied across units and could be improved. The sample sizes for each diagnostic group were small, but indicated that platelet count and alanine transaminase had low sensitivity for adverse outcomes. Other markers
(creatinine, blood pressure and early gestational presentation) were predictive of development of pre-eclampsia, early delivery or fetal growth restriction; however the researchers recorded that a third of women referred were normotensive at day unit assessment yet 16% of this cohort still went on to develop pre-eclampsia, suggesting a latent disease phase. Anumba’s study adds to the notion that current assessment practices are disparate and poorly predict disease outcome.

Anumba and colleagues’ work is supported by other research into the parameters currently used as aids both to diagnosis of pre-eclampsia and predictors of adverse outcome. A systematic review, including 13 studies involving over 3000 women with diagnosed pre-eclampsia, into the utility of liver function derangement as a predictor of maternal adverse outcome suggested a higher probability of complications with raised liver enzymes but reported poor test sensitivity. (Thangaratinam et al., 2011b). Raised uric acid concentrations were also found to be a poor predictor of any complication associated with pre-eclampsia. (Thangaratinam et al., 2006b) The same research group also explored other indicators of imminent complications and found similarly disappointing study statistics. Reported maternal symptoms of headache, epigastric pain and visual disturbance did correlate to a higher incidence of maternal poor outcome, yet a lack of symptoms did not enable convincing rule-out for adverse outcomes. (Thangaratinam et al., 2011a)

Proteinuria is one of the defining features of pre-eclampsia, with dipstick testing being performed regularly throughout the antenatal period and included as a screening tool in asymptomatic women. Once in hospital, more accurate protein
measurement is usually carried out (24 hour urinary protein collection or spot random protein:creatinine ratio). It has been hypothesised that the severity of protein loss may be linked to worst outcomes. (Chan et al., 2005) However, a large systematic review including over 6000 participants found proteinuria to be a poor determinant of adverse maternal or fetal outcomes, including perinatal death and to be of ‘very little clinical value.’ (Thangaratinam et al., 2009b) The review included two test accuracy studies conducted thirty years earlier and studies involving a range of test methods, leading to heterogeneity of comparators, yet its findings highlight the diversity of test methodology and proteinuria interpretation across units, (Chappell and Shennan, 2008) and raise questions as to the usefulness of proteinuria as an essential diagnostic criterion. (Hofmeyr and Belfort, 2009) It is notable that the newer extended definitions of pre-eclampsia from the ISSHP and ACOG make proteinuria one of the multi-organ features that defines the disease, rather than the sole additional criterion (to hypertension) as previously given.

1.7.3 Prognostic testing

Once a woman has a diagnosis of confirmed pre-eclampsia, a test may then become prognostic, in that it aims to identify those who will go on to develop adverse outcome (as defined by the PROGRESS series of papers on prognostic research). (Hemingway et al., 2013) A 2004 systematic review of 87 cohort or cross-sectional studies found only questionable clinical utility of screening tests for pre-eclampsia. (Conde-Agudelo et al., 2004a) Similar results were found by a 2013 systematic review and meta-analysis exploring the value of 37 novel biomarkers for the prediction of growth restricted babies; none gave sufficient predictive power. (Conde-
The test performance statistics, taken from this systematic review, of commonly researched biomarkers and uterine artery assessments are shown, in graphical form, below.

Figure 1.14: ROC curves for a) PlGF/sEng and b) PlGF/VEGFR-1. (Conde-Agudelo et al., 2013)

Tests have been used in this way, in each trimester, to predict certain downstream events. In the PELICAN study, PlGF was tested as to its ability to predict women requiring delivery within 14 days of testing. When the PELICAN study was conceived and designed, in 2010, there were no prospective observational cohort studies published reporting diagnostic accuracy. However, the table below shows some of the landmark studies in this field published subsequently, focussing on second/third trimester disease.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>No. of participants (n)</th>
<th>Target population</th>
<th>Time of sampling</th>
<th>Biomarker measured</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaiworapongsa, 2011</td>
<td>87</td>
<td>Women with suspected PE</td>
<td>20-36 weeks</td>
<td>PIGF, VEGFR-1, VEGFR-2, sEng, PIGF/VEGFR-1 ratio, PIGF/sEng ratio</td>
<td>Development of mild PE, severe PE requiring pre-term delivery (&lt;34 weeks)</td>
</tr>
<tr>
<td>Rana, 2012</td>
<td>616</td>
<td>Suspected PE</td>
<td>28-38 weeks</td>
<td>PIGF/sFlt</td>
<td>Diagnosed hypertensive disease</td>
</tr>
<tr>
<td>Verlohren, 2012</td>
<td>630</td>
<td>Confirmed PE</td>
<td>24-34 weeks</td>
<td>sFlt/PIGF ratio</td>
<td>Imminent delivery: time from test to delivery</td>
</tr>
<tr>
<td>Chaiworapongsa, 2013</td>
<td>1269</td>
<td>Normal pregnancy at time of first enrolment (6-22 weeks)</td>
<td>30-34 weeks</td>
<td>PIGF, VEGFR</td>
<td>Stillbirth, late PE, SGA</td>
</tr>
<tr>
<td>Nicolaides, 2013</td>
<td>300</td>
<td>50 with PE, 250 normal pregnancy</td>
<td>30-33 weeks</td>
<td>PIGF, BhcG, PAPP-A</td>
<td>Development of PE</td>
</tr>
</tbody>
</table>

Table 1.7: Summary of studies investigating third trimester PIGF testing on pregnancy outcome

<table>
<thead>
<tr>
<th>Author, year</th>
<th>No. of participants (n)</th>
<th>Target population</th>
<th>Time of sampling</th>
<th>Biomarker measured</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meler, 2014</td>
<td>84</td>
<td>Confirmed PE</td>
<td>20-37 weeks</td>
<td>PIGF</td>
<td>Prediction of maternal complication</td>
</tr>
<tr>
<td>Chappell, 2013</td>
<td>625</td>
<td>Suspected PE</td>
<td>20-40 weeks</td>
<td>PIGF</td>
<td>PE requiring delivery in 14 days</td>
</tr>
<tr>
<td>Droge, 2015</td>
<td>341</td>
<td>Twin pregnancies with suspected or confirmed PE, compared with matched singleton pregnancies</td>
<td>&gt;25 weeks</td>
<td>sFlt/PIGF</td>
<td>Diagnosis of PE/normal outcome</td>
</tr>
</tbody>
</table>

Table 1.7: Summary of studies investigating third trimester PIGF testing on pregnancy outcome
Identifying a test close to the usual onset of symptoms has merit for women presenting in the third trimester but there is an obvious need also to find a means of predicting pre-eclampsia early, to allow timely intervention. A meta-analysis of studies involving pregnant women in the first and second trimesters showed low PIGF concentrations, and high sFlt-1 and sEng concentrations in women with pre-eclampsia (Kleinrouveler et al., 2012). These changes occurred below 16 weeks, as well as above 19 weeks but did not reach significance, and test sensitivity and specificity did not reach a level that could allow recommendation for introduction into clinical use.

1.7.4 PIGF as a marker of disease

In 2011, PIGF and other biomarkers were found in significantly different concentrations in women with an eventual diagnosis of pre-eclampsia that required imminent delivery, compared with those who delivered at term. (Chaiworapongsa et al., 2011b) It is hypothesised that testing of this kind could enable accurate diagnosis of pre-eclampsia during pregnancy, at a time when expedition of delivery is required, due to disease severity, but delayed if possible, to optimise fetal outcome. (Rana et al., 2012b) It is possible that biomarker testing may provide improved evidence of disease status, in women with atypical presentations of pre-eclampsia, who may otherwise go on to undergo hospitalisation for observation or even premature delivery unnecessarily.
The potential of sFlt/PIGF ratio has also been investigated as a means of diagnosing and risk stratifying women with pre-eclampsia and HELLP syndrome. A study including 630 women found a significantly increased ratio in women with pre-eclampsia compared with controls. The ratio was higher in women presenting before 34 weeks’ gestation and in women at greatest risk of requiring imminent delivery. (Verlohren et al., 2012) This ratio is also higher in twin pregnancies complicated by pre-eclampsia (and PIGF lower) compared with singleton pregnancy. (Droge et al., 2015) Theories to justify these findings include increased placental mass or maternal blood volume associated with twin pregnancy, but this is still under debate.

Chaiworapongsra and colleagues explored the role of PIGF in predicting stillbirth or late severe pre-eclampsia in women in the third trimester, via a prospective cohort study. (Chaiworapongsra et al., 2014b) This was the first study of its kind, looking at predictive properties of anti/angiogenic factors at a fixed time-point at later gestations. Reduced concentrations of PIGF/sEng at 30-34 weeks’ gestation were associated with severe late pre-eclampsia. Similar results were reported at earlier gestations and the test appeared to work best at 30-34 weeks. (Chaiworapongsra et al., 2013a) A low PIGF/sFlt-1 ratio was associated with an increased likelihood of stillbirth. However, it remains a limitation of these studies that test accuracy statistics were not reported, making it difficult to compare against other studies or extrapolate into other populations with varying prevalence of disease.

Results of a recent study (Sibiude et al., 2012) showed that, despite a small sample size, PIGF was notably lower, not only in women who went on to develop pre-
eclampsia but also those who experienced adverse outcome and severe adverse outcome. The graph below, taken from this study, shows that higher PIGF concentrations are associated with a lack of adverse outcome (blue squares), particularly in women <35 weeks’ gestation.

Figure 1.15: PIGF concentrations according to gestation and pregnancy outcome, taken from (Sibiude et al., 2012)

However, Meler and colleagues question the test’s role in clinical practice, due to a 65.5% false positive rate in their recent prospective cohort study. (Meler et al., 2014) Despite a small cohort size (84 women), a low plasma PIGF concentration did predict maternal complications with a sensitivity of 76.9% (Meler et al., 2014) and a very low PIGF concentration was associated with pre-eclampsia, particularly in women diagnosed prior to 28 weeks.
In summary, a variety of tests have been evaluated, and reviewed in clinical practice: antenatal assessment of blood pressure and proteinuria, serum assessment of liver and renal function and Doppler ultrasound (Conde-Agudelo et al., 2004a) as well as novel biomarker analysis. There is potential to develop a two-stage screening process that may yield improved risk stratification in high risk women and reduce perinatal complications. (Lai et al., 2013b) Further research is necessary to translate findings into clinically relevant testing.

1.7.5 Combined potential of biomarkers

The complex aetiological basis of pre-eclampsia implies that a combination of clinical parameters and biomarkers could improve predictive accuracy; a prognostic model in pre-eclampsia including gestational age, serum creatinine, platelet count, aspartate aminotransferase, oxygen saturation and chest pain or dyspnoea had an area under the curve of 0.88 for adverse maternal outcomes within 48 hours. (von Dadelszen et al., 2011a) Whilst none of the parameters discussed so far have, in isolation, been recommended for use in clinical practice as a means of identifying the pregnancy at risk of pre-eclampsia, promising combinations are being explored. The findings of a systematic review evaluating the predictive capabilities of combinations of serum biomarkers measured in the late first and early second trimesters report low test performance. (Hui et al., 2012) Despite a ten-fold increase in soluble endoglin being associated with growth restriction (Rana et al., 2012a), the addition of other biomarkers (PIGF/sFlt-1 ratio) did not improve sensitivity.
Chapter 4 of this thesis discusses the basis of this research in more detail and describes a planned secondary analysis on samples from the PELICAN study, comparing the predictive performance of PIGF and other selected serum biomarkers.

1.7.6 **Point of care testing**

Tests capable of predicting downstream complications, such as the need for preterm delivery, have the potential to direct resources and on-going intervention appropriately, including transfer to tertiary units with neonatal intensive care facilities, if required.

1.8 **Health economic implications of diagnostic testing**

Diagnostic tests are required to: identify women with the disease (sensitivity), identify women without the disease (specificity) and perform adequately in practice, perhaps in combination with other clinical assessments or testing strategies. Most importantly, the test results need to translate into patient benefit or improved decision making. (Ferrante di Ruffano et al., 2012) It is clear that further testing is required to assure the feasibility and diagnostic yield of PIGF in clinical practice but there are also more unpredictable aspects, including the perceptions of both clinician and patient. (Ferrante di Ruffano et al., 2012) PIGF is a minimally invasive, bedside blood test, likely to be carried in secondary care, so may yield additive placebo effects, (Goodacre and Nicholl, 2004) giving women perceived reassurance of specialist investigation.
1.8.1 Health economic implications of PlGF

Before any diagnostic test can become a clinical reality, cost effectiveness should be demonstrated. Previous decision analytical models of diagnostic tests for pre-eclampsia have demonstrated cost-savings as a result of better identification of true positives and negatives (Hadker et al., 2010) (Schnettler et al., 2013b). The later chapters in this thesis provide additional information about the potential costs and cost savings of implementing the Triage® PLGF test, as a result of improved identification and clinical management of pregnant women with suspected pre-eclampsia. A hypothetical clinical algorithm was developed, comparing current assessment of women with suspected pre-eclampsia (National Institute for Health and Clinical Excellence, 2010) with a pathway incorporating PlGF. This analysis, is explained in chapter 5.

1.9 Patient reported outcome measures

Patient reported outcome measures assess the self-reported health and quality of life of an individual patient, via questionnaires or surveys and have become more commonplace in clinical practice, (2009b) since their recommendation in The Department of Health’s Next Stage Review in 2009. There are an extensive number of surveys now available to assess cost effectiveness (Appleby et al., 2013), to assess the impact of disease or surgical intervention (Snyder and Aaronson, 2009) or to measure healthcare provider performance. Within maternity care, patient reported outcome measures are of increasing interest to Service Commissioners (Tyler S, 2012) and as a measure of patient perceived outcome in practice.
al., 2013) However, surveys validated for pregnancy or diseases of pregnancy are not readily available for use.

Assessment of qualitative variables, such as quality of life, mood and functional status, is an increasingly recognised means of recording the impact of medical intervention. (Black, 2013) Unlike patient reported experience measures (PREMs), they do not focus on national standards of patient care (Black, 2013) but on the patient’s perspective and self-reported health status. As well as being of use in economic evaluation, the King’s Fund suggest Patient Reported Outcome Measures (PROMs) promote improved patient choice, better clinical decision making and enhanced regulation of healthcare services. (Devlin NJ and Appleby J, 2010) Their use is supported by Clinical Commissioning Groups (Health and Social Care Information Centre, 2013) and patient support groups alike.(Arthritis Research UK, 2013)

PROMs are now used routinely in orthopaedic surgery (Browne et al., 2013, Hunt and Hurwit, 2013) and have modified service delivery. (Keurentjes et al., 2013) They are recognised to be of benefit in assessing competing interventions in terms of clinical effectiveness as well as cost; (Suk et al., 2008) for example, in the calculation of quality adjusted life years following joint replacement. (Appleby et al., 2013) PROMs have also been used in paediatric intensive care,(Andersen et al., 2013) gastroenterology,(Bodger et al., 2013), psychiatry (Hunter et al., 2009) and urogynaecology (Nilsson et al., 2012, Srikrishna et al., 2010, Bjelic-Radisic et al.,
2011) but uncertainty exists as to whether these surveys perform equitably when translated and used in patients across cultural groups. (Treszezamsky et al., 2013)

The PEARLS (Perineal Assessment and Repair Longitudinal Study) used PROMs to assess its primary outcome; pain reduction. It could be argued that highly subjective, retrospective variables, such as pain, should not be compared in this way. However, evidence exists to support the role of patient surveys to inform appropriate types of pain relief for labouring women. (Jimenez et al., 2012, Ahmad Shirvani and Ganji, 2013) At the time of study design, there were no other PROMs in use within pre-eclampsia. Many current pre-eclampsia studies focus exclusively on outcomes chosen by researchers, without reference to measures that pregnant women may rate as important. Chapter 6 describes the first stage in the design of a PROM for use in pre-eclampsia as an additional tool for future use in evaluating performance of diagnostic tests.
CHAPTER 2

Aims
2.1 Aim

The overall aim of this thesis is to address the following research questions: in women presenting with suspected pre-eclampsia, what is the diagnostic accuracy of PIGF in determining pre-eclampsia requiring delivery within 14 days; how does the test performance of PIGF compare with other candidate biomarkers, suggested in literature as potential targets; what would the economic impact be of introducing PIGF testing as a diagnostic adjunct within NHS antenatal triage units; and what alternative patient reported outcome measures might be developed in hypertensive diseases of pregnancy?

2.1.2 Objectives

1. To evaluate the diagnostic accuracy of plasma PIGF concentration in women presenting with suspected preeclampsia between 20 and 35 weeks of gestation (with women recruited up to 40+6 weeks as a secondary analysis) in determining need for delivery for preeclampsia within 14 days of testing (preeclampsia-D14).

2. To determine how the predictive power of PIGF compares with other currently used biological tests and with other selected biomarkers, or combined biomarker targets, in recent medical literature.

3. To determine whether the introduction of PIGF as a point of care test in high risk women represents a realistic, cost effective strategy.

4. To explore the development of a patient reported outcome measure for women diagnosed with pre-eclampsia or suspected pre-eclampsia.
CHAPTER 3

Diagnostic accuracy of Placental Growth Factor in women with suspected pre-eclampsia
3.1 Diagnostic accuracy of Placental Growth Factor in women with suspected preeclampsia: a prospective multicentre study.

This chapter discusses the methodology, results and main conclusions from the PELICAN study, a prospective observational study designed to investigate the diagnostic accuracy of PIGF in high risk women.

3.1.1 Introduction

Preeclampsia is characterised by placental and maternal vascular dysfunction and associated adverse outcomes. (Steegers et al., 2010b) Diagnosis is based on traditional but unreliable and nonspecific clinical markers, most commonly blood pressure and urinary protein excretion; both are subject to observer error and poor test accuracy for identifying women and infants at risk of adverse outcome. (Menzies et al., 2007b) This clinical uncertainty leads to over-utilisation of ancillary testing and intervention, with associated expense of antenatal monitoring and in-patient admissions, placing considerable burden on pregnant women and their families. In the US, preeclampsia is the most common reason for iatrogenic preterm delivery. (Meis et al., 1998a) While biomarkers and imaging techniques have been evaluated, none have adequate sensitivity, specificity, and convenience for diagnosis or prediction of preeclampsia or its complications, (Meads et al., 2008, Menzies et al., 2007b) the majority identifying advanced disease with established end-organ damage.
Recent advances in understanding of preeclampsia and fetal growth restriction have elucidated important biological roles for placentally-derived angiogenic and anti-angiogenic factors. (Maynard and Karumanchi, 2011) In normal pregnancy, placental growth factor (PIGF), synthesised by placental syncytiotrophoblast, (Shore et al., 1997) increases with gestation in the maternal circulation, with concentrations peaking at 26-30 weeks (Knudsen et al., 2011) and declining towards term. PIGF is abnormally low in women with preeclampsia compared to gestational age-matched controls (Levine et al., 2004) and is reduced further in severe preeclampsia. (Robinson et al., 2006)

Development of a test for preeclampsia using a pathophysiologically relevant biomarker, such as PIGF, may have advantages over the traditional measurement of blood pressure and urinary protein which are consequences of established disease. As earlier gestation of preeclampsia onset is associated with greater maternal and perinatal risks, (Steegers et al., 2010b) and the difference in PIGF concentrations between normal and preeclamptic pregnancies is most marked prior to 35 weeks, PIGF has the potential to aid diagnosis of hypertensive disorders of pregnancy at gestations critical to clinical outcome.

The most useful test for health professionals would identify women with preeclampsia associated with clinically relevant and deteriorating disease requiring iatrogenic delivery. As women with suspected hypertensive disease are routinely monitored two-weekly, a clinically useful test should be applicable for a subsequent window of 14 days from testing, to impact on management strategies.
3.1.2 Methods

Participants

The PELICAN study was a prospective observational study, undertaken between January 2011 and February 2012 in seven consultant-led maternity units in the United Kingdom and Ireland. Women were eligible if they presented or were referred with symptoms or signs of suspected preeclampsia between $20^{+0}$ and $40^{+6}$ weeks of gestation, had a singleton or twin pregnancy, and were aged $\geq 16$ years. Symptoms or signs included headache, visual disturbances, epigastric or right upper quadrant pain, increasing oedema, hypertension, dipstick proteinuria and/or suspected fetal growth restriction. Participants were included if the healthcare provider deemed that the woman required evaluation for suspected preeclampsia. Any woman already meeting diagnostic criteria for confirmed preeclampsia at enrolment was not eligible. Baseline demographic and pregnancy-specific information were entered onto the study database (finalised prior to the first participant being enrolled). Fifteen mls of blood (additional to routine blood samples) were drawn into ethylenediamine tetra-acetic acid, transported to the laboratory within 1 hour, and plasma stored until analysis ($\leq 80^\circ$C). Pregnancy outcome details for the mother and infant were obtained from case note and electronic database review.

Outcomes

Definitions and outcomes were pre-specified in the study protocol. The primary analysis was of diagnostic accuracy of low plasma PI GF ($< 5^{th}$ centile for gestational age) to predict need to deliver for preeclampsia within 14 days of testing, in women with suspected, but unconfirmed, preeclampsia before 35 weeks’ gestation. The pre-specified secondary analyses included women presenting later ($35^{+0}$ to $36^{+6}$; $\geq 37$
weeks), or using a lower threshold (<12 pg/ml). All hypertensive disorders of pregnancy including superimposed and severe preeclampsia, were defined according to the American College of Obstetricians and Gynaecologists practice bulletin.(2002) Atypical preeclampsia was defined by the International and Australasian Societies for the Study of Hypertension in Pregnancy(Brown et al., 2001) as gestational hypertension without proteinuria but with other multi-organ involvement or fetal growth restriction (<10th customised birthweight centile). The latter(Gardosi and Francis, 2009) was calculated using the Gestation Related Optimal Weight (GROW) method.

Final adjudicated diagnosis of pregnancy outcome was the reference standard for evaluating PIGF test accuracy. This was determined by two independent senior obstetricians or obstetric physicians requiring documentation of endpoints required to fulfil the diagnostic criteria; disagreement was resolved by a third adjudicating physician. All adjudicators were masked to PIGF values when assigning a final diagnosis; PIGF measurements were not revealed until all subject adjudication was complete.

**PIGF measurement**

Plasma samples were tested, using the Triage® PIGF Test (Alere, San Diego, California), at each study centre according to the manufacturer’s instructions. All meters were programmed for the study duration to produce a masked result, determining satisfactory test completion only, without revealing the value. All laboratory staff were unaware of clinical outcomes. To determine assay reproducibility, replicate samples were also tested at a central laboratory. The assay uses fluorescently-labelled recombinant murine monoclonal antibodies and detects
PIGF specifically and quantitatively, in the range of 12-3000 pg/mL, in approximately 15 minutes. The Total Precision (coefficient of variation) on plasma controls at concentrations of 85 pg/mL and 1300 pg/mL is 12.8% and 13.2%, respectively.

**Statistical analysis**

Women were classified according to: the gestation of the test (<35, 35+0 to 36+6, and ≥37 weeks); the test result: normal (≥5th centile for gestation), low (<5th centile but ≥12 pg/ml), and very low (<12 pg/ml); and the principal outcome: preeclampsia-D14. A positive test was PIGF concentration <5th centile for gestational age for normal controls (calculated from a study of 247 women with normal pregnancies contributing 1366 samples between 20 to 40 weeks’ gestation. (Saffer et al., 2013) Test performance was evaluated as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and ROC areas. Kaplan-Meier survival curves of gestational age at delivery were produced. Median and inter-quartile ranges for the time from PIGF test to delivery were calculated. Comparison of PIGF with other standard tests for preeclampsia (systolic and diastolic blood pressure, uric acid, alanine transaminase) was carried out for the primary outcome using unadjusted PIGF concentrations; proteinuria was excluded as it forms a confirmatory component of that outcome. For implementation to clinical practice in women under 37 weeks’ gestation, an exploratory analysis was conducted for use of a single threshold (independent of gestation), with properties similar to 5th centile cut-off. We evaluated the biochemical reproducibility of the test by analysing all samples a second time in one central laboratory. The required sample sizes were calculated for accurate estimation of the sensitivity and specificity of PIGF in determining the primary endpoint. We assumed a sensitivity of 0.90,
specificity 0.90, and 95% confidence intervals (2-tailed), requiring 62 preeclampsia cases and 150 non-preeclamptic women. As adjudication of final diagnosis (some weeks after delivery) lagged behind enrolment, 287 women were recruited prior to 35 weeks’ gestation before enrolment was stopped.

3.1.3 Results
Between January 2011 and February 2012, 649 women were recruited at 20+0 to 40+6 weeks in seven centres across the UK and Ireland. Of consented women, 24 did not have a valid baseline sample (17) or were lost to follow-up (7). The characteristics of the remaining 625 women are shown overleaf in table 3.1.

There were 287 women recruited below 35 weeks gestation, 137 between 35+0 and 36+6 weeks and 201 recruited over 37 weeks gestation. Women were recruited as a result of self-reported symptoms or due to signs observed at routine antenatal appointments, as shown in table 3.2 overleaf.

Table 3.3 shows final adjudicated diagnoses, medical intervention, onset of delivery and outcome. Nearly a half of episodes of adverse maternal outcome occurred in women who delivered <35 weeks’ gestation.
Table 3.1: Characteristics at booking and enrolment, of all women

<table>
<thead>
<tr>
<th>Gestation at enrolment</th>
<th>$&lt; 35^{\text{th}}$</th>
<th>$35^{\text{th}}$ to $36^{\text{th}}$</th>
<th>$\geq 37^{\text{th}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>287</td>
<td>137</td>
<td>201</td>
</tr>
<tr>
<td><strong>Median (IQR) age (years)</strong></td>
<td>31.9 (27.0 to 35.9)</td>
<td>32.4 (27.5 to 35.4)</td>
<td>32.1 (27.5 to 36.0)</td>
</tr>
<tr>
<td><strong>Median (IQR) body mass index (kg/m²)</strong></td>
<td>28.6 (24.2 to 33.6)</td>
<td>28.6 (24.4 to 32.7)</td>
<td>26.9 (23.1 to 31.2)</td>
</tr>
<tr>
<td><strong>Nulliparous</strong></td>
<td>123 (43%)</td>
<td>60 (44%)</td>
<td>89 (44%)</td>
</tr>
<tr>
<td><strong>Singleton pregnancy</strong></td>
<td>275 (96%)</td>
<td>123 (90%)</td>
<td>198 (99%)</td>
</tr>
<tr>
<td><strong>White ethnicity</strong></td>
<td>187 (65%)</td>
<td>88 (64%)</td>
<td>151 (75%)</td>
</tr>
<tr>
<td><strong>Black ethnicity</strong></td>
<td>70 (24%)</td>
<td>27 (20%)</td>
<td>25 (12%)</td>
</tr>
<tr>
<td><strong>Asian ethnicity</strong></td>
<td>19 (7%)</td>
<td>13 (9%)</td>
<td>12 (6%)</td>
</tr>
<tr>
<td><strong>Other ethnicity</strong></td>
<td>11 (4%)</td>
<td>9 (7%)</td>
<td>13 (7%)</td>
</tr>
<tr>
<td><strong>Median (IQR) highest 1st trimester systolic BP (mmHg)</strong></td>
<td>120 (110 to 130)</td>
<td>118 (110 to 127)</td>
<td>120 (108 to 123)</td>
</tr>
<tr>
<td><strong>Median (IQR) highest 1st trimester diastolic BP (mmHg)</strong></td>
<td>74.0 (66.0 to 81.0)</td>
<td>70.0 (65.0 to 80.0)</td>
<td>72.0 (65.0 to 80.0)</td>
</tr>
<tr>
<td><strong>Current smoking</strong></td>
<td>24 (8%)</td>
<td>10 (7%)</td>
<td>19 (9%)</td>
</tr>
<tr>
<td><strong>Quit smoking</strong></td>
<td>52 (19%)</td>
<td>22 (17%)</td>
<td>30 (15%)</td>
</tr>
<tr>
<td><strong>Never smoked</strong></td>
<td>204 (73%)</td>
<td>101 (76%)</td>
<td>151 (76%)</td>
</tr>
<tr>
<td><strong>Previous medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Previous pre-eclampsia:</strong></td>
<td>55 (20%)</td>
<td>17 (12%)</td>
<td>30 (15%)</td>
</tr>
<tr>
<td><strong>Requiring delivery &lt;34/40</strong></td>
<td>30 (11%)</td>
<td>6 (4.4%)</td>
<td>9 (4.5%)</td>
</tr>
<tr>
<td><strong>Chronic hypertension</strong></td>
<td>45 (17%)</td>
<td>10 (7.9%)</td>
<td>8 (4.5%)</td>
</tr>
<tr>
<td><strong>Systemic lupus</strong></td>
<td>12 (4.5%)</td>
<td>0</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td><strong>erythematous/antiphospholipid syndrome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre-gestational diabetes</strong></td>
<td>6 (2.2%)</td>
<td>4 (3.2%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Renal disease</strong></td>
<td>19 (7.1%)</td>
<td>4 (3.2%)</td>
<td>6 (3.4%)</td>
</tr>
</tbody>
</table>
Table 3.2: Characteristics at study enrolment, by gestational age

<table>
<thead>
<tr>
<th>Gestation at enrolment (weeks, days)</th>
<th>&lt; 35(^{\circ})</th>
<th>35(^{\circ}) to 36(^{\circ})</th>
<th>≥37(^{\circ})</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=287</td>
<td>N=137</td>
<td>N=201</td>
<td></td>
</tr>
<tr>
<td>At enrolment in assessment unit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQR) gestational age</strong></td>
<td>31.0 (27.9 to 33.4)</td>
<td>36.0 (35.4 to 36.4)</td>
<td>38.4 (37.6 to 39.6)</td>
</tr>
<tr>
<td>(weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Signs/ symptoms of suspected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pre-eclampsia (non- exclusive):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New onset of hypertension</td>
<td>155 (54%)</td>
<td>92 (67%)</td>
<td>133 (66%)</td>
</tr>
<tr>
<td>Worsening of underlying hypertension</td>
<td>56 (20%)</td>
<td>21 (15%)</td>
<td>39 (19%)</td>
</tr>
<tr>
<td>New onset of dipstick proteinuria</td>
<td>18 (6%)</td>
<td>8 (6%)</td>
<td>13 (6%)</td>
</tr>
<tr>
<td>Persistent epigastric/ right upper quadrant pain</td>
<td>84 (29%)</td>
<td>44 (32%)</td>
<td>77 (38%)</td>
</tr>
<tr>
<td>Headaches</td>
<td>25 (9%)</td>
<td>4 (3%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Suspected fetal growth restriction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQR) highest systolic BP</strong></td>
<td>144 (131 to 159)</td>
<td>144 (132 to 153)</td>
<td>145 (135 to 155)</td>
</tr>
<tr>
<td><strong>Median (IQR) highest diastolic BP</strong></td>
<td>92 (82 to 100)</td>
<td>94 (86 to 100)</td>
<td>95 (87 to 100)</td>
</tr>
<tr>
<td><strong>Dipstick proteinuria:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not done</td>
<td>38 (13%)</td>
<td>19 (14%)</td>
<td>15 (8%)</td>
</tr>
<tr>
<td>Negative</td>
<td>103 (36%)</td>
<td>34 (25%)</td>
<td>81 (40%)</td>
</tr>
<tr>
<td>Present (1+ or greater)</td>
<td>146 (51%)</td>
<td>84 (61%)</td>
<td>105 (52%)</td>
</tr>
<tr>
<td><strong>Median (IQR) Alanine transaminase U/L</strong></td>
<td>14 (11 to 20)</td>
<td>15 (11 to 21)</td>
<td>14 (11 to 19)</td>
</tr>
<tr>
<td>(n=248)</td>
<td>(n=123)</td>
<td>(n=177)</td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQR) creatinine (µmol/ L)</strong></td>
<td>51 (44 to 62)</td>
<td>55 (47 to 66)</td>
<td>55 (49 to 64)</td>
</tr>
<tr>
<td>(n=267)</td>
<td>(n=128)</td>
<td>(n=194)</td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQR) uric acid (µmol/ L)</strong></td>
<td>257 (189 to 330)</td>
<td>315 (237 to 360)</td>
<td>310 (253 to 380)</td>
</tr>
<tr>
<td>(n=188)</td>
<td>(n=96)</td>
<td>(n=149)</td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQR) platelet count (10⁹/L)</strong></td>
<td>233 (196 to 271)</td>
<td>213 (175 to 263)</td>
<td>215 (177 to 270)</td>
</tr>
<tr>
<td>(n=269)</td>
<td>(n=132)</td>
<td>(n=194)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3: Final diagnoses following expert adjudication

<table>
<thead>
<tr>
<th>Gestation at enrolment (weeks, days)</th>
<th>&lt; 35\textsuperscript{\textdegree}C</th>
<th>35\textsuperscript{\textdegree}C to 36\textsuperscript{\textdegree}C</th>
<th>≥37\textsuperscript{\textdegree}C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of women</td>
<td>N=287</td>
<td>N=137</td>
<td>N=201</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>176 (61%)</td>
<td>81 (59%)</td>
<td>89 (44%)</td>
</tr>
<tr>
<td>Mild pre-eclampsia</td>
<td>25 (9%)</td>
<td>24 (18%)</td>
<td>40 (20%)</td>
</tr>
<tr>
<td>Severe pre-eclampsia</td>
<td>76 (26%)</td>
<td>31 (23%)</td>
<td>23 (11%)</td>
</tr>
<tr>
<td>Superimposed pre-eclampsia</td>
<td>40 (11%)</td>
<td>10 (6%)</td>
<td>7 (3%)</td>
</tr>
<tr>
<td>Atypical pre-eclampsia</td>
<td>32 (14%)</td>
<td>15 (12%)</td>
<td>19 (9%)</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1 (0%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>2 (1%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>27 (9%)</td>
<td>14 (10%)</td>
<td>42 (21%)</td>
</tr>
<tr>
<td>Chronic hypertension only</td>
<td>28 (10%)</td>
<td>9 (7%)</td>
<td>18 (9%)</td>
</tr>
<tr>
<td>Isolated proteinuria only</td>
<td>10 (3%)</td>
<td>6 (4%)</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>Isolated SGA (&lt;10\textsuperscript{th} customised birthweight centile)</td>
<td>8 (3%)</td>
<td>3 (2%)</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Transient hypertension</td>
<td>14 (5%)</td>
<td>17 (12%)</td>
<td>24 (12%)</td>
</tr>
<tr>
<td>Normal</td>
<td>22 (8%)</td>
<td>5 (4%)</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1%)</td>
<td>2 (1%)</td>
<td>1 (0%)</td>
</tr>
<tr>
<td>Antihypertensive use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 drug</td>
<td>51 (18%)</td>
<td>31 (23%)</td>
<td>42 (21%)</td>
</tr>
<tr>
<td>2 drugs</td>
<td>53 (18%)</td>
<td>9 (7%)</td>
<td>16 (8%)</td>
</tr>
<tr>
<td>≥3 drugs</td>
<td>19 (7%)</td>
<td>8 (6%)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>Magnesium sulfate use</td>
<td>6 (2%)</td>
<td>4 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Onset of labour:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous labour</td>
<td>42 (15%)</td>
<td>25 (19%)</td>
<td>59 (29%)</td>
</tr>
<tr>
<td>Induced labour</td>
<td>108 (38%)</td>
<td>75 (55%)</td>
<td>111 (55%)</td>
</tr>
<tr>
<td>Pre-labour Caesarean section</td>
<td>134 (47%)</td>
<td>36 (26%)</td>
<td>31 (16%)</td>
</tr>
<tr>
<td>Adverse maternal outcome</td>
<td>122 (43%)</td>
<td>44 (32%)</td>
<td>53 (26%)</td>
</tr>
</tbody>
</table>
Using pre-specified thresholds of <5\textsuperscript{th} centile (low PlGF) and <12 pg/ml (very low PlGF), low PlGF had high sensitivities and negative predictive values for women tested before 35 weeks, declining at later gestations. For implementation into clinical practice for women presenting before 37 weeks’ gestation, an exploratory analysis determined that a PlGF threshold of <100pg/mL predicted preeclampsia-D14 or before 37 weeks’ gestation (whichever was sooner) with sensitivity and negative predictive values similar to diagnostic accuracy estimates obtained using a <5\textsuperscript{th} centile cut-off. PlGF <5\textsuperscript{th} centile also had good test accuracy for predicting subsequent delivery of a small for gestational age infant <1\textsuperscript{st} centile, at any time point after enrolment (not restricted to diagnosis within 14 days of testing). The majority of women recruited below 35 weeks delivered by caesarean section, whereas vaginal delivery was more likely at later gestations. Adverse perinatal outcomes were also more likely at earlier gestations.
### Table 3.4: Mode of delivery and early pregnancy outcome

<table>
<thead>
<tr>
<th>Gestation at enrolment (weeks, days)</th>
<th>&lt; 35(^{nd})</th>
<th>35(^{nd}) to 36(^{6th})</th>
<th>≥37(^{th})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of women</td>
<td>N=287</td>
<td>N=137</td>
<td>N=201</td>
</tr>
<tr>
<td>Total number of babies</td>
<td>N=299</td>
<td>N=151</td>
<td>N=204</td>
</tr>
<tr>
<td>Median (IQR) gestation at delivery (weeks)</td>
<td>36.7 (33.6 to 38.6)</td>
<td>37.3 (36.6 to 38.4)</td>
<td>39.4 (38.6 to 40.3)</td>
</tr>
<tr>
<td>Preterm delivery &lt;37/40</td>
<td>158 (53%)</td>
<td>55 (36%)</td>
<td>0</td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous vaginal delivery</td>
<td>72 (27%)</td>
<td>54 (41%)</td>
<td>98 (50%)</td>
</tr>
<tr>
<td>Assisted vaginal delivery</td>
<td>31 (11%)</td>
<td>13 (9.9%)</td>
<td>29 (15%)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>169 (62%)</td>
<td>64 (49%)</td>
<td>70 (35%)</td>
</tr>
<tr>
<td>Fetal death</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median (IQR) birth weight (g)</td>
<td>2420 (1620 to 3125)</td>
<td>2820 (2340 to 3340)</td>
<td>3278 (2980 to 3560)</td>
</tr>
<tr>
<td>SGA (&lt;10(^{th}) customised birth weight centile)</td>
<td>142 (47%)</td>
<td>57 (38%)</td>
<td>52 (25%)</td>
</tr>
<tr>
<td>SGA (&lt;3(^{rd}) customised birth weight centile)</td>
<td>108 (36%)</td>
<td>39 (26%)</td>
<td>25 (12%)</td>
</tr>
<tr>
<td>SGA (&lt;1(^{st}) customised birth weight centile)</td>
<td>78 (26%)</td>
<td>19 (13%)</td>
<td>15 (7.3%)</td>
</tr>
<tr>
<td>Adverse perinatal outcome †</td>
<td>69 (23%)</td>
<td>13 (8.6%)</td>
<td>13 (6.4%)</td>
</tr>
</tbody>
</table>
For women presenting prior to 35 weeks’ gestation, there were three cases with false negative results (≥5th centile), all with an additional indication for early delivery; four cases with very low PIGF (<12 pg/ml) were delivered after 37 weeks with severe preeclampsia, three of whom delivered infants ≤5th customised birthweight centile suggesting placental disease. PIGF was <5th centile in all cases and <12 pg/ml in four of the seven cases of antepartum fetal death. Low PIGF predicted intrauterine fetal death with sensitivity 1.00 (95% CI 0.71 to 1.00); specificity 0.48 (0.44 to 0.52); positive predictive value 0.03 (0.02 to 0.05); negative predictive value 1.00 (0.99 to 1.00).
Table 3.5: a) False negatives, cases with very low PIGF and term delivery

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gestation (sampling)</th>
<th>Gestation (delivery)</th>
<th>[PIGF] (pg/ml)</th>
<th>Birth weight</th>
<th>BW centile</th>
<th>Final adjudicated diagnosis and other details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>False negative (PIGF normal and delivered within 14 days of sampling with final diagnosis of pre-eclampsia)</td>
</tr>
<tr>
<td>A</td>
<td>28+2</td>
<td>29+5</td>
<td>1224</td>
<td>1330</td>
<td>29</td>
<td>Superimposed pre-eclampsia; SPPROM, spontaneous labour, Caesarean section</td>
</tr>
<tr>
<td>B</td>
<td>29+6</td>
<td>30+0</td>
<td>160</td>
<td>1095</td>
<td>1</td>
<td>Atypical pre-eclampsia; reduced fetal movements and pre-labour Caesarean section</td>
</tr>
<tr>
<td>C</td>
<td>33+2</td>
<td>34+4</td>
<td>218</td>
<td>2020</td>
<td>5</td>
<td>Severe pre-eclampsia; previous history of early onset pre-eclampsia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PIGF very low and not delivered pre-term &lt;37/40</td>
</tr>
<tr>
<td>D</td>
<td>33+6</td>
<td>37+5</td>
<td>&lt;12</td>
<td>2900</td>
<td>34</td>
<td>Severe pre-eclampsia</td>
</tr>
<tr>
<td>E</td>
<td>34+1</td>
<td>38+1</td>
<td>&lt;12</td>
<td>2350</td>
<td>3</td>
<td>Severe pre-eclampsia</td>
</tr>
<tr>
<td>F</td>
<td>34+2</td>
<td>37+0</td>
<td>&lt;12</td>
<td>2310</td>
<td>5</td>
<td>Severe pre-eclampsia</td>
</tr>
<tr>
<td>G</td>
<td>34+2</td>
<td>37+2</td>
<td>&lt;12</td>
<td>1805</td>
<td>0</td>
<td>Severe pre-eclampsia</td>
</tr>
</tbody>
</table>
b) Antepartum deaths in women presenting <35 weeks’ gestation.

<table>
<thead>
<tr>
<th>Enrolment gestation</th>
<th>Delivery gestation</th>
<th>PIGF concentration (pg/ml)</th>
<th>Final diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>23+0</td>
<td>23+1</td>
<td>374</td>
<td>Severe pre-eclampsia</td>
</tr>
<tr>
<td>25+3</td>
<td>26+6</td>
<td>690</td>
<td>Severe pre-eclampsia with placental abruption</td>
</tr>
<tr>
<td>27+5</td>
<td>29+4</td>
<td>570</td>
<td>Superimposed pre-eclampsia</td>
</tr>
<tr>
<td>28+0</td>
<td>30+2</td>
<td>480</td>
<td>Twin pregnancy; severe pre-eclampsia and discordant FGR</td>
</tr>
<tr>
<td>28+0</td>
<td>35+4</td>
<td>2210</td>
<td>Chronic hypertension with increase in blood pressure; FGR not suspected antenatally</td>
</tr>
<tr>
<td>30+4</td>
<td>35+5</td>
<td>2220</td>
<td>Chronic hypertension with placental abruption</td>
</tr>
<tr>
<td>33+2</td>
<td>38+6</td>
<td>1900</td>
<td>Gestational hypertension; FGR not suspected antenatally</td>
</tr>
</tbody>
</table>

The area under the ROC curve for low PIGF in predicting preeclampsia-D14 was greater than all other commonly utilised tests, either singly or in combination (p<0.001 for all comparisons). Addition of blood pressure or other blood tests currently utilised did not increase the ROC area further compared to PIGF alone.
Figure 3.1: ROC areas (standard error) for PI GF compared to five other signs/ tests (systolic and diastolic blood pressure, uric acid, alanine transaminase and proteinuria) in determining PE D14 in 176 women presenting <35+0 weeks gestation measured using parameters singly (panel A) or in combination (panel B).

Panel A:

Panel B:
The times (in days) to delivery for the three groups (very low, low and normal PlGF) are presented by Kaplan-Meier curves, below:

**Figure 3.2: Kaplan-Meier curves showing PlGF concentration by gestational age**

- Gestation of delivery by PlGF concentration measured at 24 to 34+6 weeks.
- Gestation of delivery by PlGF concentration measured at 35 to <37 weeks.
- Gestation of delivery by PlGF concentration measured at >=37 weeks.
The following table shows PIGF concentrations according to final diagnosis, stratified by gestational age. It is apparent that PIGF concentrations were lower in cases with severe pre-eclampsia, than with mild and in pregnancy associated with adverse outcome. As discussed in chapter 1, PIGF concentrations are naturally lower with increasing gestational age.
<table>
<thead>
<tr>
<th></th>
<th>&lt;35th</th>
<th>35th to 36th</th>
<th>≥37th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>N=287</td>
<td>N=137</td>
<td>N=201</td>
</tr>
<tr>
<td>PIGF concentrations (pg/ml) Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild pre-eclampsia</td>
<td>51 (20 to 228)</td>
<td>n=25</td>
<td>29 (15 to 65)</td>
</tr>
<tr>
<td>Severe pre-eclampsia</td>
<td>10 (10 to 25)</td>
<td>n=79</td>
<td>16 (10 to 28)</td>
</tr>
<tr>
<td>Superimposed pre- eclampsia</td>
<td>43 (10 to 432)</td>
<td>n=40</td>
<td>54 (28 to 100)</td>
</tr>
<tr>
<td>Atypical pre- eclampsia</td>
<td>29 (10 to 106)</td>
<td>n=32</td>
<td>14 (12 to 52)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>153 (59 to 407)</td>
<td>n=27</td>
<td>29 (23 to 97)</td>
</tr>
<tr>
<td>All other diagnoses</td>
<td>291 (143 to 542)</td>
<td>n=84</td>
<td>104 (36 to 273)</td>
</tr>
<tr>
<td>By adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No event</td>
<td>107 (20 to 365)</td>
<td>n=168</td>
<td>40 (15 to 146)</td>
</tr>
<tr>
<td>Systolic BP &gt;=160mmHg only</td>
<td>32 (10 to 140)</td>
<td>n=80</td>
<td>25 (14 to 51)</td>
</tr>
<tr>
<td>All other adverse events</td>
<td>19 (10 to 132)</td>
<td>n=39</td>
<td>36 (15 to 100)</td>
</tr>
</tbody>
</table>
Standard test performance statistics were calculated to evaluate the performance of PIGF and these are summarised in the table below, suggesting a low PIGF is a robust predictor of adverse outcome.

**Table 3.7: Test performance statistics for low PIGF in prediction of adverse outcomes**

<table>
<thead>
<tr>
<th>Enrolment gestation (weeks)</th>
<th>&lt; 35&lt;sup&gt;th&lt;/sup&gt;</th>
<th>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</th>
<th>≥37&lt;sup&gt;th&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=287</td>
<td>73/76</td>
<td>47/67</td>
<td>49/86</td>
</tr>
<tr>
<td>N=137</td>
<td>115/211</td>
<td>45/70</td>
<td>88/115</td>
</tr>
<tr>
<td>N=201</td>
<td>190/211</td>
<td>64/70</td>
<td>102/115</td>
</tr>
</tbody>
</table>

**PlGF <5<sup>th</sup> centile for gestation**

<table>
<thead>
<tr>
<th></th>
<th>Pre-eclampsia requiring delivery within 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.96 (0.89 to 0.99)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.70 (0.58 to 0.81)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.57 (0.46 to 0.68)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.55 (0.48 to 0.61)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.64 (0.52 to 0.75)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.77 (0.68 to 0.84)</td>
</tr>
<tr>
<td><strong>Positive Predictive Value</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.43 (0.36 to 0.51)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.65 (0.53 to 0.76)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.65 (0.53 to 0.75)</td>
</tr>
<tr>
<td><strong>Negative Predictive Value</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.98 (0.93 to 0.995)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.69 (0.57 to 0.80)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.70 (0.62 to 0.78)</td>
</tr>
<tr>
<td><strong>Positive Likelihood Ratio</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.1 (1.8 to 2.5)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.0 (1.4 to 2.8)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.4 (1.7 to 3.5)</td>
</tr>
<tr>
<td><strong>Negative Likelihood Ratio</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.63 (0.51 to 0.74)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.22 (0.13 to 0.34)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.26 (0.17 to 0.36)</td>
</tr>
<tr>
<td><strong>PlGF &lt;12 pg/ml</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.90 (0.85 to 0.94)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.91 (0.82 to 0.97)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.89 (0.81 to 0.94)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.48/69</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.70 (0.57 to 0.80)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.71 (0.48 to 0.89)</td>
</tr>
<tr>
<td><strong>Positive Predictive Value</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.70 (0.57 to 0.80)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.71 (0.48 to 0.89)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.63 (0.45 to 0.79)</td>
</tr>
<tr>
<td><strong>Negative Predictive Value</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.87 (0.82 to 0.91)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.55 (0.46 to 0.64)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.61 (0.54 to 0.69)</td>
</tr>
<tr>
<td><strong>Positive Likelihood Ratio</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4.1 (1.0 to 9.9)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.6 (1.1 to 6.3)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.3 (1.2 to 4.2)</td>
</tr>
<tr>
<td><strong>Negative Likelihood Ratio</strong></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td></td>
</tr>
<tr>
<td>&lt;35&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.41 (0.30 to 0.55)</td>
</tr>
<tr>
<td>35&lt;sup&gt;th&lt;/sup&gt; to 36&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.85 (0.73 to 0.98)</td>
</tr>
<tr>
<td>≥37&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.84 (0.73 to 0.97)</td>
</tr>
</tbody>
</table>
The following figure demonstrates that time from test to delivery was significantly lower in women with a very low PIGF. The difference is most marked in women under 35 weeks' gestation.

Figure 3.3: Time to delivery (median, IQR) stratified by PIGF concentration for all participants and for pre-eclampsia cases. Red line: very low PIGF (<12 pg/ml); orange line: low PIGF (<5th centile); green line normal PIGF (≥5th centile). The numbers in the table below relate to the figure bars.
3.1.4 Discussion

This study suggests that PlGF testing presents a realistic and innovative adjunct to the management of women with suspected preeclampsia, especially those presenting preterm. Low PlGF concentration (<5th centile or ≤100pg/ml) has high sensitivity and negative predictive value, in determining which women presenting with suspected disease at less than 35 weeks’ gestation are likely to need delivery for preeclampsia within 14 days. Time to delivery is markedly different for women with very low, low and normal PlGF values, facilitating stratified management strategies with appropriate surveillance. PlGF was more predictive of need for delivery than other commonly utilised signs and tests, either singly or in combination, in current clinical practice. Sensitivity and negative predictive values were also high for delivery of an SGA infant <1st centile; this indicator is most likely to equate to fetal growth restriction of placental origin and be associated with adverse perinatal outcomes. Although diagnostic accuracy is greatest for women presenting before 35 weeks’ gestation, the test may still benefit those presenting up to 37 weeks’ gestation (using a threshold of <100pg/ml) for whom stratified surveillance is also advantageous and the risks/benefits of delivery remain uncertain.

The strengths of this study include use of multiple centres encompassing a wide demographic and ethnic profile and a pragmatic approach to enrolment with minimal exclusion criteria, enabling generalisability. The main research question was chosen to be clinically relevant, utilising a primary outcome where delivery was indicated for the mother or infant, despite being preterm. Final diagnoses were independently adjudicated by two senior clinicians following database record review, using strict criteria. PlGF concentrations were not revealed until all diagnoses had been
adjudicated, so that the test result could not influence decisions for delivery. Laboratory staff were also unaware of the diagnosis. The analysis followed pre-specified methods and outcomes, with subsequent transparent evaluation of a single PIGF threshold (rather than using a variable 5th centile threshold dependent on gestational age) to enable easier adoption into clinical practice.

The optimal choice of primary outcome was difficult. When the study was planned, there was no validated composite measure of adverse outcome for women with preeclampsia. The fullPIERS model subsequently published used a composite outcome determined by iterative Delphi consensus; (von Dadelszen et al., 2011a) components of this composite (other than blood transfusion) are reported in our study. Maternal plasma PIGF normally declines in the latter part of the third trimester, reducing test performance above 35 weeks' gestation; an ideal test would maintain separation between preeclamptic cases and other women, which is probably unachievable using a single biomarker at all gestations. More accurate determination of very low PIGF values (less than the current limit of detection of 12 pg/ml) could be useful; however, the high clinical sensitivity reported in this study relates to the pre-specified threshold of <5th centile (low PIGF, or PIGF ≤100 pg/ml) rather than very low PIGF.

This is the largest, and the first prospective multicentre, study to evaluate PIGF in women with suspected preeclampsia. Other studies have evaluated PIGF and other factors including soluble Flt-1 (sFlt-1; soluble fms-like tyrosine kinase-1), a trophoblast derived anti-angiogenic factor that is increased in plasma from preeclamptic women. A retrospective study of 87 women gave promising results for
sensitivity (0.93) of the ratio, PIGF/sFlt-1 (R&D Systems immunoassay, Minneapolis, USA) in identifying need for delivery within two weeks; (Chaiworapongsa et al., 2011b) however a more recent study using a different assay for sflt-1/ PIGF ratio (Elecsys platform, Roche, Penzburg, Germany), found considerably lower sensitivity (0.73) and negative predictive value (0.87) at high specificity (0.94) in predicting maternal adverse outcome in women presenting at less than 34 weeks’ gestation, (Rana et al., 2012b) a level of sensitivity which is unlikely to be useful in clinical practice. A case-control study (Knudsen et al., 2011) and a small prospective observational study (Sibiude et al., 2012) using the Triage assay reported promising test performance. Another report of a direct comparison of assays in 128 pregnant women (44 with preeclampsia) confirmed higher sensitivity of the Triage test than the sflt-1/ PIGF ratio (Elecsys) in diagnosing early-onset preeclampsia. (Benton et al., 2011) This may relate to different target epitopes of PIGF used in the Triage test compared to others available. Other studies have not reported sensitivity and specificity (recommended measures of diagnostic accuracy), making direct comparison difficult, (Verlohren et al., 2012) but have compared assays in women with established disease (Sunderji et al., 2010) or have tested at a fixed time-point rather than at presentation. (Lai et al., 2013a)

Suspected preeclampsia is the most frequent clinical presentation to obstetric day care assessment units, and those with early onset disease are at greatest risk. Current signs and tests do not perform well in predicting need for delivery or adverse outcomes. We hypothesise that adding PIGF measurement to current clinical assessment of women with suspected preeclampsia before 37 (and particularly before 35) weeks’ gestation could improve risk stratification, achieve an earlier
diagnosis based upon underlying pathophysiology, enable individualised management of women with the disease, with the potential to reduce associated maternal morbidity and reduce unnecessary health service usage. There may be double benefit: targeting of resources to those at highest risk, while minimising excessive assessment and intervention in women at lower risk. One decision-analytic modelling analysis has estimated $1400 cost saving associated with introduction of PlGF testing (based on sensitivity of 0.82) for management of pregnant women in a United Kingdom setting. (Hadker et al., 2010) Cost savings may be greater when the Triage platform has been adapted to test whole blood at point-of-care. We would propose that further assessment of PlGF should be undertaken in the context of a randomised controlled trial, as recommended for all new diagnostic tests, to measure the impact on the health of mother and baby through changing diagnostic/treatment decisions, time to treatment, as well as potential harms. (Ferrante di Ruffano et al., 2012)

Hypertensive disorders of pregnancy remain a challenge worldwide, as indicated by the recent Global Burden of Disease Study; (Lozano et al., 2012) improved detection and management have also been strongly recommended for reduction of stillbirths. (Bhutta et al., 2011) Whilst current strategies focus on blood pressure measurement and assessment of end-organ damage, this study provides evidence for the recently proposed concept that better diagnosis results from measuring secondary rather than tertiary features of preeclampsia. (Staff et al., 2013b)
CHAPTER 4

PIGF compared with other biomarker targets
4.1 Biomarkers predicting pre-eclampsia requiring delivery within 14 days

4.1.1 Introduction

Presenting symptoms of pre-eclampsia are often subjective and non-specific with clinical findings based on features of advanced disease or markers of end organ involvement. (Gomez-Arriaga et al., 2013, Benton et al., 2012a, Conde-Agudelo et al., 2013) High blood pressure and urinary protein excretion are typically used to diagnose the disease but both are subject to error and poor test accuracy. (Menzies et al., 2007b, Benton et al., 2012a, Conde-Agudelo et al., 2013) It is currently difficult to distinguish pre-eclampsia of a severity that requires early delivery, from other less serious phenotypes (von Dadelszen et al., 2011b), nor is it possible to risk discriminate in the large number of women who present with suspected disease. An accurate biomarker (or panel of biomarkers) to enable diagnosis and prognosis of perinatal complications could have substantial impact on management strategies with the aim of minimising adverse maternal and fetal outcomes. (Myers et al., 2013)

Recent research suggests an imbalance of placentally-derived factors could hold diagnostic potential in these women and encompass feto-placental involvement.

Using samples from women recruited to the PELICAN study, this planned analysis evaluated the performance of a further 57 biomarkers, (including those prevalent in current medical literature and reflecting the heterogeneous components of the disease pathogenesis) in isolation, as a ratio or in combination with PlGF, to determine pre-eclampsia requiring delivery within 14 days in women presenting with suspected pre-eclampsia <35 weeks’ gestation, and presenting between 35\(^0\) and 36\(^6\) as a secondary analysis. Plasma concentrations were obtained from 397 women for measurement of a targeted biomarker panel. Factor analysis and
stepwise logistic regression were conducted to determine whether any biomarkers added to PIGF for the determination of subsequent pre-eclampsia requiring delivery within 14 days.

4.1.2 Materials and Methods

The PELICAN study was a prospective multicentre cohort study, undertaken between January 2011 and February 2012 in seven consultant-led maternity units in the United Kingdom and Ireland. Women were eligible for the study if they had signs or symptoms of pre-eclampsia, were over 20+0 weeks’ gestation with a singleton or twin pregnancy and were aged ≥16 years. Primary analysis (as pre-specified) was performed on those presenting prior to 35 weeks’ gestation, with analysis also reported for those between 35+0 and 36+6 weeks’ gestation.

We undertook a planned analysis reported here on two groups of women: Group 1: presenting prior to 35 weeks of gestation, and Group 2: presenting between 35+0 and 36+6 weeks of gestation. These gestational age groupings were pre-specified, based on known differences in pathophysiological pathways associated with preterm pre-eclampsia and our prior knowledge of gestational changes of biomarker concentrations related to these pathways. Written informed consent was obtained and baseline demographic and pregnancy-specific information, including blood pressure readings, were entered onto the study database. Blood pressure was taken according to unit guidelines. Blood samples were drawn into ethylenediamine tetra-acetic acid, with consent, at the time of enrolment. The samples were labelled, transported to the laboratory and the plasma was stored until analysis at -80°C.
Pregnancy outcomes were determined by case note review with independent adjudication (masked to all biomarker concentrations) for final maternal diagnosis.

Independent adjudication was undertaken by two senior physicians, masked to biomarker measurements, requiring documentation of end points required to fulfil the diagnostic criteria; disagreement was resolved by a third adjudicator. All sites managed women (including decision for delivery) in line with the Hypertension in Pregnancy recommendations from the National Institute for Health and Care Excellence and local guidelines.

**Biomarker selection**

An initial panel of biomarkers was selected based on either *a priori* knowledge of an association with pre-eclampsia, a biological role in placentation or a role in cellular mechanisms involved in the pathogenesis of pre-eclampsia e.g., angiogenesis, inflammation, coagulation. (Myers et al., 2013) The full list of biomarkers (Table S1) was generated following a review of the literature, appraisal of selected bibliographies and consultation with medical experts.

**Biomarker measurement**

Plasma samples were tested for Placental Growth Factor (PlGF) using the Triage® PlGF Test (Alere, San Diego, CA) by trained laboratory staff at the study site where the sample was taken (as previously published). Samples were labelled, and transported to the laboratory where they were spun at 3000 rpm for 10 minutes. The additional 57 biomarker assays were analysed in a central laboratory facility (Alere, San Diego, CA). A list of biomarker assay information (low and high cut-offs, assay
coefficient variable and assay format) is given in table S2. All laboratory staff were masked to clinical outcomes. Samples were stored at -80 °C and thawed prior to the assays being performed at room temperature.

Immunoassays utilizing human plasma were performed in 384-well microtitre plates using Perkin-Elmer Minitrak robotic liquid handling system for all liquid handling steps. Assays were variations of antibody sandwich assays or competitive assays using biotinylated antigen. All assays were heterogeneous and required multiple washes. Test samples were added to the 384-well plate, containing wells for a calibration curve consisting of multiple analyte concentrations and control samples. Calibration curves were prepared gravimetrically in plasma from healthy donors. For sandwich assays, one concentration in each set of calibrators included neutralizing antibody for correction of endogenous antigen present in the plasma pool. All participants had delivered and pregnancy outcomes recorded before biomarker concentrations were analysed and revealed.

**Statistical analysis**

Standard distributional checks showed high levels of skewness for all 57 additional biomarkers, which were consistent with underlying log normal distributions. Logged values of these biomarkers were therefore used. Before considering the pregnancy outcomes factor analysis of biomarker data from all the women enrolled was undertaken, reducing the 57 biomarkers into a smaller group of factors. Consideration of scree plots and Eigen-values (> two) identified the most important factors for further analysis. (Schnittler et al., 2013a) These factors were rotated
(orthogonal varimax method) so that each factor related strongly (correlation >0.6) to a small number of biomarkers only.

The principal outcome for this analysis was pre-eclampsia requiring delivery within 14 days and the factor scores were entered into a multiple logistic regression model for determination of this outcome. Two factors (and their biomarkers) were identified for further investigation. Stepwise logistic regression (a parametric method) was used to determine which of these biomarkers appeared to provide additional information beyond that derived from PIGF and prediction scores were extracted for the best combinations. A comparison of Receiver Operated Curves (ROC) areas (a non-parametric method) of individual biomarkers and combinations was made to see if any of the additional information was both consistent and large enough to be clinically useful.

Some biomarkers, with high uniqueness scores, were not strongly associated with any factor. To investigate whether any of these biomarkers had diagnostic power in addition to that provided by PIGF and biomarkers identified earlier, stepwise logistic regression was undertaken. To avoid excluding a biomarker that may be of potential value, we did not use a standard multiple-testing correction to p-values, such as Bonferroni. However, for a biomarker to be considered useful, it had to pass a series of tests, so that the chance of a false positive was greatly reduced. These included: being a component of a significant factor, being a significant predictor in logistic regression both alone and after allowing for PIGF, having a ROC area for the combined score significantly greater than PIGF alone, being a useful determinant for
pre-eclampsia requiring delivery within 14 days and provide an improvement over PIGF alone that was judged to be clinically useful.

### 4.1.3 Results

423 women with enrolment samples and outcome data available were recruited to the study in seven centres across the UK and Ireland between January 2011 and February 2012; 286 women in Group 1 (presenting at 20\(^{+0}\) to 34\(^{+6}\) weeks of gestation) and 137 women in Group 2 (presenting at 35\(^{+0}\) to 36\(^{+6}\) weeks of gestation).

For the 286 women who were enrolled prior to 35\(^{+0}\) weeks of gestation, characteristics of the study population at antenatal booking are shown in table 4.1, subdivided into those that met the primary outcome (pre-eclampsia requiring delivery within 14 days) and all others. Table 4.2 shows characteristics of delivery and maternal and neonatal outcome. Table 4.3 shows the test performance for the most promising individual biomarkers, depicted by ROC areas. PIGF had the highest ROC area (0.87) for determining preeclampsia requiring delivery within 14 days; the ROC areas for sflt-1 (0.83) and endoglin (0.83) were not significantly different.

Addition of further biomarkers to PIGF increased the area under the ROC curve by a small, non-significant increment only. The highest test performance for preeclampsia requiring delivery within 14 days was found using a combination of PIGF, podocalyxin, soluble endoglin and procalcitonin, with a ROC area of 0.90, not significantly greater than the ROC area for PIGF alone (0.87; p=0.43).
For women presenting between 35*0 and 36*6 weeks of gestation (n=137), the results follow a similar pattern as for women presenting at earlier gestations. The ROC area for PIGF alone (0.75; 95% CI (0.67 to 0.83)) in determining need for delivery for preeclampsia within 14 days was lower than that achieved in earlier gestations. Integration of soluble fms-like tyrosine kinase-1 (sFlt-1) with PIGF (as a ratio) increased the ROC to 0.77 (95% CI (0.69 to 0.84). The combination of PIGF, pregnancy-associated plasma protein A and cystatin yielded the highest ROC area of 0.81 (95% CI (0.74 to 0.88) (table 4). Both increments were small and not significant.

**Figure 4.1: Participant flow diagram for women <35 weeks**

- **Total recruited <37 weeks’ gestation n=423**
  - Total for analysis 20*0 to 34*6 weeks gestation n=286
    - Women with preeclampsia requiring delivery within 14 days n=76
  - All other participants n=210
- Total recruited 35*0 to 36*6 weeks gestation n=137
  - Women with preeclampsia requiring delivery within 14 days n=67
  - All other participants n=70
Table 4.1: Characteristics of participants at booking and enrolment in women <35 weeks’ gestation (according to diagnosis of pre-eclampsia) Values given are median (quartiles) or n (%) as appropriate.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women with PE D-14 n=76</th>
<th>All other participants n=210</th>
<th>P value</th>
<th>All women n=286</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At booking:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.1 (26.8 to 35.6)</td>
<td>32.0 (27.3 to 35.9)</td>
<td>0.84</td>
<td>31.9 (27.0 to 35.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 (22.8 to 30.1)</td>
<td>29.1 (25.0 to 34.7)</td>
<td>&lt;0.001</td>
<td>28.6 (24.2 to 33.6)</td>
</tr>
<tr>
<td>White ethnicity</td>
<td>50 (65.8)</td>
<td>137 (65.2)</td>
<td>0.62</td>
<td>187 (65.4)</td>
</tr>
<tr>
<td>Singleton</td>
<td>71 (93.4)</td>
<td>203 (96.7)</td>
<td>0.27</td>
<td>274 (95.8)</td>
</tr>
<tr>
<td>Highest SBP (mmHg)</td>
<td>120 (110 to 130)</td>
<td>121 (110 to 130)</td>
<td>0.32</td>
<td>120 (110 to 130)</td>
</tr>
<tr>
<td>Highest DBP (mmHg)</td>
<td>70 (65 to 80)</td>
<td>75 (66 to 84)</td>
<td>0.04</td>
<td>74 (66 to 81)</td>
</tr>
<tr>
<td>Smoker at booking</td>
<td>11 (14.9)</td>
<td>42 (20.5)</td>
<td>0.30</td>
<td>58 (19.0)</td>
</tr>
<tr>
<td>Quit smoking</td>
<td>7 (9.5)</td>
<td>27 (13.2)</td>
<td>0.41</td>
<td>34 (12.2)</td>
</tr>
<tr>
<td><strong>Previous medical history:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-eclampsia &lt;34/40</td>
<td>10 (13.3)</td>
<td>20 (9.7)</td>
<td>0.20</td>
<td>30 (10.7)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>7 (10.1)</td>
<td>38 (19.0)</td>
<td>0.08</td>
<td>45 (16.7)</td>
</tr>
<tr>
<td>Known SLE or APS</td>
<td>2 (2.9)</td>
<td>10 (5.0)</td>
<td>0.44</td>
<td>12 (4.5)</td>
</tr>
<tr>
<td>Pre-existing DM</td>
<td>2 (2.9)</td>
<td>4 (2.0)</td>
<td>0.71</td>
<td>6 (2.2)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>5 (7.2)</td>
<td>14 (7.0)</td>
<td>0.98</td>
<td>19 (7.1)</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure in <12 weeks; DBP: diastolic blood pressure in <12 weeks;

DM: diabetes mellitus; SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women with PE D-14 n=76</th>
<th>All other participants n=210</th>
<th>P value</th>
<th>All women n=286</th>
</tr>
</thead>
<tbody>
<tr>
<td>At enrolment:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New onset hypertension</td>
<td>53 (70)</td>
<td>101 (48)</td>
<td>0.03</td>
<td>154 (54)</td>
</tr>
<tr>
<td>Worsening of hypertension</td>
<td>14 (18)</td>
<td>42 (20)</td>
<td>&lt;0.001</td>
<td>56 (20)</td>
</tr>
<tr>
<td>Proteinuria (+1 or greater)</td>
<td>51 (67)</td>
<td>94 (45)</td>
<td>0.77</td>
<td>145 (51)</td>
</tr>
<tr>
<td>Highest systolic BP (mmHg)</td>
<td>150 (140 to 165)</td>
<td>141 (129 to 156)</td>
<td>&lt;0.001</td>
<td>143 (131 to 159)</td>
</tr>
<tr>
<td>Highest diastolic BP (mmHg)</td>
<td>97 (88 to 102)</td>
<td>90 (80 to 98)</td>
<td>&lt;0.001</td>
<td>91 (82 to 100)</td>
</tr>
<tr>
<td>Suspected SGA (customised birth weight centiles)</td>
<td>40 (42)</td>
<td>40 (31)</td>
<td>&lt;0.001</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>16 (12 to 21)</td>
<td>14 (11 to 19)</td>
<td>0.10</td>
<td>14 (11 to 20)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>60 (50 to 73)</td>
<td>49 (42 to 57)</td>
<td>&lt;0.001</td>
<td>51 (44 to 62)</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>327 (256 to 410)</td>
<td>240 (180 to 289)</td>
<td>&lt;0.001</td>
<td>257 (190 to 330)</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>221 (179 to 269)</td>
<td>238 (204 to 274)</td>
<td>0.06</td>
<td>234 (197 to 271)</td>
</tr>
</tbody>
</table>
Table 4.2: Characteristics of delivery and maternal and neonatal outcome. Values given are median (quartiles) or n (%) as appropriate.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women with PE-D14 days n=76</th>
<th>All other participants n=210</th>
<th>P value</th>
<th>All women n=286</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset of labour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>3 (4%)</td>
<td>38 (18%)</td>
<td>0.01</td>
<td>41 (14%)</td>
</tr>
<tr>
<td>Induced</td>
<td>13 (17%)</td>
<td>95 (45%)</td>
<td>&lt;0.001</td>
<td>108 (38%)</td>
</tr>
<tr>
<td>Pre-labour caesarean section</td>
<td>59 (78%)</td>
<td>75 (36%)</td>
<td>&lt;0.001</td>
<td>134 (47%)</td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>3 (4%)</td>
<td>67 (32%)</td>
<td>&lt;0.001</td>
<td>70 (25%)</td>
</tr>
<tr>
<td>Assisted vaginal delivery</td>
<td>4 (5%)</td>
<td>27 (13%)</td>
<td>&lt;0.001</td>
<td>31 (11%)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>67 (91%)</td>
<td>116 (55%)</td>
<td>&lt;0.001</td>
<td>183 (64%)</td>
</tr>
<tr>
<td>Adverse maternal outcome*</td>
<td>37 (49%)</td>
<td>84 (40%)</td>
<td>0.11</td>
<td>121 (42%)</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>32.9 (30 to 34.4)</td>
<td>37.9 (36 to 39.3)</td>
<td>&lt;0.001</td>
<td>36.9 (33.6 to 38.7)</td>
</tr>
<tr>
<td><strong>Neonatal outcomes</strong></td>
<td>n=71</td>
<td>n=203</td>
<td></td>
<td>n=274</td>
</tr>
<tr>
<td>Fetal death</td>
<td>3 (4)</td>
<td>3 (2)</td>
<td>0.19</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1460 (1030 to 1740)</td>
<td>2900 (2320 to 3350)</td>
<td>&lt;0.001</td>
<td>2500 (1620 to 3170)</td>
</tr>
<tr>
<td>SGA</td>
<td>55 (78)</td>
<td>75 (37)</td>
<td>&lt;0.001</td>
<td>130 (47)</td>
</tr>
<tr>
<td>Adverse perinatal outcome†</td>
<td>34 (48)</td>
<td>26 (13)</td>
<td>&lt;0.001</td>
<td>60 (22)</td>
</tr>
</tbody>
</table>

* Adverse maternal outcome defined as presence of any of the following complications: Maternal death, Eclampsia, Stroke, Cortical blindness or retinal detachment, Hypertensive encephalopathy.
Systolic blood pressure ≥160mmHg, Myocardial infarction, Intubation (other than for caesarean section), Pulmonary oedema, Platelets <50×10⁶/L (without transfusion), Disseminated intravascular coagulation, Thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome, Hepatic Dysfunction (Alanine transaminase ≥70IU/L), Hepatic haematoma or rupture, Acute fatty liver of pregnancy, Creatinine >150 μmol/L, Renal dialysis, Placental abruption, Major postpartum haemorrhage, Major infection.

SGA: small for gestational age (<10th centile for birth weight)

† Adverse perinatal outcome defined as: presence of any of the following complications: Antepartum/intrapartum fetal or neonatal death, Neonatal unit admission for >48 hrs at term, Intraventricular haemorrhage, Periventricular leucomalacia, seizure, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising enterocolitis.
Table 4.3: ROC areas (95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to determine pre-eclampsia requiring delivery within 14 days of sampling in women presenting before 35 weeks’ gestation.

[ ] indicates low concentration of biomarker/ratio correlated to disease.

<table>
<thead>
<tr>
<th>Biomarkers or combinations</th>
<th>ROC areas (95% confidence intervals)</th>
<th>P value (vs. PIGF alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PAPP-A]</td>
<td>0.65 (0.57 to 0.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGAL</td>
<td>0.67 (0.61 to 0.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin</td>
<td>0.68 (0.61 to 0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP</td>
<td>0.75 (0.69 to 0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST2</td>
<td>0.76 (0.70 to 0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>0.83 (0.78 to 0.88)</td>
<td>0.08</td>
</tr>
<tr>
<td>Endoglin</td>
<td>0.83 (0.79 to 0.88)</td>
<td>0.07</td>
</tr>
<tr>
<td>[PIGF]</td>
<td>0.87 (0.83 to 0.92)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PIGF/sFlt-1 ratio]</td>
<td>0.88 (0.83 to 0.91)</td>
<td>1.00</td>
</tr>
<tr>
<td>PIGF, C-Met</td>
<td>0.88 (0.83 to 0.91)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF/sEng ratio]</td>
<td>0.88 (0.84 to 0.92)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], sEng</td>
<td>0.88 (0.84 to 0.92)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], [CPA-4], [C-Met]</td>
<td>0.88 (0.84 to 0.92)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], [CPA-4]</td>
<td>0.89 (0.84 to 0.92)</td>
<td>0.86</td>
</tr>
<tr>
<td>[PIGF], Cystatin, PAPP-A</td>
<td>0.89 (0.85 to 0.93)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], Podocalyxin, BNP, [CPA-4]</td>
<td>0.90 (0.86 to 0.93)</td>
<td>0.23</td>
</tr>
<tr>
<td>[PIGF], Podocalyxin, sEng, [CPA-4]</td>
<td>0.90 (0.86 to 0.93)</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Table 4.4: ROC areas (95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to determine preeclampsia requiring delivery within 14 days of sampling in women presenting between 35\(^{+0}\) and 36\(^{+6}\) weeks’ gestation. [ ] indicates low concentrations of biomarker correlated to disease.

<table>
<thead>
<tr>
<th>Biomarkers or combinations</th>
<th>ROC areas (95% confidence intervals)</th>
<th>P value (vs. PIGF alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin</td>
<td>0.64 (0.55 - 0.73)</td>
<td>0.11</td>
</tr>
<tr>
<td>[Pregnancy specific plasma protein A] (PAPP-A)</td>
<td>0.66 (0.58 - 0.75)</td>
<td>0.12</td>
</tr>
<tr>
<td>Neutrophil gelatinase-associated lipocalin (NGAL)</td>
<td>0.67 (0.59 - 0.76)</td>
<td>0.22</td>
</tr>
<tr>
<td>Brain natriuretic peptide (BNP)</td>
<td>0.70 (0.61 - 0.78)</td>
<td>0.35</td>
</tr>
<tr>
<td>Interleukin-1 receptor-like 1 (ST2)</td>
<td>0.71 (0.63 - 0.79)</td>
<td>0.50</td>
</tr>
<tr>
<td>Endoglin</td>
<td>0.71 (0.63 - 0.80)</td>
<td>0.60</td>
</tr>
<tr>
<td>Soluble fms-like tyrosine kinase-1 (sFlt-1)</td>
<td>0.75 (0.67 - 0.83)</td>
<td>0.88</td>
</tr>
<tr>
<td>[Placental growth factor] (PIGF)</td>
<td>0.75 (0.67 - 0.83)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PIGF], procalcitonin</td>
<td>0.73 (0.65 - 0.81)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], endoglin</td>
<td>0.75 (0.67 - 0.83)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], Podocalyxin, BNP, procalcitonin</td>
<td>0.76 (0.68 - 0.84)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], Podocalyxin, sEng, procalcitonin</td>
<td>0.76 (0.68 - 0.83)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF/sFlt-1 ratio]</td>
<td>0.77 (0.69 - 0.84)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF/endoglin ratio]</td>
<td>0.77 (0.66 - 0.82)</td>
<td>1.00</td>
</tr>
<tr>
<td>[PIGF], Cystatin, [PAPP-A]</td>
<td>0.81 (0.74 - 0.88)</td>
<td>0.40</td>
</tr>
</tbody>
</table>
4.1.4 Discussion

This prospective multicentre study is a comprehensive direct comparison of diagnostic biomarkers for preeclampsia. The results demonstrate that in women with suspected preeclampsia presenting preterm, use of a single angiogenesis-related biomarker (PlGF, sflt-1 or endoglin) alone represents a useful diagnostic test for determining preeclampsia requiring delivery within 14 days, a relevant endpoint indicating that a clinician has considered that the risks of adverse outcomes associated with ongoing expectant management are outweighed by the risks of delivery.

Suspected hypertensive disorders in pregnancy are the commonest reason for presentation for obstetric assessment in the third trimester of pregnancy. Diagnostic uncertainty is common when women present to obstetric assessment units with one or more signs suggestive of preeclampsia. Women undergo a series of investigations, many of which are poor predictors of the need for delivery or likely adverse outcome. In practice, obstetricians require a test that enables a woman to be triaged, to determine those that require increased surveillance, and those where the likelihood of needing delivery for preeclampsia within fourteen days is very low and outpatient care may be appropriate. Such a test would enable development of safe clinical algorithms and avoid inappropriate intervention or unnecessary maternal anxiety.

PlGF is an angiogenic factor synthesised by the trophoblast, a marker of associated placental dysfunction in pre-eclampsia, with known low plasma concentrations in the
disease. Whilst combining PIGF with some of the other 46 biologically plausible biomarkers marginally improved the ROC area, the combinations added little to the diagnostic performance of PIGF alone. This important negative result demonstrates the diagnostic option of using a single biomarker (over and above a combination of biomarkers) in preterm preeclampsia. These findings are more marked in women presenting prior to 35 weeks of gestation, and are similar, with lesser diagnostic efficacy, in women presenting between 35\textsuperscript{+0} and 36\textsuperscript{+6} weeks of gestation. This probably reflects the inclusion of women who meet the primary outcome definition (preeclampsia with delivery within 14 days) who were delivered routinely at 37 weeks of gestation following national guideline recommendations and not because of a clinician concern over a potential placentally-mediated adverse event.

Strengths of this study include use of seven study sites and a large participant cohort, encompassing a wide demographic and ethnic profile including women with underlying maternal disease. Plasma testing was carried out in a central laboratory ensuring that results were obtained with rigorous quality control. Progressive statistical analysis explored single biomarker predictive power, and compared the impact of combining groups of markers, or using biomarker ratios. A limitation was that test results were not validated in a repeat sample or by comparative testing at a second laboratory.

Previous studies have described other pathophysiologically relevant third trimester markers, including soluble endoglin (Rana et al., 2012a), or measurement of a ratio such as PIGF/sFlt-1. (Chaiworapongs et al., 2011a, Rana et al., 2012b) However, studies have usually been small or from a single centre, in established disease,
using a case-control design and have shown lower sensitivity for a clinically relevant endpoint. Several additional biomarkers, in combination with PIGF yielded the highest ROC area, but the increase in test performance was only marginal.

Pre-eclampsia and its related conditions represent a diagnostic challenge for clinicians. The lack of a reliable diagnostic test results in poorly targeted antenatal monitoring and hospitalisation. (Conde-Agudelo et al., 2004b, Schnettler et al., 2013a, von Dadelszen et al., 2011b) Women with pre-eclampsia and a viable fetus present difficult management decisions; iatrogenic preterm delivery may avoid further maternal complications but may result in morbidity for the infant. Previous studies have suggested that individual biomarkers for diagnosis are promising, but often in case-control studies in which women with established pre-eclampsia are compared to healthy women with uncomplicated pregnancies, leading to inevitable over-fitting. We are not aware of previous reports of this size in a clinically relevant cohort (women with suspected pre-eclampsia) that has included such a panel of biomarkers that reflects the heterogeneity of the disease.

Development of a diagnostic test, using pathophysiologically relevant biomarkers where concentrations correlate with need for imminent delivery, and therefore with clinically relevant disease severity, may have advantages over traditional diagnostic measures. (Anumba et al., 2010, Steegers et al., 2010a) Early onset pre-eclampsia is associated with greater maternal and perinatal risks; a test that predates signs of established disease would be advantageous allowing targeted surveillance and facilitating appropriate reassurance. Systematic reviews have indicated that currently utilised tests such as proteinuria, (Thangaratinam et al., 2009a) transaminases
(Thangaratinam et al., 2011c) and uric acid (Thangaratinam et al., 2006a) are not good predictors of maternal or fetal complications in women with suspected pre-eclampsia.

This study supports the utility of PlGF (without additional biomarkers) for diagnostic use in women with suspected pre-eclampsia and confirms other smaller or single-centre studies. (Chaiworapongsa et al., 2013b, Meis et al., 1998b, von Dadelszen et al., 2011b, Moore et al., 2012) Women with low or very low PlGF concentrations experienced adverse perinatal outcomes (Chappell et al., 2013b) and suggest that increased surveillance should be considered for these women. Evidence now supports the use of PlGF across a range of demographic settings (Thangaratinam et al., 2009a) in the prediction of pre-eclampsia, (Chappell et al., 2013b, Thangaratinam et al., 2011c) adverse outcome (Conde-Agudelo et al., 2013) and placentally related stillbirth. (Moore et al., 2012)

Suspected hypertension in pregnancy is the commonest reason for presentation for obstetric assessment in the third trimester of pregnancy. This study demonstrates that PlGF measurement alone is a very good diagnostic biomarker for determining need for imminent delivery for pre-eclampsia and that other biomarkers add minimal increment to its performance. Improved risk stratification would facilitate diagnosis and subsequent management decisions, allowing appropriate intervention and timely delivery. We suggest that PlGF measurement, as an adjunct to physical assessment and existing markers of disease, could improve outcomes for women and their babies. We further hypothesise that testing of this kind may improve the experience of women and the allocation of health resources. Improved detection of placental
disease remains a global health priority. Further research is essential to assess revealed PIGF measurements in real-time management of women with suspected pre-eclampsia through appropriate clinical algorithms, with the scope to determine disease prior to tertiary features of end organ damage. (Stepan et al., 2007, Staff et al., 2013c)

The lack of reliable diagnostic tests results in poorly targeted antenatal monitoring and hospitalisation. A test performed at presentation that enables targeted surveillance for those at increased risk of maternal or fetal complications and provides appropriate reassurance to those who test negative has the potential to assist in the allocation of health resources. Biomarkers such as PIGF can be analysed quickly, representing a test that could aid risk stratification of women with suspected preterm preeclampsia. Further research, through randomised controlled trials, is essential to assess how these biomarker measurements can assist in determining (or refuting) diagnosis in preeclampsia, and how this can improve outcomes for mother and baby through optimal tailored clinical management. Further work is also needed on prognosis of multi-organ maternal complications in established preeclampsia.
4.1.5 Conclusions

Improved detection of placental disease remains a global health priority. In women presenting with suspected pre-eclampsia before 37 weeks’ gestation, use of a single angiogenesis-related biomarker may be clinically useful as a diagnostic test without the need for combinations (which entail additional cost and complexity). Biomarkers such as PlGF can be analyzed quickly, representing a test that could aid risk stratification of women with suspected preterm preeclampsia. PlGF represents a potentially useful diagnostic adjunct and may prove to be a valid clinical tool in the future.
CHAPTER 5

Budget Impact Evaluation
5.1 Placental Growth Factor (PlGF) in women with suspected pre-eclampsia prior to 35 weeks' gestation: a budget impact analysis.

The aim of a budget impact analysis is to estimate the financial consequences to a health-care decision maker of implementing a new technology, including the number of patients treated, the effectiveness of the new technology, the costs and rate of implementation and the overall impact on resource use compared to the current technology. In the NHS, national policy and clinical practice is influenced by clinical guidelines published by the National Institute for Health and Clinical Excellence (NICE) and guidelines by specialist groups, such as the Pre-eclampsia Community Guideline (PRECOG) (Milne et al 2005). Providers have the responsibility of ensuring that they provide evidence based health care, including diagnostic and screening tests. The NHS Clinical Commissioning Groups (CCGs) are responsible for improving practice in their respective area so as to ensure the best patient outcomes within the resource envelope available.

One of the biggest challenges for the management of pre-eclampsia is early, reliable identification, and risk stratification. The current method for identifying pre-eclampsia is based on insensitive and unspecific clinical markers: 20% of women who have suspected pre-eclampsia do not meet the criteria for a clinical diagnosis prior to developing the end-stage, eclampsia (Altman et al., 2002) and only 0.7 to 5% of those who meet the diagnostic criteria will go on to experience any pre-eclampsia related adverse outcome. (Menzies et al., 2007a) A diagnostic test that could reliably aid clinical diagnosis and direct clinical management by risk stratifying women into low-, intermediate-, and high-risk groups for adverse outcome would
facilitate clinical management. Such a test would need very high sensitivity and Negative Predictive Value (NPV), allowing better differentiation of those women who could benefit from more intensive management, from women who often receive inappropriate and resource-intensive management, but do not progress to a clinical diagnosis of pre-eclampsia or an adverse.

Establishing a diagnosis of pre-eclampsia can be time consuming and resource intensive. In women with suspected pre-eclampsia, current clinical management requires high-cost monitoring, fetal surveillance, (Steegers et al., 2010b) and medical management, which increases the likelihood of antenatal admission and possible iatrogenic preterm delivery. (Meis et al., 1998a) In the US in 1992, $20 billion was spent on managing women with pre-eclampsia and their babies.(Schnettler et al., 2013b) The next chapter of this thesis describes the development of a management algorithm, modelling the resource implications of PIGF testing in women with suspected pre-eclampsia prior to 35 weeks’ gestation compared with current practice.
5.1.1 Introduction

In the absence of a reliable test, clinical uncertainty leads to over-utilisation of ancillary testing and intervention, with associated expense of antenatal monitoring and in-patient admissions, placing considerable burden on pregnant women and their families. While biomarkers and imaging techniques have been evaluated, none have adequate sensitivity, specificity, and convenience for diagnosis or prediction of pre-eclampsia or its complications, (Meads et al., 2008) the majority identifying advanced disease with established end-organ damage. The PELICAN study identified PIgf as an important and reliable predictor of pre-eclampsia, in women below 35 weeks’ gestation. Test performance statistics revealed high sensitivity (0.96) and negative predictive value (0.98). The introduction of PIgf could target those women at greatest risk for increased surveillance, whilst avoiding unnecessary intervention and resource use in those with subsequent normal outcomes.

Current clinical guidelines (American College of Obstetricians and Gynecologists. Task Force on Hypertension in Pregnancy and American College of Obstetricians and Gynecologists, National Institute for Health and Clinical Excellence, 2010) support the differentiation of pre-eclampsia into mild and severe categories; entities which are treated differently, particularly at preterm gestations. Diagnostic uncertainty and imperfect risk stratification leads to treatment delays and poorer outcomes (for women at high risk of disease who may need imminent delivery), or over-management and high costs for the health service and women (through unnecessary admissions for women at low risk of adverse outcomes or requiring delivery).
Time to delivery is markedly different for women with <12, ≥12<100, and ≥100 pg/mL PlGF values, (9 days, 23 days and 62 days respectively), (Chappell et al., 2013a) facilitating stratified management strategies with appropriate surveillance. Pre-eclampsia is diagnosed by the presence of hypertension and new onset proteinuria. Clinical management, according to the NICE Guideline CG107 (August 2010), is determined by the severity of hypertension (mild, moderate, and severe) and by the presence of other concerning findings (e.g., small for gestation age foetus or concerning maternal blood anomalies).

Performing a budget impact evaluation provided an opportunity to supplement data from the literature with actual resource use to calculate the cost of current practice and model the savings if PlGF were used in management decisions. A treatment algorithm, to be used alongside the PlGF test, allowed us to hypothesise how pregnant women might be managed based on PlGF and other clinical characteristics. The aim of this analysis was to evaluate the cost impact on local NHS budgets of introducing PlGF testing in this cohort of women if management were based on revealed PlGF results. Costs for current treatment, without PlGF, are taken from women recruited as part of the PELICAN study. The cost of PlGF plus a treatment algorithm is calculated using a decision analytic model.
5.1.2 Methods

Participants

We undertook a prospective observational, cohort study investigating the role of PIGF testing in women with suspected pre-eclampsia, between January 2011 and February 2012, in seven centres across the UK and Ireland (PELICAN study). (Chappell et al., 2013a) Women were eligible for the study if they had signs and/or symptoms of suspected pre-eclampsia, were between 20+0 and 40+6 weeks of gestation with a singleton or twin pregnancy and were aged ≥16 years. Women with confirmed pre-eclampsia at the time of presentation were not eligible. Written informed consent was obtained and baseline demographic and pregnancy-specific information were entered onto the study database. As part of the budget impact analysis we conducted a detailed case note review of the resource use and pregnancy outcomes of 132 women enrolled in the PELICAN study prior to 35 weeks’ gestation from two sites (London and Oxford), selected from the 625 women enrolled in the PELICAN study. A sample of women presenting prior to 35 weeks’ gestation was based on (i) a random sample of 109 women from a large inner city hospital so that all 18 diagnostic groups associated with hypertension and proteinuria were represented in the model, and all women with no hypertension, no proteinuria (protein: creatinine ratio <30mg/mmol) and with no diagnosis of pre-eclampsia prior to delivery were included and (ii) 23 women from a smaller site. Detailed retrospective case note review was carried out to record health service usage, including outpatient appointments, day assessment attendance, hospital admissions and ultrasound surveillance during the two week period after their enrolment to the study. Participants gave informed consent and the study followed institutional guidelines.
Plasma samples were tested for PIGF using the Triage® PIGF Test (Alere, San Diego, California) by trained laboratory staff at the UK site where the sample was taken. All participants had delivered and had pregnancy outcomes recorded before biomarker concentrations were analysed and revealed. Using a threshold cut-off of the 5th centile, a PIGF concentration below this was classed as ‘low PIGF’. A PIGF concentration above 100pg/ml (equivalent to the 5th centile) was classed as ‘normal PIGF’. A PIGF concentration below 12pg/ml was categorised as ‘very low PIGF’. Diagnoses of mild, moderate, and severe hypertension were made using criteria dictated by National Institute for Health and Care Excellence guidelines for the management of hypertension in pregnancy; (National Institute for Health and Clinical Excellence, 2010) diagnosis of preeclampsia was made through adjudication by senior physicians using international definitions. (Brown et al., 2001)

PIGF treatment algorithm

The National Institute for Health and Care Excellence guidelines on the management of hypertensive disorders in pregnancy advocate admission for all women diagnosed with pre-eclampsia, with severity of hypertension and fetal well-being directing management and timing of delivery; timing of delivery is dependent on maternal and fetal condition and neonatal intensive care availability. (National Institute for Health and Clinical Excellence, 2010) This guideline was used to inform the ‘current treatment’ algorithm.

Actual resource use, extracted from retrospective case note review, was applied to the treatment model, allowing theoretical comparison of economic burden. We
hypothesised that additional measurement of PIGF could aid clinical decision-making as to appropriate place of care and frequency of monitoring. Figure 1 shows a clinical management pathway, based on data from the PELICAN study, that uses measurement of PIGF alongside blood pressure and proteinuria to risk stratify women with suspected pre-eclampsia.

**Decision analytic model**

A decision model was developed to assess the budget impact of introducing PIGF testing as a prognostic adjunct compared with current practice. The model used a hypothetical cohort of 1,000 women who are assumed to have the same characteristics as 1,000 consecutive pregnant women presenting to an antenatal service in England. Using the proportions derived from our study data we calculated (i) the number of women who would be tested for pre-eclampsia using PIGF (ii) the number of women who fall into each of the three PIGF categories (iii) the number of women who will eventually have a diagnosis of pre-eclampsia or not in each of the resulting branches (iv) the number of women with no, mild to moderate or severe hypertension in each of the resulting branches. Of the 1,000 women, it is assumed that only women presenting with suspected pre-eclampsia undergo PIGF testing. Given that the treatment for women who do not present with suspected pre-eclampsia remains the same in both arms of the model their costs have not been included in the model.
Figure 5.1: Summary of proposed algorithm, based on current NICE guidance
Health care resource use

Health care resource use for the current treatment group presenting with suspected pre-eclampsia prior to 35 weeks’ gestation was calculated from women in the case note review. Women were divided by the three different PlGF test thresholds: <12 pg/ml PlGF; PlGF ≥12<100 pg/ml; or PlGF ≥100 pg/ml and into three different groups of hypertension: normotensive or mild hypertension; moderate hypertension; or severe hypertension for a total of nine groups. As clinicians in the study were not aware of the PlGF test result the resource use for each group represents current practice based on clinical impression only, with no knowledge of PlGF concentrations.

Resource use was evaluated by (i) percentage of women that accessed the service (ii) the mean number (and standard deviation) of times women accessed the service or average length of stay in the case of inpatient admissions (sub-divided into those that had fewer than five days length of stay and those with greater length of stay to reflect the different tariff payments for long and short stay women).

Health care resource use for the ‘PlGF test plus treatment’ algorithm was based on the current treatment algorithm (figure 1) and the National Institute for Health and Care Excellence Hypertension in Pregnancy Guideline. (National Institute for Health and Clinical Excellence, 2010) Health care resource use was calculated in the same way as for the ‘current treatment’ arms, except that a weighted average was included for the proportion of women in each group with proteinuria (given that this would increase the likelihood of women being admitted). The proportion of women in each group with proteinuria was calculated from the 288 records in the PELICAN study.
where the baseline measurement of PlGF, proteinuria and blood pressure was taken prior to 35 weeks’ gestation (table 2).

The cost of routine diagnostic tests (such as serum transaminases, urinary protein estimation) and medication was not included as they represent a negligible proportion of total cost of care and reliable recorded data were not readily available. Most of these costs would be included in the tariff and hence would not represent an additional cost to the payer.

Cost Perspective
The model is from the budget perspective of a commissioner, the organisation responsible for buying health care, within the National Health Service (NHS) in England. All costs are for the 2013/2014 financial year. Costs were obtained from 2013-2014 NHS tariffs and 2011-2012 reference costs (table 3). Reference costs were converted to 2013-2014 values using the average last two years (2011/12 and 2012/2013) Hospital and Community Health Services price increase index.(Curtis, 2013)

Confidence intervals
Confidence intervals were calculated using Monte Carlo simulation for 1,000 iterations of the model to calculate the Monte Carlo error and associated 95% confidence intervals. The percentage of iterations where the model reported a cost saving are also reported. All percentages were modelled using a beta distribution and health care resource using a gamma distribution. Point estimates only were used for health care resource use associated with the treatment algorithm. The
impact of different assumptions about health care resource use for the treatment algorithm on cost savings was tested as part of the deterministic sensitivity analysis. It was assumed that tariff, reference and PIGF costs were constant and hence these were also not varied.

**Sensitivity Analysis**

Studies have reported different point estimates for the incidence of pre-eclampsia and the presentation of risk factors indicative of pre-eclampsia in a pregnant population. We conducted two sensitivity analyses using the point estimates reported by Hadker et al (2010) (Hadker et al., 2010) and Meads et al (2008). (Meads et al., 2008) The ‘PIGF plus treatment’ algorithm is based on guidelines for women presenting with suspected pre-eclampsia in whom there was additional information available on PIGF concentrations. There are no data directly available for actual resource use following implementation of the PIGF test and treatment algorithm as no trial has been conducted and formal implementation of the test has not been comprehensively reported. As a result we tested a range of best and worst case scenarios of health care resource use to assess the impact on potential cost savings from the PIGF test and treatment algorithm. The final price for PIGF test has not been confirmed. Additional analyses using the cost of £30 and £70 per test have been conducted.
5.1.3 Results

The resource cost (per 1,000 women) for two weeks following the PI GF test, according to diagnostic group, is summarised in Tables 5.1 and 5.2. Of 1,000 women, 60 presented with suspected pre-eclampsia prior to 35 weeks' gestation and 18 (30%) had a final diagnosis of pre-eclampsia. In the model, one woman with a final diagnosis of pre-eclampsia had a PI GF concentration greater than 100 pg/ml (false negative). 19 women without pre-eclampsia had a PI GF concentration below 100 pg/ml PI GF threshold (false positives) and hence were managed using the PI GF algorithm even though they did not have a final diagnosis related to pre-eclampsia.

The mean cost saving associated with the PI GF test (in the PI GF plus treatment arm) was £35,087 (95% CI -£33,181 to -£36,992) per 1,000 women. For each woman tested this equated to a cost saving of £582 (95% CI -£552 to -£613). In 94% of iterations, PI GF testing was associated with cost saving compared to current practice. (Hadker et al., 2010) used an incidence of pre-eclampsia of 4.0% with 15% of pregnant women presenting with symptoms indicative of pre-eclampsia. If these figures are used in the model, holding all other variables at the baseline values, the mean cost saving per 1,000 women is £24,324 (95% CI -£22,876 to -£27,785) with 77% of the iterations of the model demonstrating a cost saving.

These assumptions produce a mean cost saving per woman, (with inclusion of the PI GF test), of £543 (95% CI -£493 to -£594), with the assumption that 45 pregnant women will present with suspected pre-eclampsia prior to 35 weeks' gestation and hence PI GF concentrations will be measured. If the incidence of pre-eclampsia reported previously (Meads et al., 2008) is used, the result is similar to the baseline
result at a cost-saving of £22,342 (95% CI -£20,320 to -£24,362) and 78% of iterations of the model are cost saving. Most cost savings were found in the moderate hypertension diagnostic group with a saving of £37,413 across 35 women. Women with a PIGF of ≥12<100 pg/ml had a total cost saving of £33,491 (across 21 women). In the ‘current treatment’ group, 60% of women were admitted, 28% for longer than five days. This was high compared to women with no to mild hypertension, where 39% were admitted, with 4% being admitted for fewer than 5 days.

The following diagram represents these assumptions, showing final diagnosis in 60 women with suspected pre-eclampsia, according to their PIGF concentrations.
Figure 5.2: Algorithm (+PIGF) showing hypothetical diagnostic outcome for 60 women <35 weeks’ gestation with suspected pre-eclampsia
Table 5.1: Two-week costs of PlGF plus treatment algorithm compared to current practice for 1,000 pregnant women, based on correct identification of women with a final diagnosis of pre-eclampsia (deterministic).

<table>
<thead>
<tr>
<th>PIGF (pg/ml)</th>
<th>Hypertension</th>
<th>No. of Women</th>
<th>PIGF + Algorithm Total Cost</th>
<th>Current Practice Total Cost</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGF≥100</td>
<td>No to mild hypertension</td>
<td>0.2</td>
<td>£139</td>
<td>£135</td>
<td>£4</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>0.5</td>
<td>£395</td>
<td>£576</td>
<td>-£181</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>0.1</td>
<td>£103</td>
<td>£35</td>
<td>£68</td>
</tr>
<tr>
<td>Total PIGF≥100</td>
<td>Total</td>
<td>0.7</td>
<td>£637</td>
<td>£747</td>
<td>-£110</td>
</tr>
<tr>
<td>PIGF≥12&lt;100</td>
<td>No to mild hypertension</td>
<td>1.5</td>
<td>£1,334</td>
<td>£1,523</td>
<td>-£190</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>4</td>
<td>£5,678</td>
<td>£17,001</td>
<td>-£11,323</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>0.5</td>
<td>£1,764</td>
<td>£1,099</td>
<td>£665</td>
</tr>
<tr>
<td>Total PIGF≥12&lt;100</td>
<td>Total</td>
<td>6</td>
<td>£8,775</td>
<td>£19,623</td>
<td>-£10,848</td>
</tr>
<tr>
<td>PIGF&lt;12</td>
<td>No to mild hypertension</td>
<td>2.7</td>
<td>£2,898</td>
<td>£5,101</td>
<td>-£2,203</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>7.7</td>
<td>£20,888</td>
<td>£19,764</td>
<td>£1,125</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>0.9</td>
<td>£4,425</td>
<td>£1,950</td>
<td>£2,475</td>
</tr>
<tr>
<td>Total PIGF&lt;12</td>
<td>Total</td>
<td>11.3</td>
<td>£28,212</td>
<td>£26,815</td>
<td>£1,397</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>18</td>
<td>£37,624</td>
<td>£47,185</td>
<td>-£9,560</td>
</tr>
</tbody>
</table>
Table 5.2: Two-week costs of PlGF cost plus treatment algorithm compared to current practice for 1,000 pregnant women based on correct identification of women without a final diagnosis of pre-eclampsia (deterministic).

<table>
<thead>
<tr>
<th>PlGF (pg/ml)</th>
<th>Hypertension</th>
<th>Number of Patients</th>
<th>PIGF + Algorithm Total Cost</th>
<th>Current Practice Total Cost</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGF≥100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No to mild hypertension</td>
<td>9.5</td>
<td>£7,634</td>
<td>£6,858</td>
<td>£775</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>12.7</td>
<td>£10,240</td>
<td>£14,946</td>
<td>-£4,706</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>0.9</td>
<td>£1,652</td>
<td>£566</td>
<td>£1,085</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>23</strong></td>
<td><strong>£19,525</strong></td>
<td><strong>£22,370</strong></td>
<td><strong>-£2,845</strong></td>
</tr>
<tr>
<td>PIGF≥12&lt;100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No to mild hypertension</td>
<td>6</td>
<td>£5,638</td>
<td>£6,439</td>
<td>£801</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>8</td>
<td>£11,365</td>
<td>£34,029</td>
<td>-£22,664</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>1</td>
<td>£2,183</td>
<td>£1,360</td>
<td>£822</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
<td><strong>£19,186</strong></td>
<td><strong>£41,829</strong></td>
<td><strong>-£22,643</strong></td>
</tr>
<tr>
<td>PIGF&lt;12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No to mild hypertension</td>
<td>1.7</td>
<td>£1,834</td>
<td>£3,228</td>
<td>-£1,394</td>
</tr>
<tr>
<td></td>
<td>Moderate hypertension</td>
<td>2.3</td>
<td>£6,257</td>
<td>£5,920</td>
<td>£337</td>
</tr>
<tr>
<td></td>
<td>Severe hypertension</td>
<td>0.2</td>
<td>£819</td>
<td>£361</td>
<td>£458</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>4.2</strong></td>
<td><strong>£8,911</strong></td>
<td><strong>£9,509</strong></td>
<td><strong>-£599</strong></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>42</strong></td>
<td><strong>£47,622</strong></td>
<td><strong>£73,709</strong></td>
</tr>
</tbody>
</table>
The following tables show presumed parameters inputted into the model.

**Table 5.3: Presumed population parameters**

<table>
<thead>
<tr>
<th>Diagnosis per 1000 women</th>
<th>Percentage (95% CI)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected pre-eclampsia</td>
<td>20% (10%-30%)</td>
<td>Clinical expert</td>
</tr>
<tr>
<td>Suspected pre-eclampsia &lt;35 weeks</td>
<td>6% (4%-8%)</td>
<td>Clinical expert</td>
</tr>
</tbody>
</table>

**Disease Incidence**

<table>
<thead>
<tr>
<th>Incidence of pre-eclampsia</th>
<th>1.8% (0.8%-2.5%)*</th>
<th>Clinical expert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage with moderate hypertension in women diagnosed with pre-eclampsia</td>
<td>68% (60%-76%)</td>
<td>Anumba et al (2010)</td>
</tr>
<tr>
<td>Percentage with severe hypertension in women diagnosed with pre-eclampsia</td>
<td>8% (4%-12%)</td>
<td>Anumba et al (2010)</td>
</tr>
<tr>
<td>Percentage with moderate hypertension in women without a diagnosis of pre-eclampsia</td>
<td>55% (50%-60%)</td>
<td>Anumba et al (2010)</td>
</tr>
<tr>
<td>Percentage with severe hypertension in women without a diagnosis of pre-eclampsia</td>
<td>4% (2%-6%)</td>
<td>Anumba et al (2010)</td>
</tr>
</tbody>
</table>

**PIGF test characteristics (<35 weeks predictive for the next two weeks)**

<table>
<thead>
<tr>
<th>Sensitivity PIGF&gt;100pg/ml</th>
<th>96% (89%-99%)</th>
<th>Chappell et al (2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity PIGF&gt;100pg/ml</td>
<td>55% (48%-61%)</td>
<td>Chappell et al (2013)</td>
</tr>
<tr>
<td>Sensitivity PIGF&lt;12pg/ml</td>
<td>63% (51%-74%)</td>
<td>Chappell et al (2013)</td>
</tr>
<tr>
<td>Specificity PIGF&lt;12pg/ml</td>
<td>90% (85%-94%)</td>
<td>Chappell et al (2013)</td>
</tr>
<tr>
<td>Cost of PIGF test</td>
<td>£50</td>
<td>Alere</td>
</tr>
</tbody>
</table>

*There is no good estimate of the prevalence of pre-eclampsia in women <35 weeks’ gestation with estimations varying widely. We have used a conservative estimate at the lower end of the potential prevalence based on clinical opinion, as using a greater percentage increases cost-savings.
Table 5.4: Percentage of pregnant women with PCR>30 mg/mmol by hypertensive category, on all 625 PELICAN pregnant women

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>Normotensive to mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlGF≥100 pg/ml</td>
<td>26%</td>
<td>27%</td>
<td>29%</td>
</tr>
<tr>
<td>PlGF≥12&lt;100 pg/ml</td>
<td>42%</td>
<td>30%</td>
<td>59%</td>
</tr>
<tr>
<td>PlGF&lt;12 pg/ml</td>
<td>76%</td>
<td>64%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Table 5.5: Cost parameters

<table>
<thead>
<tr>
<th>Cost parameter</th>
<th>Cost per unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital admission – length of stay up to 5 days</td>
<td>£789</td>
<td>NHS PbR Tariff</td>
</tr>
<tr>
<td>Hospital admission – cost per day after 5 days</td>
<td>£377</td>
<td>NHS PbR Tariff</td>
</tr>
<tr>
<td>Outpatient appointments</td>
<td>£284</td>
<td>NHS PbR Tariff</td>
</tr>
<tr>
<td>Additional specialised ultrasound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference cost – 2011/12</td>
<td>£121</td>
<td>Reference costs</td>
</tr>
<tr>
<td>% increase</td>
<td>7%</td>
<td>Curtis (2013)</td>
</tr>
<tr>
<td>2013/2014 figure</td>
<td>£129</td>
<td></td>
</tr>
<tr>
<td>Day unit cost (not admitted)</td>
<td>£378</td>
<td>NHS PbR Tariff</td>
</tr>
</tbody>
</table>
Table 5.6: Sensitivities using Monte Carlo simulation (1000 women)

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Cost of PIGF plus algorithm</th>
<th>Cost of current treatment</th>
<th>Difference</th>
<th>% Simulations PIGF + algorithm cost saving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algorithm admits all women with PIGF&lt;100 pg/ml (assumes length of stay &lt;5 days)</td>
<td>£106,261</td>
<td>£120,894</td>
<td>-£14,633</td>
<td>71%</td>
</tr>
<tr>
<td>Increase length of stay for all women admitted PIGF + algorithm by 3 days</td>
<td>£95,132</td>
<td>£120,894</td>
<td>-£25,761</td>
<td>81%</td>
</tr>
<tr>
<td>Algorithm admits all women with PCR&gt; 30 mg/mmol</td>
<td>£95,182</td>
<td>£120,894</td>
<td>-£25,712</td>
<td>87%</td>
</tr>
<tr>
<td>Admission to inpatient ward costs 50% more</td>
<td>£92,403</td>
<td>£147,320</td>
<td>-£54,917</td>
<td>97%</td>
</tr>
<tr>
<td>Admission to inpatient ward costs 50% less</td>
<td>£78,089</td>
<td>£94,467</td>
<td>-£16,378</td>
<td>85%</td>
</tr>
<tr>
<td>PIGF test costs £30 per test</td>
<td>£84,046</td>
<td>£120,894</td>
<td>-£36,847</td>
<td>95%</td>
</tr>
<tr>
<td>PIGF test costs £70 per test</td>
<td>£86,446</td>
<td>£120,894</td>
<td>-£34,447</td>
<td>94%</td>
</tr>
</tbody>
</table>


5.1.4 Discussion

Main findings

The results of the decision analytic model suggest that, based on the best information available, there is a greater than 90% chance that PIGF testing plus a treatment algorithm represents a cost saving for a commissioner’s budget compared to current practice. This cost saving is likely to be around £582 per woman presenting prior to 35 weeks’ gestation with clinical characteristics indicative of pre-eclampsia over two weeks or £35,087 per 1,000 pregnant women. These results are relatively robust to changes made to the assumptions in the model, although changes in the incidence of pre-eclampsia reduce the probability that PIGF plus a treatment algorithm is cost-saving.

Strengths and limitations

The main strength of this study is the comprehensive comparison of resource use in women undergoing PIGF testing for suspected pre-eclampsia. With most savings associated with pregnant women presenting with moderate hypertension, the ‘PIGF plus management’ algorithm potentially provides clinicians with the ability to stratify these women into risk groups more appropriately. Data were extracted from our recent prospective study, including participants encompassing a wide demographic and ethnic profile and a pragmatic approach to enrolment with minimal exclusion criteria, enabling generalisability. Final diagnoses were independently adjudicated by two senior clinicians following database record review, using strict criteria. PIGF concentrations were not revealed until all diagnoses had been adjudicated.
The model has a number of limitations. PIGF has not yet been tested as part of a randomised controlled trial, meaning that there is uncertainty about what resource use pregnant women with suspected pre-eclampsia, tested with PIGF and managed using the treatment algorithm, would actually use. An improvement in health outcomes for women and their infants is not yet proven, although results of our study suggest that PIGF has the potential to aid diagnosis and assist decision-making, with subsequent impact on maternal and perinatal outcomes. Resource use may have varied costs in different settings, and so the cost savings presented here need to be reproduced in other settings.

The predictive potential of PIGF testing is optimal below 35 weeks’ gestation, with outcomes reliably predicted in the two week period after testing (the primary outcome of our study). For the purposes of this analysis, therefore, we did not evaluate women presenting after 35 weeks’ gestation or assess resource use beyond the two week test period. It is now common practice to routinely deliver women with pre-eclampsia at 37 weeks. (Koopmans et al., 2009) This implies costs are likely to decline towards term, as hospital admission demands the greatest economic burden. (American College of Obstetricians and Gynecologists and Task Force on Hypertension in Pregnancy, 2013) It was not possible to include additional diagnostic tests and therapeutic medications in the model, due to the lack of availability of this information. We believe, however, that this would produce a marginal change to the total costs and may well be captured as part of the tariff.
The results suggest that PlGF plus a treatment algorithm presents a realistic and innovative adjunct to the management of women with suspected pre-eclampsia. Previous decision analytical models of screening tests for pre-eclampsia have demonstrated cost savings as a result of better identification of true positives and negatives. (Hadker et al., 2013) The weakness of these previous models is that health care resource use data were not reported with PlGF test results, and hence there was no information on the current cost implications of different PlGF thresholds.

**Conclusions**

PlGF testing is associated with improved predictive performance, in the diagnosis of preeclampsia, compared with current diagnostic practice in high risk women. It is likely that PlGF testing with linked treatment algorithm is cost-saving compared to current practice from the perspective of a health care commissioner over a two week period. Some uncertainties still remain that warrant further research with a prospective analysis of costs with actual implementation of PlGF.
Figure 5.3: Schematic findings of budget impact analysis, suggesting how PlGF could be implemented to inform management of women presenting with suspected pre-eclampsia.

<table>
<thead>
<tr>
<th>LEVEL 1 CARE:</th>
<th>LEVEL 2 CARE:</th>
<th>LEVEL 3 CARE:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BP up to 149/99 mmHg</strong></td>
<td><strong>Moderate hypertension (BP 150/100–159/109 mmHg)</strong></td>
<td><strong>Severe hypertension (BP ≥ 160/110 mmHg)</strong></td>
</tr>
<tr>
<td>- Do not admit to hospital.</td>
<td>- Do not admit to hospital.</td>
<td>- Admit to hospital until BP ≤159/109 mmHg and treat hypertension to keep BP &lt; 150/80–100 mmHg.</td>
</tr>
<tr>
<td>- BP up to 149/99 mmHg</td>
<td>- Treat hypertension to keep BP &lt;150/80–100 mmHg.</td>
<td>- Measure BP at least x4/ day</td>
</tr>
<tr>
<td>- Do not treat hypertension.</td>
<td>- Measure BP at least x2/ wk.</td>
<td>- Test kidney function, electrolytes, FBC, transaminases, bilirubin.</td>
</tr>
<tr>
<td>- Measure BP no more than x1/wk</td>
<td>- Test for proteinuria at each visit</td>
<td>- Test for proteinuria daily</td>
</tr>
<tr>
<td>- Test for proteinuria at each visit</td>
<td>- Test kidney function, electrolytes, FBC, transaminases, bilirubin.</td>
<td>- Test kidney function, electrolytes, FBC, transaminases, bilirubin at presentation &amp; then weekly.</td>
</tr>
<tr>
<td><strong>Carry out routine antenatal blood tests.</strong></td>
<td><strong>No further blood tests if no subsequent proteinuria.</strong></td>
<td></td>
</tr>
<tr>
<td><strong>If presenting before 32/40, or at high risk of pre-eclampsia, test for proteinuria &amp; measure BP x2/ wk.</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PLGF**<br>**<12 VERY LOW**<br>**ADMIT FOR ASSESSMENT**

**PLGF LOW 12-100**<br>**Move to level 2 care**

**PLGF >100 NORMAL**<br>**Continue with level 1 care**

**PLGF <12 VERY LOW**<br>**ADMIT FOR ASSESSMENT**

**PLGF LOW 12-100**<br>**Continue with level 2 care**

**PLGF >100 NORMAL**<br>**Return to level 1 care**

**PLGF <12 VERY LOW**<br>**ADMIT FOR ASSESSMENT**

**PLGF LOW 12-100**<br>**ADMIT for BP control then level 2 care**

**PLGF >100 NORMAL**<br>**ADMIT for BP control then level 1 care**
CHAPTER 6

Patient Reported Outcome Measures
6.1 Patient reported outcome measures (PROMs) in obstetrics

The application of patient reported measures of health outcome is becoming increasingly relevant in the evaluation of health care and outcomes in clinical trials. The use of such measures in obstetrics, particularly pre-eclampsia, remains developmental but could represent an important step in understanding the issues important to women throughout their care pathway.

6.1.1 Introduction

Patient reported outcome measures (PROMs) assess the health, functional status and quality of life of an individual patient, usually via a standardised, validated questionnaire or survey. (Darzi, 2008) The initial design enabled comparative feedback following elective surgery. Their use is now commonplace in intervention-specific surgical specialities and chronic disease (Devlin NJ and Appleby J, 2010). Over the last decade, PROMs have been used in a number of ways: as a diagnostic tool, (Kroenke et al., 2001) to assess burden of disease (Snyder and Aaronson, 2009), or as means of measuring healthcare provider performance. (Devlin NJ and Appleby J, 2010) The short form 36 (SF-36) health survey, for example, is a generic questionnaire that assesses self perceived health status by using 36 questions relating to eight broad areas (or “domains”) of wellbeing. (Dawson et al., 2010) The European Quality of Life index (EQ-5D) is another generic health questionnaire that uses five domains and a visual analogue scale to calculate a ‘utility’ value of health status. There are also several examples of disease-specific questionnaires, allowing appropriate enquiry, specific to the patient demographic and unique disease characteristics.
There is a paucity of these tools within obstetrics, but their development could provide useful information to guide future commissioning (Tyler S, 2012) and enhance understanding of perceived outcomes and experiences in clinical practice. (Ismail et al., 2013) There has been a marked shift internationally, towards acknowledging the patient’s perspective when monitoring health and health-related intervention, (Devlin NJ and Appleby J, 2010) which recognises the patient’s agenda may not match that of the clinician involved with their medical care. PROMs may elicit a more accurate reflection of the issues important to service users and ensure their inclusion in future management tools. For this part of the project, we used both the SF-36 and EQ-5D as a starting point from which to develop a PROM focussing on obstetric care. These survey formats had been used to introduce PROMs in other clinical domains; their development in obstetrics seemed feasible.

Trials evaluating clinical interventions across all fields often report a wide range of outcomes, potentially leading to reporter bias or difficulty in reliably comparing results across trials. One response has been to produce and use a core outcome set, an agreed minimum set of outcomes to be measured and reported in trials. Work is currently underway to develop this idea further and provide recommendations, for researchers, on how these outcomes should be recorded and assessed. Such information was not available at the time of conception of the PELICAN study. Core outcome sets may variably include PROMS, and it remains unclear whether outcomes chosen truly represent those pertinent to pregnant women. It is likely that events such as preterm delivery, admission to hospital or neonatal unit admission are also likely to be important to women, but this aspect of the work explored the
development of PROMs from an alternative perspective previously utilised and recommended in the generation of PROMs in other fields of medicine.

6.1.2 Methods
A first draft survey, created by obstetric clinicians and social scientists, was circulated among twenty pregnant women. These women were recruited from antenatal clinics, the antenatal day assessment unit, hospital antenatal classes and the inpatient antenatal ward. All participants were (i) over the age of 16 years, (ii) able and willing to give consent, and (iii) over twenty weeks’ gestation. The only exclusion criterion was known lethal fetal abnormality. Their feedback was used to amend the initial survey, before it was distributed to a wider cohort of 100 women: 51 pregnant women with hypertensive pregnancy disorders (forty-six pre-eclampsia or suspected pre-eclampsia, three gestational hypertension and one chronic hypertension) and 49 pregnant women with no significant medical disorder in the second half of pregnancy.

The resulting questionnaire was based on the 36-item short-form health survey (SF-36) and EuroQol EQ-5D. Following collection of one hundred surveys, data were anonymised. All questions had answers on numerical scales (a five or six point Lickert scale) or were attributed numerical values for the purposes of analysis. It was pre-specified that the report would include the two most extreme values (1 and 2, compared with 5 and 6 on the scale). Initial analysis led to the production of a five question survey, using an unpaired student t-test, based on the questions that yielded the most comparative scores. For the small number of missing data and
questions that were unanswered were given the median score for the group (cases or controls). The results of these questions were isolated and analysed to provide a simplified overview of the psychosocial impact of pre-eclampsia. All answers were allocated a score of 1 to 5 or 6, with a low score indicating minimal disruption.

6.1.3 Results

Results were analysed to enable the research group to draw comparison between hypertensive women (women with a diagnosis of suspected or confirmed pre-eclampsia) and healthy pregnant women.

6.1.3.1 Development work

The first draft questionnaire comprised of thirty-two questions; only eight domains were found to be significantly associated with hypertension during pregnancy. Three of these related to reported symptoms during pregnancy (swelling), two to demographic characteristics (parity, children living with participant, and fulltime employment), one to perceived quality of life (ability to continue usual employment), and one’s attitudes towards pregnancy (desire for subsequent pregnancy). The early developmental stages highlighted the following survey domains as generating the most divergent scores:

- quality of life
- mood
- attitude towards pregnancy

Early results suggested that women with a diagnosis of hypertension during pregnancy were more likely to report difficulty in maintaining employment and a
reduced desire for subsequent pregnancy than healthy controls. There was a trend towards reduced enjoyment of life but no significant differences between mood in women with pre-eclampsia compared with healthy pregnancy. However, there were increased numbers of women responding negatively to questions relating to sleep and anxiety, so these topics were also included.
### Demographics of 100 women

**Table 6.1: Demographic characteristics of participants [percentage]**

<table>
<thead>
<tr>
<th>Demographic Characteristic</th>
<th>Controls [%] (n=50)</th>
<th>Hypertensive women [%] (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>33 years</td>
<td>33.5 years</td>
</tr>
<tr>
<td>White</td>
<td>38 [76]</td>
<td>18 [36]</td>
</tr>
<tr>
<td>Asian</td>
<td>4 [8]</td>
<td>4 [8]</td>
</tr>
<tr>
<td>Black</td>
<td>6 [12]</td>
<td>24 [48]</td>
</tr>
<tr>
<td>Arab</td>
<td>0 [0]</td>
<td>1 [2]</td>
</tr>
<tr>
<td>Other</td>
<td>0 [0]</td>
<td>1 [2]</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>43 [86]</td>
<td>24 [48]</td>
</tr>
<tr>
<td>1</td>
<td>4 [8]</td>
<td>13 [26]</td>
</tr>
<tr>
<td>2</td>
<td>2 [4]</td>
<td>8 [16]</td>
</tr>
<tr>
<td>&gt;3</td>
<td>0 [0]</td>
<td>3 [6]</td>
</tr>
<tr>
<td>Full time employment</td>
<td>31 [62]</td>
<td>18 [36]</td>
</tr>
<tr>
<td>Unemployed</td>
<td>5 [10]</td>
<td>10 [20]</td>
</tr>
<tr>
<td>Primary school leaver</td>
<td>1 [2]</td>
<td>4 [8]</td>
</tr>
<tr>
<td>GCSE</td>
<td>5 [10]</td>
<td>10 [20]</td>
</tr>
<tr>
<td>A level</td>
<td>8 [16]</td>
<td>9 [18]</td>
</tr>
<tr>
<td>Degree</td>
<td>14 [24]</td>
<td>8 [16]</td>
</tr>
<tr>
<td>Higher degree/professional</td>
<td>22 [24]</td>
<td>18 [36]</td>
</tr>
</tbody>
</table>
6.1.3.3 Results of 100 women

Overall, 75% of hypertensive women reported deterioration in how they rated their current health compared to 59% of healthy women (not significant). Hypertensive disease correlated with a significantly lower number of hypertensive women (18%) returning to employment as compared with healthy controls (45%; p=0.003). There were no differences reported in relationship status, mood, or anxiety, with similar incidence recorded in hypertensive and healthy women. 39% of healthy women stated that they were enjoying being pregnant compared to 41% of hypertensive women. Financial concerns were more prevalent in the control group, perhaps representative of expected parental concern at times of domestic change, in the absence of overriding physical health concerns. The similarity between hypertensive and healthy women was unexpected. This possibly indicates the high baseline anxiety and uncertainty experienced even in healthy pregnancy, or perhaps that hypertension in pregnancy does not have a striking negative impact on most self-reported pregnancy outcomes. Compared to normal pregnancy, women with a diagnosis of hypertension in pregnancy were more likely to report a past medical history of hypertension (6% of controls and 46% of cases, p<0.001) and previous pre-eclampsia (0 controls and 10% of cases, p=0.001). However, they were no more likely to report a history of diabetes (p=0.118) or renal disease (p=1). Women with hypertension during pregnancy were also more likely to report inpatient stays during their current pregnancy as compared to controls (p<0.001); but were not more likely to report consultant care (p=0.070). This is shown in the comparative table below:
Table 6.2: Health before and during pregnancy [percentage]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls [%] (n=50)</th>
<th>Cases [%] (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-existing hypertension</td>
<td>3 [6]</td>
<td>23 [46]</td>
</tr>
<tr>
<td>Pre-existing diabetes</td>
<td>0 [0]</td>
<td>4 [8]</td>
</tr>
<tr>
<td>Previous pre-eclampsia</td>
<td>0 [0]</td>
<td>10 [10]</td>
</tr>
<tr>
<td>Under care of consultant</td>
<td>18 [36]</td>
<td>28 [56]</td>
</tr>
<tr>
<td>Inpatient stay during current pregnancy</td>
<td>4 [8]</td>
<td>35 [70]</td>
</tr>
</tbody>
</table>

The majority of healthy women were recruited to the study between 33 and 35 weeks’ gestation, perhaps because this represents the stage in normal pregnancy when women are most likely to engage with hospital services, such as antenatal classes. The hypertensive cases were recruited across a range of gestational ages, increasing closer to term, as shown in the graph below:
Women with hypertension in pregnancy were significantly more likely to report swelling (p=0.001), and in particular hand and foot swelling (p=0.026) or facial swelling (p=0.006) than controls. They were no more likely to report sickness, back pain, urinary problems, hip or pelvis pain, bleeding, abdominal pain, headaches or visual disturbances than healthy controls. Cases were more likely to report that they had suffered disruption to their employment as a result of their pregnancy (16% of controls and 32% of cases reported disruption, p=0.032) than their healthy
counterparts. However, they were less likely to report increased financial concerns as a result of their pregnancy (p=0.040)
Table 6.3: Perceived quality of life among participants [percentage]

<table>
<thead>
<tr>
<th>Quality of life indicator</th>
<th>Healthy [%] (n=50)</th>
<th>Hypertensives [%] (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How would you rate your current health?</td>
<td>12 [24]</td>
<td>29 [58]</td>
</tr>
<tr>
<td><strong>A little worse – worse ever</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compared with one month ago, how often have you felt unwell?</td>
<td>13 [26]</td>
<td>18 [36]</td>
</tr>
<tr>
<td><strong>Almost all of the time – about half of the time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How has this pregnancy affected your ability to continue your usual employment?</td>
<td>8 [16]</td>
<td>16 [32]</td>
</tr>
<tr>
<td><strong>A lot of disruption – stopped working</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How has pregnancy affected your relationship with your partner?</td>
<td>9 [18]</td>
<td>4 [8]</td>
</tr>
<tr>
<td><strong>Suffering – ended relationship</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Suffering – ended relationship</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please record how this pregnancy has affected your relationship with your children.</td>
<td>2 [4]</td>
<td>4 [8]</td>
</tr>
<tr>
<td><strong>Suffering – ended relationship</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please record how this pregnancy has affected your ability to enjoy being pregnant.</td>
<td>14 [28]</td>
<td>14 [28]</td>
</tr>
<tr>
<td><strong>Reduced moderately – no longer want to be pregnant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How has this pregnancy affected your ability to enjoy life?</td>
<td>28 [56]</td>
<td>38 [76]</td>
</tr>
<tr>
<td><strong>Reduced slightly – extremely unhappy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please record how this pregnancy has affected your ability to manage your finances.</td>
<td>18 [36]</td>
<td>8 [16]</td>
</tr>
<tr>
<td><strong>Moderate impact – cannot afford pregnancy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please record how this pregnancy has affected your ability to prepare for a new baby.</td>
<td>9 [18]</td>
<td>12 [24]</td>
</tr>
<tr>
<td><strong>Much harder to prepare – completely unprepared</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.3 above includes some of the questions included in the final draft of the study survey. Women were asked to choose an answer from a defined scale (e.g. ranging from ‘not affected at all’ to ‘extremely unhappy’). Selections of the answers are included in bold, with the question above. The table highlights the difference between hypertensive and healthy women; the presence of pre-eclampsia led to a 76% of cases reporting reduced enjoyment of life compared with 56% of healthy controls and demonstrates difficulty continuing normal function and employment. Interestingly, many more healthy women revealed difficulties in their relationship during pregnancy than pre-eclamptic women. Hypertensive women did not report any significant differences in mood as compared with healthy controls, in terms of sadness, tearfulness or anxiety (p=1, p=1 and p=0.624 respectively), although 18% of healthy and 24% of hypertensive women reported feeling anxious at least half the time.

There was no significant difference in ability to sleep (p=0.512). Women with pre-eclampsia were not more likely to use anti-depressants (p=0.362) or alcohol than healthy women (p=0.617) and admission to hospital was found not to reduce anxiety in either cohort. Furthermore, admission to hospital did not improve women’s understanding of pre-eclampsia with 38% of healthy and 54% of hypertensive women reporting that their knowledge was either unchanged or only slightly improved (p=0.816). Women with hypertensive disease of pregnancy were more likely to report that their disease experience had reduced their desire to be pregnant again, with 14% of controls and 44% of cases stating less inclination towards
subsequent pregnancy ($p=0.002$). Overall, there was less difference between hypertensive and healthy pregnancy than expected.
Table 6.4: Perceived mood among participants

<table>
<thead>
<tr>
<th>Quality of life indicator (showing negative responses on scale)</th>
<th>Healthy [%] (n=50)</th>
<th>Hypertensive [%] (n=50)</th>
<th>Fisher's (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How has this pregnancy affected your anxiety?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel anxious half of the time – I have anxiety attacks</td>
<td>9 [18]</td>
<td>12 [24]</td>
<td>0.62</td>
</tr>
<tr>
<td>How has this pregnancy affected how sad you feel?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel sad all of the time – I am depressed</td>
<td>4 [8]</td>
<td>3 [6]</td>
<td>1</td>
</tr>
<tr>
<td>How has this pregnancy affected your sleeping?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have problems sleeping half of the time – I never sleep well</td>
<td>18 [36]</td>
<td>12 [24]</td>
<td>0.51</td>
</tr>
<tr>
<td>How has this pregnancy affected how often you feel tearful?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel tearful most of the time – I cry every day</td>
<td>3 [6]</td>
<td>3 [6]</td>
<td>1</td>
</tr>
<tr>
<td>How often do you take medications for low mood?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How often do you use alcohol or other substances to improve your mood?</td>
<td>3 [6]</td>
<td>1 [2]</td>
<td>0.62</td>
</tr>
<tr>
<td>Occasionally – when not pregnant (answers 2-6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has being seen at hospital improved our anxiety?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all – improved a little</td>
<td>20 [40]</td>
<td>27 [54]</td>
<td>0.76</td>
</tr>
<tr>
<td>How has your understanding of pre-eclampsia changed?</td>
<td>19 [38]</td>
<td>27 [54]</td>
<td>0.82</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Unchanged – a bit better</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has this pregnancy made you less likely to want another?</td>
<td>7 [14]</td>
<td>22 [44]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Somewhat – I will never have another pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.1.4 Discussion

The lack of significant difference in findings between hypertensive women and their healthy counterparts suggests that, although the ED-5Q and SP-30 have proven to be an effective basis to develop PROMs for orthopaedics and other surgical specialities, they may not adequately capture clinical picture of pregnancy.

6.1.4.1 Strengths and weaknesses

The main strengths of the study lie in its novelty including its use of healthy controls with co-morbidities, rather than pre-selected healthy controls and its objective to establish a PROM in pregnancy. Limitations of the study include the small sample size and the fact women were selected from a demographic that was unlikely to be representative of the wider population due to the high number of primiparous women in the control group and the fact the study was single centred. The inclusion of women with hypertensive diseases of pregnancy other than pre-eclampsia may have skewed the results further. The questionnaire itself faced limitations given that several of the answers provided were subjective and did not relate directly to the questions asked. Further development of questions would be required to allow reliable statistical conclusions, without bias according to accepted norms (eg the presumption that women are likely to wish to pursue further pregnancy).

6.1.4.2 Future development

PROMs are a patient-centric way of assessing the effectiveness of care from the patient’s perspective. Their use has been advocated by the NHS to encourage public involvement. The Francis report highlighted a magnitude of deficiencies in patient-
centered care including; neglect of patients and poor standards of care, inadequacy of regulatory systems, issues of negative culture, tolerance of poor standards and disengagement from managerial and leadership responsibilities. The inclusion of service user experience as a means of reporting clinical performance represents a unique opportunity to provide holistic feedback at individual trust level. PROMs have been becoming more widespread across many medical specialities over the last five years, including early pregnancy, anal sphincter injury and pregnancy-associated surgery. At the time of writing, however, PROMs had not been described in obstetric literature. Maternity services are used by over 650,000 women each year (Sandall et al., 2014) and generate over £3 billion annually in litigation cases (NHS Litigation Authority 2012) highlighting the potential of woman-centric quality indicators to standardise care and improve cost effectiveness.

The development of innovative data gathering techniques and performance monitoring opportunities are key aspects of healthcare management and should take into account the views of patients. Current measures of satisfaction lack validity and specificity (Devlin NJ and Appleby J, 2010) leading to the introduction of questionnaires that are defined by patients, as well as informed by them. The relevance of PROMs is underpinned by the methodology used for their development. Rising costs and the need for funding in line with the growing number of births in the UK and patients’ expectations of quality of care mean PROMs have a role in policy making and allocation of healthcare resources. (Dawson et al., 2010) Development of PROMs in maternity services could provide a quality assessment tool that can link women’s health status to outcomes and allow funds and services to be tailored according to user needs. The CQC Maternity Surveys have shown that bench-
marking services against each other is a driver for improvement and maternity
PROMS provide a much more potent benchmark that is derived entirely from the
woman’s perspective. Strategies to aid the deployment of openness, transparency
and candour, especially in the case of deliveries that have not gone well may help
with mitigating the impact of litigation.

6.1.5 Conclusion
To our knowledge, this is the first patient reported outcome measure specifically
designed for use in pre-eclampsia. Patient reported outcomes also give an
opportunity for scientists and clinicians to align their research with patients’ interest.
Pregnancy is a complex emotional and physical time for most women and its impact
if influenced on both physical wellbeing and individual psychosocial circumstances.
Development of tools to assess patient perspectives in the evaluation of health care
is an area of interest that requires further research to better direct support services
and recording of outcomes in women with pregnancy complicated by hypertensive
disease.

PROMs link the effect of different treatments to women’s health status to deliver
compassionate, patient-centric care. Development of PROMs in maternity services
will provide a novel quality assessment tool that can direct resources appropriately
and support tailored service development. Recognising the women’s perspectives of
health care delivery could improve empathy and organisational structure to support
an individual focus whilst ensuring the most effective use of NHS resources.
However, it seems unlikely that existing PROMs would translate effectively into use
in obstetric settings and so further innovative development is necessary.
CHAPTER 7

Conclusions
7.1 Conclusion

Pre-eclampsia remains an important cause of maternal and perinatal mortality. The ability to predict the disease, particularly the more severe phenotypes, at earlier gestations, would allow clinically relevant surveillance and appropriate intervention to improve outcomes. Recent advances in understanding of its pathogenesis have led to new predictive, diagnostic and prognostic tests being evaluated. The research contained in this thesis suggests that PlGF represents a practical, point of care test, with high sensitivity and negative predictive value with the potential to better direct management strategies in high risk women below 37 weeks’ gestation. Furthermore, our evidence suggests it could be effectively funded within an NHS setting. Additional research is required to assess its position in clinical practice.

7.2 Summary of findings

The initial PELICAN Study paper (chapter 3) reported high sensitivity (95-96%) and negative predictive value (95-98%) for low PIGF in determining need for delivery for confirmed pre-eclampsia within 14 days. The need for a test with high sensitivity is paramount in this setting because there is greater preference for minimizing false negatives when considering overall benefits and harms and in ensuring appropriate resource use. The clinical utility of PIGF in facilitating stratified management strategies with appropriate surveillance is further apparent by the markedly different times to delivery demonstrated in the PELICAN study for women with very low, low and normal PIGF values; as PIGF measurements were masked to the clinicians in the study, women were delivered because of deteriorating maternal or fetal parameters, not on the basis of knowledge of PIGF values.
We have also shown that PlGF is considerably better than current markers, even when currently utilised test are combined, as in clinical practice. The area under the receiver operating characteristic curve for low PlGF (0.87, standard error 0.03) for predicting pre-eclampsia within 14 days was greater than all other commonly used tests, singly or in combination (range, 0.58–0.76), in women presenting with suspected pre-eclampsia (P<0.001 for all comparisons). We hypothesised that adding PlGF measurement to current clinical assessment of women with suspected pre-eclampsia before 37 (and particularly before 35) weeks’ gestation could improve risk stratification, achieve an earlier diagnosis and enable individualised management, with the potential to reduce associated maternal morbidity and unnecessary health service usage.

One of the key research recommendations of the NICE Hypertension in Pregnancy guidelines was “What is the role of assessing haematological or biochemical parameters at diagnosis of gestational hypertension and during surveillance of gestational hypertension?” and the logical extension of this is to consider novel diagnostic tests once prospective observational studies had identified suitable candidate biomarkers (i.e. beyond current conventional haematological and biochemical parameters). A decision analytical model using data from the PELICAN study to establish the budget impact of treating women with suspected pre-eclampsia for two weeks from the date of PlGF testing demonstrated a mean cost saving associated with the clinical algorithm integrating PlGF of £34,447 per 1,000 pregnant women or a cost saving of £574 per woman given a PlGF test. Most of the
cost savings for the clinical algorithm integrating PIGF were for pregnant women with moderate hypertension, with a cost saving of £36,706.

### 7.3 Clinical application of main findings

Pre-eclampsia remains a leading cause of iatrogenic pre-term delivery and perinatal adverse outcome. Diagnostic deliberations are common when women present to obstetric assessment units with one or more signs suggestive of pre-eclampsia. Women undergo a series of investigations, many of which are poor predictors of the need for delivery or likely adverse outcome. In practice, obstetricians require a test that identifies women requiring enhanced surveillance with possible intervention and those where the likelihood of needing imminent delivery is low, meaning outpatient care is appropriate. The development of safe clinical algorithms to direct care and avoid inappropriate admission, over-utilisation of stretched resources and unnecessary maternal anxiety would be a useful outcome. Current expectant management protocols demand considerable resource input and require clinicians to make treatment decisions around mode and time of delivery without a reliable means of assessing likely maternal outcome.

PIGF analysis represents a test for pre-eclampsia that uses a biomarker implicated in its pathophysiology and has attraction over the traditional measurement of blood pressure, urinary protein and assessment of established haematological and biochemical markers which are end-organ consequences of established disease. Accurate biomarker tests will potentially have greater impact at earlier gestations on decisions for surveillance and intervention by iatrogenic delivery (currently the only
definitive treatment). The difference in PlGF concentrations is greatest between normal and pre-eclamptic pregnancies prior to 37 weeks. Using PlGF as a test therefore offers a rational and reliable method to aid the diagnosis of hypertensive disorders of pregnancy for women presenting at earlier gestations. PlGF provides a point-of-care diagnostic advantage in women presenting with suspected pre-eclampsia and out-performs other biologically plausible biomarkers of disease.

The development of a PROM questionnaire in hypertensive pregnancy demonstrates its potential to improve the patient-doctor relationship and better direct support services. It is, however, difficult at this stage to confirm whether our findings reflect women’s views on a larger scale and further work and validation is required to assess its suitability for clinical use. Wider public and patient involvement (PPI) would ensure the inclusion of outcome measures that matter to women.

7.4 Future research

The PELICAN Study adds to an increasing body of research recommending early biomarker testing in the risk stratification of women with suspected pre-eclampsia and contributes substantial evidence to support the next phase of clinical trials. There is a window of opportunity to define its impact and role in directing clinical decision making in a real-time setting, with a view to introducing it as a test across the NHS. Given the substantial health resource use associated with assessment of women with suspected pre-eclampsia, PlGF testing has potential to provide significant financial savings for the NHS in addition to clinical benefit.
PIGF should be assessed by means of a randomised controlled trial, to explore its potential as a functional clinical test. This could be undertaken by investigating the capability of PIGF to diagnose a clinically relevant endpoint (such as need for delivery within 7 or 14 days). Timely diagnosis allows appropriate surveillance level and potential prevention of adverse outcome. Currently, a consensus based definition of ‘adverse outcome’ does not exist. It is uncertain whether PIGF could predict a composite measure of maternal adverse outcomes, as events such as pulmonary oedema or post-partum haemorrhage (that are commonly included in such composite measures) are not directly linked to disease pathophysiology. A randomised controlled trial directly comparing PIGF testing (in addition to currently used variables) against current normal practice would provide useful data as to whether PIGF represents a cost effective diagnostic test. It is worth noting, however, that most diagnostic tests, including measurement of proteinuria, have been implemented without the benefit of an evidence base from randomised controlled trials, and therefore the ‘gold standard’ or ‘referent’ may be imperfect. A study of this kind could also be used to carry out a robust health economic evaluation demonstrating resource use and associated cost implications of introducing PIGF as a diagnostic adjunct.

A randomised controlled trial of a diagnostic test may have several components: (Ferrante di Ruffano et al., 2012) evaluating whether knowledge of the novel diagnostic test (e.g. PIGF) compares against usual care within a trial setting (e.g. whether knowledge of revealed PIGF results in a shorter time to confirmed diagnosis
compared to usual care) and/or whether a diagnostic test impacts on outcomes further downstream in the disease process. This latter step is dependent on implementation of a management pathway related to the diagnostic test and is far more dependent on other factors such as clinician and patient behaviour in response to the diagnostic test result.

Since the PELICAN study was undertaken and the initial results published, grant funding has been secured to undertake a stepped wedge randomised controlled trial of PlGF testing in women with suspected pre-eclampsia (the PARROT trial). The main objectives of this trial are to test the hypothesis that knowledge of plasma PlGF measurement will reduce the time to diagnosis of confirmed pre-eclampsia, compared to current management, and to evaluate whether knowledge of plasma PlGF measurement identifies women with an increased likelihood of clinically indicated need for delivery within 14 days for diagnosed pre-eclampsia and has a beneficial impact on maternal and infant health economic outcomes.
CHAPTER 8

Project Co-ordination
8.1 My contribution to the project

I was the Project Co-ordinator for the PELICAN Study and was involved in every aspect of the study that produced the three papers included in this thesis (chapters 3, 4 and 5). I developed the initial study protocol (see appendix) and produced the majority of the study literature, including participant information leaflets, consent forms and awareness posters. I wrote the application for ethical approval and ensured information submitted to the Ethics and Research and Development Committees was updated as the study progressed. At the start of the project, I formed links with selected trusts and conducted induction meetings, whereby I presented the concept and requirements of the study, to identify units that would be suitable to take part.

Once maternity units had agreed to participate, I managed the distribution and administration of contracts between the trusts and the sponsor. Throughout the project, I regularly attended meetings with the staff at all the trusts involved, presenting information to ensure awareness of the project and trouble-shooting as issues arose. I ensured that all aspects of the project were being managed and monitored uniformly and in line with the protocol instructions.

I was based at St Thomas' Hospital and recruited the majority of participants at this site, meaning that I was able to explain the project to the women taking part and gain a useful insight into their experience. I designed and managed the database used to collate anonymous demographic details of each participant, in a meaningful way, as
well as overseeing laboratory sampling using unique numerical identifiers. I trained appropriate members of the study team at each site as to how to use the database and record necessary information.

The Triage testing kit, used to measure PIGF, was produced by Alere, San Diego. All plasma samples were sent for repeat testing and storage in San Diego. I participated in regular teleconferences between UK Project Leads and stakeholders in the US, to resolve problems and ensure a unified, coordinated approach to all aspects of the project. I remained research active throughout the duration of the project and was able to share in the team’s achievement once the study reached completion and results were ready for analysis. Alongside the main PELICAN study, I led on development of a questionnaire survey, used to develop a Patient Reported Outcome Measure for pregnancy. This piece of work provided me with a useful perspective which, I hope, is reflected in the discussion, as a powerful reminder of the potential of a point of care test, such as PIGF.

The first paper, published in Circulation, presented the initial findings from the PELICAN Study. I contributed by writing the first draft of the introduction and methods but went on maternity leave during the final stages of PELICAN and for some of the results writing phase of this paper. As such, I am the second author and my supervisor, the first. On my return, I played a major role in the required revisions of the final version and presented this work at various national and international obstetric and maternal medicine conferences. I was then closely involved with the in
depth statistical analysis and production of the second paper included in this thesis (for which I am the primary author).

The budget impact analysis was an important and carefully planned further analysis. I worked closely with a Health Economist to plan its design and structure. I conducted the data extraction at both participating sites, requiring me to produce a robust database and collect all necessary information in an appropriate format. After attending a training course in Health Economics, I wrote the paper with the Health Economist, which has since been presented at a national conference.

8.2 Associated publications

8.2.1 Oral presentations

2012: Blair Bell scientific meeting, London
2013: BMFMS international conference, Dublin
2013: Registrars’ academic meeting, Cambridge

8.2.2 Papers and manuscripts arising from this work

   Prognostic biomarkers in women with suspected preeclampsia in a prospective multicentre study *Obstet Gynaecol* (accepted, in press).

3. Duckworth S, Chappell LC, Seed PT, Mackillop L, Shennan AH, Hunter R.
   Placental Growth Factor (PlGF) in women with suspected pre-eclampsia prior to 35 weeks’ gestation: a budget impact analysis *PLoS ONE* (submitted, under review).
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APPENDICES
PRE-ECLAMPSIA: CLINICAL APPLICATION OF PLGF

Assessment of the Clinical Utility of Placental Growth Factor in Women Presenting with Signs and Symptoms of Pre-eclampsia

13 May 2010-Amendment 1 November 2010

Version 4 (July 2011)

Synopsis

PELICAN is a multi-center, prospective, observational study to support the development of clinical management guidelines for the interpretation and use of Placental Growth Factor (PIGF) in pregnant women with clinical signs and/or symptoms of pre-eclampsia (measured in EDTA anti-coagulated human plasma.)

The goals of the study are to validate PIGF as an aid to the diagnosis of pre-eclampsia and as a predictor of final pregnancy outcome. The results of this study will be used to develop clinical management guidelines, to be validated by a follow-up study. The primary analysis will use the Triage® PIGF Test.

Pregnant women over 16 years of age and between 20 to 37 weeks of gestation with signs or symptoms of pre-eclampsia will be enrolled. Baseline demographics and medical/gestational history will be recorded, as well as the participant’s symptoms, signs and laboratory data relevant to the evaluation of pre-eclampsia.

Study blood samples will be collected for the measurement of PLGF on the Triage system at the following timepoints:

- Upon initial study enrollment with signs or symptoms of pre-eclampsia;
- If the participant is not believed to have pre-eclampsia upon the initial evaluation but returns with new or worsening symptoms or signs of pre-eclampsia;
• At follow up consultations as appropriate, (samples more than a week apart);
• At the time of hospital admission for further diagnostic testing or for expectant management;

EDTA-anti-coagulated venous whole blood (~10-20ml) will be collected at each of the aforementioned time points, processed to plasma and evaluated on the Triage PLGF assay.

All measurements of PLGF in plasma samples will be performed at the enrolling sites. PlGF measurements will be blinded to all attending clinicians and not used in the routine clinical management of participants.

1 Background and Rationale

1.1 Pre-eclampsia

While most pregnancies are reassuringly healthy, up to 20% of women have obstetric or medical complications requiring additional treatment and evaluation. The most common obstetric complication is preterm labour, followed closely by the various hypertensive disorders of pregnancy.

Hypertension complicates 6-12% of all pregnancies[3], and includes two relatively benign conditions (namely, chronic and gestational hypertension), and the more severe conditions of pre-eclampsia or eclampsia. Pre-eclampsia complicates 3-5% of all pregnancies, and is characterized by placental and maternal vascular dysfunction which may lead to adverse outcomes such as severe hypertension, stroke, seizure (eclampsia), renal and hepatic injury, hemorrhage, fetal growth restriction, or even death.[4] Pre-eclampsia may develop from 20 weeks gestation through six weeks post-partum.

The diagnosis of pre-eclampsia, and hence the prediction of adverse events, is based on traditional but unreliable and nonspecific clinical markers such as blood pressure, urine protein excretion, and symptoms. For example, more than 20% of women who have eclampsia will fail to meet the common diagnostic criteria of pre-eclampsia prior to their event, making the prediction of this adverse outcome extremely difficult.[5] Conversely, only 0.7 to 5.0% of women with classically defined pre-eclampsia will experience any composite adverse outcomes.[6] Thus, the traditional criteria for pre-eclampsia perform poorly in identifying women and infants at risk of adverse outcome, and consequently this clinical uncertainty leads to significant overutilization of ancillary testing and intervention. Not surprisingly, the suspicion of pre-eclampsia is the most common reason for iatrogenic preterm delivery and labour induction in the U.S.[7, 8] Multiplied by 4 million births per year, the potential for unintentional harm and economic waste is obvious.

There is a clear need for rational improvement in the diagnosis of pre-eclampsia to 1) improve the evaluation of women and infants at risk for adverse outcomes, and 2) reduce unnecessary testing,
intervention, and expense in cases with a benign prognosis. While many biomarkers and imaging
techniques have been evaluated for this purpose, none have adequate sensitivity, specificity, and
cost-benefit for the diagnosis or prediction of pre-eclampsia.[9,10] Furthermore, very few of these
markers have been independently evaluated for their ability to separately predict the timing or
severity of specific adverse outcomes such as placental abruption, severe hypertension, neurological
injury, fetal growth restriction, etc. The reason for these disappointing results is that, until recently,
the unique pathophysiology of pre-eclampsia was not understood, and the biomarkers previously
studied were mostly generic indicators of vascular activation and dysfunction which arise late in the
disease process, and which are not specific to pre-eclampsia - or even to pregnancy. However,
recent advances have identified a class of pregnancy-specific angiogenic and anti-angiogenic factors
which are produced by the placenta, and which closely correlate with the preclinical and clinical
stages of pre-eclampsia.[11,12] The possibility now exists to develop assays for these biomarkers
that would finally offer a rational and reliable way to aid in the diagnosis of the hypertensive
disorders of pregnancy.

1.2 PlGF as a Marker of Pre-eclampsia

The placenta plays a central role in the pathogenesis of pre-eclampsia [13], as evidenced by the
rapid disappearance of clinical signs or symptoms following delivery of the placenta.

The maternal syndrome of hypertension, proteinuria and oedema is part of a severe systemic
inflammatory response that includes leukocyte and endothelial cell activation. Although the origins
of pre-eclampsia remain unclear, a major cause is the failure to develop an adequate blood supply to
the placenta, leading to placental oxidative stress.[14]

Current evidence suggests that the clinical signs or symptoms of pre-eclampsia may be mediated, in
part, by an imbalance of circulating angiogenic factors of placental origin. Placental growth factor
(PlGF) is made by the placenta and circulates at high concentration in normal pregnancy. In pre-
eclampsia, there is increased expression of soluble fms-like tyrosine kinase-1 (sFlt1) which binds to
circulating PlGF.[15-17] Consequently, concentrations of plasma PlGF are found to be decreased in
pre-eclampsia.[18-20]

Gestational age also affects circulating levels of PlGF. PlGF concentrations peak at 26 to 30 weeks
and then decline as term approaches.[21] PlGF levels are abnormally low in patients with pre-
eclampsia compared to controls of approximately the same gestational age and PlGF is lower in
severe pre-eclampsia compared with mild pre-eclampsia.[22] Thus, a diagnostic test utilizing PlGF to
aid in the diagnosis of pre-eclampsia may be optimized by the use of different PlGF cut-off levels at
different gestational ages.
1.3 The Triage® PlGF Test

The Triage® PlGF Test is a fluorescence immunoassay to be used with the Triage® Meter for the quantitative determination of Placental Growth Factor (PlGF) in EDTA anti-coagulated plasma specimens. The test is intended for use as an aid in the diagnosis of pre-eclampsia in conjunction with other diagnostic and clinical information.

The Triage® PlGF Test device is designed to be compatible with EDTA plasma samples for measuring the concentration of PlGF present in the sample. The results (PlGF pg/ml concentration) can be displayed, printed, and temporarily stored. The test device contains a positive and negative internal control mechanism. The system is provided with compatible external liquid QC controls which are supplied separately.

The test procedure involves the addition of a specified volume of EDTA anti-coagulated plasma to the test device using a disposable transfer pipette, insertion of the inoculated device into the Meter, reading of the result from the display screen or printout, and storage of the result. Results are typically obtained within 15 minutes.

2 Study Objectives

2.1 Primary Study Objective

The primary study objective is to assess the clinical utility of plasma PlGF in the diagnosis and management of pre-eclampsia.

3 Study Design

3.1 Intended Use

The Triage® PlGF Test is a fluorescence immunoassay to be used with the Triage® Meter for the quantitative determination of Placental Growth Factor (PlGF) in EDTA anti-coagulated plasma specimens. The test is intended for use as an aid in the diagnosis of pre-eclampsia in conjunction with other diagnostic and clinical information.

3.2 Overview of Study Design

Pregnant women over 16 years of age and between 20 to 37 weeks of gestation with signs or symptoms of pre-eclampsia will be enrolled. Baseline demographics and medical and gestational history will be recorded, as well as the participant’s symptoms, signs and laboratory data relevant to the evaluation of pre-eclampsia.

Study blood samples can be collected for the measurement of PLGF on the Triage system at the following timepoints:
• Upon initial study enrollment with signs or symptoms of pre-eclampsia;
• If the participant is not believed to have pre-eclampsia upon the initial evaluation but returns with new or worsening symptoms or signs of pre-eclampsia;
• At follow up consultations as appropriate, (samples more than a week apart);
• At the time of hospital admission for further diagnostic testing or for expectant management);

EDTA-anti-coagulated venous whole blood (~20ml) can be collected at any/each of the aforementioned time points; these blood draws will occur in parallel with other clinical testing being performed as routine standard care. These study blood samples will be processed to plasma and tested on the Triage System.

The final diagnosis and adverse maternal, fetal and neonatal outcomes will be recorded.

The utilization of healthcare resources associated with the diagnosis and management of pre-eclampsia will also be recorded.

3.3 Blinding
The results of the Triage PlGF Tests will be blinded to the medical personnel at the site involved in care of the participant during the study and will not impact medical management of the participants.

3.4 Study Duration
Each participant’s involvement in the study will last from enrollment to delivery. Post delivery outcome data will also be recorded.

4 Study Participants
4.1 Number of Participants
Approximately 500 pregnant women between 20 and 40 weeks gestation will be initially enrolled, during a pilot phase, at multiple clinical centers in the UK and Ireland. Further women (20-37 weeks gestation) will be recruited across an additional five UK centres from July 2011.

The table below shows the breakdown, per gestational age, of the expected and targeted groups of women enrolled in the study:
Assumptions for UK Training Study

<table>
<thead>
<tr>
<th>GA Bin</th>
<th>%Population</th>
<th>non-PE</th>
<th>PE</th>
<th>Total (in Bin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>7.0%</td>
<td>25</td>
<td>11 (target)</td>
<td>35 (target)</td>
</tr>
<tr>
<td>24-29</td>
<td>13.0%</td>
<td>46</td>
<td>20</td>
<td>65</td>
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<tr>
<td>29-32</td>
<td>20.0%</td>
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<td>70</td>
<td>30</td>
<td>100</td>
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<tr>
<td>35-37</td>
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<td>70</td>
<td>30</td>
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</tr>
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<td>37-40</td>
<td>20.0%</td>
<td>70</td>
<td>30</td>
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</tr>
<tr>
<td>Total</td>
<td>100.0%</td>
<td>350</td>
<td>150</td>
<td>500</td>
</tr>
</tbody>
</table>

Minimum Enrollment Criteria

(1) target of 10 PE cases per GA bin.

(2) minimum of 60 PE cases for GA < 35 weeks.

(3) minimum of 40 PE cases for GA >= 35 weeks.

(4) minimum of 25 non-PE cases per GA bin.

(5) minimum of 150 non-PE cases for GA < 35 weeks.

(6) minimum of 100 non-PE cases for GA >= 35 weeks.

Sample Size

For a sensitivity of 0.90 and a sample size of 60 PE, the 95% confidence interval is 0.795 to 0.962.

For a specificity of 0.90 and a sample size of 150 non-PE, the 95% confidence interval is 0.840 to 0.943.

(Further breakdown of statistical considerations can be seen in section 7.)

4.2 Inclusion / Exclusion Criteria

To be enrolled in the study, each participant must meet all of the following inclusion criteria and none of the following exclusion criteria:
4.2.1 Inclusion Criteria
   a. Age 16 or over at enrollment;
   b. Pregnancy at 20+0 to 40+6 weeks gestation;
   c. Signs or symptoms of pre-eclampsia.
   d. Able to give informed consent
   e. Singleton or twin pregnancy

4.2.2 Exclusion Criterion
   a. Unable to give informed consent
   b. Gestation with 3 or more viable fetuses at the time of enrollment

4.3 Targeted Participants for Enrollment
Any woman suspected of having pre-eclampsia may be included. The following section is a guide as to who this may include.

Sites are encouraged to enroll participants with a variety of presenting symptoms, signs and findings suggestive of the diagnosis of pre-eclampsia, or where there is clinical uncertainty.

Appropriate study participants include women with signs or symptoms suggestive of the presence of pre-eclampsia with onset after gestational week 20+0, such as:

- New onset of increases in blood pressure – such as:
  o Resting systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90; or
  o ≥ 30 mmHg increase in systolic and/or ≥ 15 mmHg increase in diastolic BP compared to average of 1st trimester values (≤ 14+0 weeks).

- Worsening of underlying hypertension – defined as pre-existing hypertension now with any one of the following:
  o New onset of resting systolic BP ≥ 160 mmHg or diastolic BP ≥ 105 mmHg; or
  o Need for doubling of pre-20 week dose of antihypertensive; or
  o Need for addition of second antihypertensive agent.

- New onset of protein in urine – defined as any one of the following:
  o ≥ 1+ proteinuria by dipstick in the absence of urinary tract infection
  o ≥ PCR 30mg/mmol
  o 24 hour collection ≥300 mg (or 165mg/12hr collection)

- New onset symptoms including:
  o Persistent epigastric or right upper quadrant pain;
- Nausea and vomiting;
- Headaches, visual disturbances or migraines (in participant without a history of migraines).

- Unexplained laboratory anomalies including:
  - Thrombocytopenia (platelets ≤ 100,000);
  - Aspartate aminotransferase (AST) ≥ ULN;
  - Alanine aminotransferase (ALT) ≥ ULN;
  - Lactate dehydrogenase ≥ ULN;
  - Serum creatinine ≥ 80 IU/dL;
  - Uric acid ≥ 4 mg/dL;

- Evidence of abnormal fetal growth or placental function:
  - Fetal growth restriction – defined as ultrasound estimated fetal weight ≤ 3rd percentile for gestational age;
  - Fetal or placental hydrops;
  - Uterine artery Doppler notching or increased resistance;
  - Suspected placental abruption.

- Other unexplained clinical events:
  - Suspected pulmonary oedema;
  - Suspected or possible seizure activity.

As PIGF levels vary with gestational age, it is desired that enrolled participants span the entire range of gestational ages from 20 to 37 weeks, with participants enrolled in each of the following strata:

- Gestational age 20+0 to 23+6 weeks;
- Gestational age 24+0 to 28+6 weeks;
- Gestational age 29+0 to 31+6 weeks;
- Gestational age 32+0 to 34+6 weeks;
- Gestational age 35+0 to 36+6 weeks;

Participants with twins may be enrolled.

Enrollment of participants with signs or symptoms of pre-eclampsia with underlying chronic hypertension and gestational hypertension (mild and severe) who meet study criteria are also encouraged so that the utility of the Triage PIGF Test in this subset of participants can be assessed.
Enrollment of participants with signs or symptoms of pre-eclampsia where either the diagnosis is unclear or the future risk to the mother or fetus remains unclear is encouraged.

Similarly, enrollment of participants with signs or symptoms of pre-eclampsia plus co-morbid conditions which can confound the diagnosis of pre-eclampsia is also encouraged, such as:

- Chronic hypertension;
- Renal disease;
- Diabetes (gestational or pre-existing);
- Lupus erythematosus;
- Anti-phospholipid antibodies;
- Solid organ transplantation.

4.4 Participant Withdrawal and Replacement
A participant will be considered inappropriate for evaluation, for the primary purposes of this study, if she:

- Withdraws consent for study participation or is lost to follow-up before information on the final diagnosis and adverse maternal and fetal outcomes can be obtained.

Participants who are inappropriate may be withdrawn from the study.

Participants may voluntarily choose to withdraw from the clinical study at any time. Participants may also be withdrawn by the Investigator due to noncompliance with study procedures.

4.5 Screening
The study must be explained to each potential study participant and written informed consent obtained prior to enrollment. All participants will be offered at least 24 hours to consider enrollment, but can choose to participate the same day if they wish.

Prior to enrollment, the potential participant’s medical history and status should be reviewed to assure they meet the study inclusion/exclusion criteria based upon available information.

No study-specific procedures should be performed (i.e. study-related blood draws) until after written informed consent has been obtained for that participant.
4.6 At Enrollment (Initial Study Visit)

Screening and enrollment may occur at the same clinic visit. Once it has been determined that the participant meets the study enrollment criteria (including signs or symptom of pre-eclampsia) and written informed consent has been obtained, the following procedures will be performed:

Demographics and baseline medical / gestational history will be recorded on the CRF, including:

- History of chronic co-morbid conditions (such as hypertension, diabetes, renal disease [including proteinuria] and auto-immune diseases) or solid organ transplantation;

- Information related to risk factors for pre-eclampsia, such as:
  - Gravidity and parity;
  - Previous history of pre-eclampsia;
  - Family history in first degree female relatives of pre-eclampsia;
  - Number of viable fetuses at enrollment;
  - Interval since previous pregnancy;
  - Anti-phospholipids antibodies;
  - Polycystic ovarian syndrome;
  - BMI >30 prior to pregnancy;
  - Tobacco use/Nicotine patch use;

- Available information on blood pressure or proteinuria prior to this pregnancy, in the first trimester, and in the second trimester, prior to gestational week 20+0.

Concomitant medications will be recorded, including any anti-hypertensive agents, aspirin, heparin, insulin and corticosteroids (with dosage).

Blood pressure obtained by the clinical staff will be recorded following local practice.

Clinical Blood Samples:

These will be taken at the discretion of the attending clinicians following local guidelines.

NB: The clinical team will be blinded to all results from the Triage System.

If any other laboratory assessments are performed to assess for possible pre-eclampsia (uric acid, liver function etc) the results of those tests will also be recorded.
Research Blood Sample:

Approximately 10-20 ml of blood will be collected in “lavender top” Vacutainer tubes with EDTA anti-coagulant. One aliquot will be tested at the study site on Triage PI GF and the result recorded.

The remaining, unused, plasma sample will be stored, in accordance with local hospital policy. Storage will be at local hospital sample laboratory, for a period of less than 12 months. The remaining frozen, plasma aliquots (each 2ml) will be shipped to The Alere Research Centre, in San Diego, United States. This transfer is to enable possible further analysis, and/or validation, co-ordinated by our international, commercial collaborators, in the event that future pre-eclampsia, (or other hypertensive disorders of pregnancy,) biomarkers are identified. The Alere Research Centre will hold the plasma samples for a period in of 25 years. The Research Centre is FDA compliant and will hold and dispose of the samples accordingly.

Clinical sites will evaluate and treat participants with signs or symptoms of pre-eclampsia according to their usual medical and institutional standards.

The decision to proceed immediately to delivery, admit the participant to the hospital for further diagnostic evaluations, or expectant management, or to manage the participant as an outpatient, will be at the discretion of the attending physician and should not be affected by study participation.

The initial disposition of the participant will be recorded.

Once the results of the initial assessments are available, the Investigator will record the participant’s initial diagnosis at the time of the enrollment assessment using the definitions as delineated in Section 5.

4.7 During the Pregnancy

If the participant did not meet diagnostic criteria for pre-eclampsia at the initial study visit but this diagnosis is again suspected at least one week later during the pregnancy due to new or worsening symptoms, signs or findings, the assessments will be repeated and recorded. The signs, symptoms and findings suggestive of pre-eclampsia at this return visit will be recorded.

If the participant is admitted to the hospital for further diagnostic testing or expectant management for pre-eclampsia after the initial study visit, an additional biomarker blood sample (~20ml each) will be collected in “lavender top” Vacutainer tubes with EDTA anti-coagulant.
The sample will be processed to plasma and PIGF will be measured using the Triage System.

NB: The clinical team will be blinded to all results from the Triage System.

Results of additional tests performed to monitor for pre-eclampsia as usual care during the remainder of the pregnancy will also be recorded.

All hospital admissions/discharges and any interventions (dialysis, intubation, etc) will be recorded. This information will be used to inform health economic analysis, to demonstrate the following:

- Day Unit attendances
- Ante-partum bed nights
- Post-partum bed nights
- Fetal monitoring by ultrasound
- Special Care Baby Unit bed nights
- Neonatal Intensive Care Unit bed nights
- Administration of corticosteroids and magnesium
- Biochemical analysis of serum and/or urine

4.8 Post-partum

Information about the delivery and neonate will be recorded, such as:

- The date/time of onset of labor and date/time of delivery;
- Mode of delivery (e.g., spontaneous or induced, labor, C-section or vaginal delivery);
- Whether epidural anesthesia was used;
- Neonatal birth weight;
- Neonatal birth defects and genetic abnormalities;
- Placental weight and pathology (infarcts, etc), if available.

The use of any medications for the treatment of hypertension, antenatal steroid or IV magnesium during the pregnancy / peripartum period will be recorded.

If any subsequent information has led to a revision of the estimated gestational age at the time the initial visit study blood samples were obtained, the revised gestational age at initial assessment will be recorded.

Any adverse maternal, fetal or neonatal outcomes, including during the peri-natal period, will be recorded.
Each participant’s clinical course and test results during the entire pregnancy (and peri-natal period, where appropriate) will be reviewed by the Investigator. The Investigator will then record the final diagnoses for each participant using the diagnostic definitions described below (with the dates diagnostic criteria were first met), taking into account the entire clinical course of the participant’s pregnancy.

Assessments of blood pressure, urine protein and other laboratory assessments to support the diagnosis will also be recorded.
5 Definitions

5.1 Diagnoses

For the purposes of this study, the following definitions will be utilised for diagnoses, based upon the American College of Obstetrics and Gynecology (ACOG) practice guidelines bulletin [23]:

**Hypertension**

- **Mild hypertension:**
  
  Systolic BP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg on two occasions 4 hours to 1 week apart;

- **Severe hypertension:**
  
  Systolic BP ≥ 160 mm Hg or DBP ≥ 110 mm Hg on two occasions at least 4 hours apart while the patient is on bed rest;

- **De novo hypertension:**
  
  New onset of hypertension after 20+0 weeks gestation (with documented non-hypertensive blood pressures prior to 20+0 weeks gestation);

- **Chronic hypertension:**
  
  Documented presence of chronic non-gestational hypertension prior to this pregnancy, or

  De novo hypertension that does not resolve by 6 weeks postpartum, or

  On anti-hypertensive medication prior to 20+0 weeks or at 6 weeks post-partum.

- **Gestational hypertension:**
  
  De novo hypertension after gestational week 20+0 or hypertension that resolves by 6 weeks postpartum - without proteinuria or markers of severe pre-eclampsia.
Mild gestational hypertension:

SBP 140-159 mm Hg and/or DBP 90-109 mmHg on two occasions 4 hours to 1 week apart presenting de novo after gestational week 20 without proteinuria or markers of severe pre-eclampsia.

Severe gestational hypertension

SBP ≥ 160 and/or DBP ≥ 110 mm Hg on two occasions 4 hours to 1 week apart presenting de novo after gestational week 20 without proteinuria or markers of severe pre-eclampsia.

Proteinuria:

Any of the following*:

- Urine protein ≥ 300 mg/24 hours (or 165mg/12hr) from a timed urine collection (preferred definition, if results from 24-hour timed urine collection available);
- Urinary protein 1+ on dipstick on two occasions at least 4 hours apart;
- Urinary protein ≥2+ on dipstick on one occasion.
- Protein: Creatinine ratio ≥ 30 (mg/mmol).

*In the absence of a symptomatic urinary tract infection.

Gestational proteinuria:

De novo proteinuria after 20+0 weeks gestation (with a negative proteinuria assessment prior to 20+0 weeks gestation).

Chronic proteinuria:

Proteinuria noted prior to 20+0 weeks; or proteinuria that fails to resolve by 6 weeks postpartum.

Pre-eclampsia (Traditional Definition):

Gestational hypertension plus gestational proteinuria.
Mild pre-eclampsia:

Mild gestational hypertension plus gestational proteinuria that does not meet the criteria for severe pre-eclampsia as stated below.

Severe pre-eclampsia:

Presence of pre-eclampsia as defined above plus one or more of the following:

- Systolic BP ≥ 160 mm Hg or DBP ≥ 110 mm Hg on two occasions at least 6 hours apart while the patient is on bed rest;
- Proteinuria of 5 g or higher in a 24-hour urine specimen or 3+ or greater on two random urine dipstick assessments collected at least 4 hours apart;
- Oliguria of less than 500 mL urine output in 24 hours;
- Cerebral or visual disturbances;
- Pulmonary edema or cyanosis;
- Epigastric or RUQ pain;
- Impaired liver function (2X ULN for AST and/or ALT);
- Thrombocytopenia (platelet count <100,000/mm³);
- Fetal growth restriction (fetal weight <5th percentile for gestational age).

Superimposed pre-eclampsia (Traditional definition):

- Chronic hypertension plus new onset proteinuria (defined as urine protein ≥ 300 mg/24 hours from a timed urine collection)

Superimposed pre-eclampsia (Atypical)[24]:

Any of the following:

- Chronic hypertension plus new onset and persistent symptom(s) (e.g., headache and/or scotomata and/or epigastric pain);
- Chronic hypertension plus abnormal laboratory test (low platelets or elevated liver enzymes).
Eclampsia:

The presence of new-onset grand mal seizures in a woman with pre-eclampsia or gestational hypertension in the absence of all of the following:

- Known seizure disorder;
- Chronic treatment with anti-seizure medications;
- Known intra-cerebral pathology.

Atypical pre-eclampsia:[25]

In the absence of proteinuria:

Gestational hypertension plus any of the following:

- Presence of symptoms of pre-eclampsia: epigastric pain, headache, nausea and vomiting, visual changes (see definitions in Section 6.2);
- Hemolysis;
- Thrombocytopenia (platelet count <100,000/mm\(^3\));
- Elevated liver function tests (2X ULN for AST and/or ALT).
- IUGR fetal weight <10%

In the absence of hypertension:

Gestational proteinuria plus any of the following:

- Presence of symptoms: epigastric pain, headache, nausea and vomiting, visual changes (see definitions in Section 6.2).
- Hemolysis;
- Thrombocytopenia (platelet count <100,000/mm\(^3\));
- Elevated liver function tests (2X ULN for AST and/or ALT).
- IUGR fetal weight <10%

HELLP syndrome:

Gestational hypertension or gestational proteinuria plus elevated liver enzymes (2X ULN), elevated LDH (2X ULN) and thrombocytopenia (platelet count <100,000/mm\(^3\)).
5.2 **Symptoms**

The following definitions of symptoms will be utilized for this study:

**Epigastric pain:**

Right upper quadrant to mid upper abdominal deep pain that is persistent and not related to dietary intake.

**Headache:**

Moderate to severe headache that persists following appropriate medication in a participant without a prior history of chronic headaches.

**Nausea and vomiting:**

Persistent, unexplained nausea or emesis unresponsive to treatment.

**Visual changes:**

Participant complains of visual impairment that is persistent.

5.3 **Other Prior / Concomitant Conditions**

**History of pre-eclampsia:**

Participant recalls a prior pregnancy affected by pre-eclampsia, toxemia, eclampsia, HELLP syndrome, or

Participant can not recall name of condition but reports a prior that was

- induced for hypertension, or
- treated with magnesium sulphate

Any known family history of the above, for a first degree relative (mother or sister.)

**Pre-existing proteinuria:**

- \( \geq 1+ \) proteinuria by dipstick in the absence of urinary tract infection
- \( \text{P:C Ratio} \geq 30\text{mg/mmol} \)
- \( \geq 165 \text{ mg/12 hour collection or } \geq 300 \text{ mg/24 hours urine protein collection} \)

**History of diabetes:**

Pre-gestational diabetes

- Type I – insulin-requiring diabetes
  - Participant taking (or prescribed) insulin prior to pregnancy
Type II – non-insulin requiring diabetes
  ▪ Participant taking (or prescribed) oral diabetic agents prior to pregnancy

Gestational diabetes
  o Prior pregnancy affected by gestational diabetes
    ▪ No interval diagnosis of diabetes
  o Current pregnancy affected by gestational diabetes

History of renal disease:
  Participant reports any history of kidney disease and documented serum Cr ≥ 80 to 20+0 weeks

History of lupus or arthritis:
  Participant reports history of
    o Lupus
    o Arthritis
    o Unspecified autoimmune disease
  and
  Documented abnormality of rheumatologic laboratory test prior to, or during pregnancy

History of anti-phospholipid antibody syndrome:
  Participant reports history of antiphospholipid syndrome
  and
  Documented laboratory abnormality by any of the following (2 occasions, 12 weeks apart):
    o Anticardiolipin IgG
    o Anticardiolipin IGM
    o Presence of lupus anticoagulant

Gravidity:
  Total number of pregnancies, including current pregnancy

Parity:
  Number of birth events, categorised as:
    o Term births (≥ 37+0 weeks),
    o Preterm births (between 20+0 and 36+6 weeks), and
    o Pregnancy loss or termination less than 20+0 weeks
5.4 Adverse Maternal, Fetal and Neonatal Outcomes

Any maternal, fetal and neonatal adverse outcomes (with date of onset) will be recorded, including the following:

**Adverse maternal outcomes:**

- Acute renal failure (≥100 micromol/L AN, or ≥130 PN)
  - Need for dialysis
- Acute myocardial ischemia
- Need for third IV agent to control blood pressure (e.g., in addition to labetolol and hydralazine)
- Hypertensive encephalopathy
  (Altered mental status with characteristic cerebral imaging)
- Cortical blindness
- Retinal detachment
- Stroke (ischemic or hemorrhagic)
  (Focal motor impairment of a sustained or permanent nature)
- Pulmonary edema/Adult respiratory distress syndrome (ARDS)
  (Characteristic pulmonary imaging in addition to oxygen requirement)
  - Need for mechanical ventilatory support (other than for Cesarean section)
- Disseminated intravascular coagulation (DIC)
- Thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome (HUS)
- Acute fatty liver
- Liver hematoma or rupture
- Placental abruption
  (Retroplacental clot or associated with pre-term delivery or fetal demise)
- Death

**Adverse Fetal Outcomes:**

- Preterm delivery (prior to 37+0 weeks gestation)
- Fetal growth restriction (fetal weight below 10th percentile for gestational age)
- Severe fetal growth restriction (fetal weight below 5th percentile for gestational age)
- Antepartum / intrapartum fetal death
- Customized SGA <10th centile

**Adverse Neonatal Outcomes:**

- Neonatal death
- Respiratory distress syndrome
- Intraventricular hemorrhage*
- Necrotising enterocolitis
- Bronchopulmonary dysplasia
- Periventricular leuomalacia*
- Retinopathy of prematurity*
- Seizure
- NICU admission for >48 hrs (for full-term infant).
  * = record stage
Severe adverse outcomes are those that result in major organ failure or death. Non-severe adverse outcomes are those that do not result in major organ failure or death.

5.5 **Health Outcome Data/Health Economics**

Data on the utilisation of health resources will be recorded including the following;

Maternal: *(Bed night is defined as a day with an overnight stay)*

- Number of out-patient visits / healthcare contact associated with the diagnosis of suspected pre-eclampsia;
- Number of out-patient visits associated with the monitoring of confirmed pre-eclampsia;
- Number of bed nights for “diagnostic testing” including investigations performed (urinalysis and serum analysis);
- Number of bed nights for “expectant management and delivery”;
- Total number of day assessment bed days;
- Intervention provision as a result of suspected pre-eclampsia eg. administration of corticosteroids or IV magnesium;
- Adverse maternal outcome and subsequent treatment;

Fetal:

- Need for admission into neonatal care;
- Need for admission for Special Care Baby Unit
- Number of bed nights in neonatal care associated with delivery for PE;
- Neonatal complications in post-natal period including seizures, intraventricular haemorrhage, respiratory distress;

6 **Biomarker Blood Sample Collection Procedures**

6.1 **Collection of Biomarker Blood Samples**

6.1.1 Study-specific venous blood samples must be obtained via direct venepuncture or via an existing central venous line, peripheral intravenous line or hep-lock (as long as the hospital staff follows their protocol for first withdrawing blood to flush the line). The route by which each blood sample was obtained and the actual date/time it was obtained must be recorded for each sample on the Case Report Form.
6.1.2 Gently invert each blood collection tube at least 8 – 10 times to allow for dissolution of the additive (if any). It is very important that the blood sample is mixed properly to ensure adequate handling and improved quality of the samples in subsequent steps.

6.1.3 Using a single sheet of barcode labels for each time point, affix:

- one barcode label from the sheet to the blood collection tube(s) and
- one barcode label from the same sheet to each of the appropriate area(s) on the Sample Collection Form.

The labels on each sheet have the same 6-digit number and are followed by a unique letter (Example: 001234A, 001234B, 001234C, etc).

Each blood draw time point should be associated with a different 6-digit number obtained from a new (unused) single sheet of barcode labels.

6.1.4 The blood sample tubes should be transported promptly (with the corresponding used sheets of barcode labels) for processing.

6.2 Processing of Blood Samples
Study blood biomarker samples from all time points will be analysed at the site for PlGF. Leftover sample will be processed to plasma, aliquoted and frozen.

Sample Replacement
A blood sample will be considered inappropriate for assessment for this study if:

- It contains an insufficient volume to perform the PlGF measurements;
- A plasma sample is grossly hemolysed;
- It was not collected in the proper type of collection tube;
- It is not properly labeled with a barcode label;
- It is not properly frozen at the site.

If the site personnel become aware that a sample is inappropriate, a replacement sample can be obtained from the participant if this can be done within 24 hours.
6.3 Disposition of Plasma Samples after Study Completion

After completion of this study, the remaining unused plasma from each sample will be stored at the relevant laboratory, according to local guidelines. These samples will NOT be submitted to a cell/DNA bank and will NOT be used for genetic testing. Each sample will be identified only by its barcode number and will not be individually identifiable.

6.4 Possible Risks & Benefits Associated with Study Procedures

There is no health benefit to participants by participating in this study. The Triage PlGF Test results will not be used in the management of patients.

The only parts of this protocol that are experimental are the measurement of biomarkers in blood specimens. All other aspects of this protocol are considered routine care.

The only study-related procedures that could impact participant safety are the blood draws to obtain blood samples required for the measurement of PlGF and other biomarkers.

The risks of drawing blood via venepuncture may include pain, bleeding, bruising or swelling at the site of the blood draw or lightheadedness or syncope. Infection at the site of the blood draw is also a rare complication. Whenever possible, study-specific blood samples will be drawn at the same time as routine blood draws to minimise the need for multiple venepunctures.

There is no risk to the participant associated with running the Triage PlGF Test, as these tests are performed in vitro on blood samples.

Also, as the results of the study-related tests will be blinded to the medical team during the study, the results of these tests will not alter participant care.

No DNA testing will be performed on any samples.
7  **Statistical Considerations (PELICAN study)**

7.1  **Objectives**

The primary objective is to measure PlGF levels in samples of EDTA-anti-coagulated plasma collected from pregnant women with signs or symptoms of pre-eclampsia to determine whether PlGF levels predict maternal and fetal adverse outcomes and might be useful as a safe and reliable parameter to guide clinical management.

7.2  **Normal and abnormal values of PlGF**

PlGF levels are known to vary with gestation in normal pregnancies (Krauss 2004, Levine 2006, Romero 2008). The reference values for the PELICAN study are not defined in the protocol.

Two approaches to defining Normal ranges will be used

(i)  Predefined standard values

(ii)  Gestation-adjusted centiles

7.2.1  **Predefined standard values**

Standard values of PlGF are by Alere with the *Triage* ® machine, corresponding to the 5\(^{th}\) centile in a healthy sample of 2207 pregnant women. (Table 1 below).

<table>
<thead>
<tr>
<th>GA Range (weeks)</th>
<th>PlGF Cut-off (pg/mL)</th>
<th>Clinical Sensitivity</th>
<th>95%LCI</th>
<th>95%UCI</th>
<th>Test Neg</th>
<th>Test Pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 ≤ GA &lt; 24</td>
<td>62.9</td>
<td>1.000</td>
<td>0.025</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>24 ≤ GA&lt; 29</td>
<td>130</td>
<td>1.000</td>
<td>0.664</td>
<td>1.000</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>29 ≤ GA &lt; 32</td>
<td>128</td>
<td>0.889</td>
<td>0.518</td>
<td>0.997</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>32 ≤ GA &lt; 35</td>
<td>70.4</td>
<td>0.944</td>
<td>0.727</td>
<td>0.999</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>GA ≥ 35</td>
<td>14.6</td>
<td>0.535</td>
<td>0.377</td>
<td>0.688</td>
<td>20</td>
<td>23</td>
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<tr>
<td>19 ≤ GA &lt; 35</td>
<td>Variable as above</td>
<td>0.946</td>
<td>0.818</td>
<td>0.993</td>
<td>2</td>
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</table>
Assessment of test performance.
Using these two systems, all PlGF measurements will be assessed as normal or abnormal. Measurements are to be repeated up to 5 times per woman, depending on gestation at trial entry. The first measurement is taken at the earliest worrying sign for preeclampsia (abnormal proteinuria or blood tests, hypertension or severe symptoms as detailed in the protocol). For analysis purposes, measurements will be grouped as: first, maximum/most abnormal, and by gestational age bands. The boundaries of the gestational age bands will be as defined above (19 ≤ GA < 24, 24 ≤ GA < 29, 29 ≤ GA < 32, 32 ≤ GA < 35, GA ≥ 35, 19 ≤ GA < 35 weeks).

Test performance will be evaluated separately for each group of tests. This will allow us to investigate whether the test is valid only at certain gestations, and whether repeated testing is useful.

Critical values will be assessed using sensitivity, specificity, positive and negative predictive values and ROC areas. 95% confidence intervals will be produced using standard techniques (the Clopper-Pearson (1934) exact method for percentages, Normal approximation for ROC areas). Analysis will be carried out in Stata (version 11.2 or later; StataCorp, College Station, Texas, USA).

8 Investigator Obligations

8.1 Guidelines for the Conduct of the Study
The investigator is responsible for ensuring that the study is conducted in accordance with the clinical protocol and is in full compliance with FDA regulatory requirements. The basic principles outlined in 21 CFR Parts 50, 54, 56 & 312, the ICH-Guidelines for Good Clinical Practice as published in the Federal Register on May 9, 1997 and the Declaration of Helsinki. The investigator is also responsible for protecting the rights, safety and welfare of patients under the investigator’s care.

8.2 Informed Consent
The investigator or designee will inform the participant of the nature, risks and purpose of the study. A written informed consent form will be provided to each participant describing this information. This form must be reviewed and approved by the Sponsor and the Institutional Review Board/Ethics Committee (IRB/EC) before its use in the study. Each participant must sign and date this form prior to their participation in the study. A signed original consent form for each participant will be kept on file at the clinical site. A copy will also be given to the participant signing the form.

For patients who are sedated and/or hemodynamically unstable, surrogate consent from a family member or legal representative will be required and may be obtained if permitted by the clinical
site’s policies and IRB/EC. In such cases, the participant will be informed of their enrollment in this study when they have become fully alert: they will be told who gave surrogate consent for their participation and that they have the right to withdraw from the study.

8.3 Confidentiality
The Principal Investigator and designees, employees and agents involved with this study will comply with relevant state and federal laws relating to the confidentiality, privacy and security of participant’s health information. They will only create, maintain, use or disclose any data that is generated by this study or other information disclosed to the Principal Investigator or their employees or agents during the course of the study to the Sponsor, IRB/EC, FDA or other authorised recipients as appropriate for the execution, analysis, review and reporting of this study. Such information shall not be used for any other purposes and will remain confidential.

8.4 Protocol Modification/Amendments
If preliminary or interim review indicates that modification should be made in the experimental design, study parameters, participant selection, etc, these changes will be made after appropriate amendment(s) to this protocol with the mutual approval of the Sponsor and the investigator. Any protocol change that may significantly affect the safety of study patients must also be submitted for review and approval by the IRB/EC and may also require FDA review and approval.

8.5 Recording & Monitoring of Study Data
All required study data will be recorded on the internet based the Case Report Forms (CRFs). The data recorded on the CRFs is derived from study specific database currently used in the department.

8.6 Direct Access to Source Data & Study Documents
The investigator and study center will permit trial-related monitoring, audits, IRB/EC review and regulatory inspection by providing authorised personnel from the Sponsor, its representatives, the IRB/EC, other appropriate regulatory agency direct access to all trial related data.

Direct access is the permission to examine, analyse, verify and reproduce any records, source documents or reports that are important to the evaluation of a clinical study. Any party with direct access should take reasonable precautions to maintain the confidentiality of the study participants.

8.7 Record Retention
Case Report Forms, ICFs, original source documents, study records, and reports must be maintained by the investigator for a period of 25 years after the investigation is terminated or completed.
Appendix 2

Patient Participation Leaflet

PELICAN: PRE-ECLAMPSIA: CLINICAL APPLICATION OF PIGF

Participation Information Leaflet

1. Study Title
   Pre-Eclampsia: Clinical Application of PIGF

2. Background
   You have been asked to take part in this study because you are being investigated for hypertensive disorders (high blood pressure) of pregnancy, or suspected 'pre-eclampsia'. You will be having blood tests in hospital and we would like to add one extra test, to use for this study. Please read the following information. Ask us if there is anything that is not clear. You do not have to take part.

   What is the purpose of the study?

   A substance called Placental Growth Factor (PIGF) is produced by the placenta in normal pregnancy but the levels of PIGF are reduced in pre-eclampsia. By carrying out this study, we are hoping to provide evidence that the test can be used to improve diagnosis of the disease.

3. What will happen to me if I take part?
   You will be seen by a doctor or midwife, who will explain the study, answer questions and obtain your consent to take part. There will be no change to your current treatment and the result will not be revealed to those caring for you. Your blood sample will be tested for Placental Growth Factor (PIGF) and the findings will be used to help women in the future.

   What do I have to do?

   All you have to do is allow us to analyse an additional blood test, taken together with your necessary samples. Later, the hospital team will collect information from your hospital notes about you and your baby’s birth. This information will help us to work out the relationship between PIGF levels and pre-eclampsia.

4. What are the other possible disadvantages and risks of taking part?
   There are no known side effects of taking part, as you will be having blood tests anyway. There may be a slight increase in the time taken to obtain the samples.
5. **What are the potential benefits of taking part?**
   None for you, but you may help future mothers at risk of pre-eclampsia.

6. **What if there is a problem?**
   Any complaint about the research will be dealt with. Please speak to any of the researchers or midwives/doctors looking after you. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure.

7. **Will my taking part in the study be kept confidential?**
   Yes. All the information about your participation in the study will be kept confidential. The data will be stored following NHS guidelines for 25 years. Only the clinical research team and independent monitor will have access to this information.

8. **What will happen if I don’t want to carry on with the study?**
   You are free to withdraw from the study at any time. Just let one of the doctors or midwives looking after you know.

9. **What will happen to any samples I give?**
   The PIGF test will be done using the blood sample. The sample may be stored afterwards, for at least 25 years. Part of this sample will be stored in the USA. The same sample may be used during this time, for future studies into the same disease. This will not require you to provide any further samples.

10. **What will happen to the results of the research study?**
    The results of the study will be published in a medical journal. You will not be identified in any report/publication.

11. **Who has reviewed the study?**
    This study was reviewed by the Proportionate Review Sub-Committee of the East London 3 Research Ethics Committee.

12. **Who should I contact?**
    If you are interested in taking part or would like further information, please contact:

    Dr Suzy Duckworth (Study Co-ordinator)

    Maternal and Fetal Research Unit

    St Thomas’ Hospital

    Telephone: 07976 951634

   Thank you for taking time to read this.
Appendix 3

First draft patient questionnaire in the development of obstetric PROMs

Date of completion

Background Information

1 Age

2 Ethnicity

<table>
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</thead>
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<td>other</td>
<td>other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How many children have you had?

3

How many children (<18 years) live with you?

4
5 Employment status:  
Please tick one box only  
- Working full-time 37 hours/week or more  
- Working part-time less than 37 hours/week  
- Carer  
- Student  
- Homemaker/carer of own children  
- Unemployed

6 Please indicate your highest educational level  
- Primary school leaver  
- GCSE  
- A-level  
- Degree  
- Higher degree/professional

Medical History

7 Have you ever been told you had:  
- High blood pressure  
- Diabetes  
- Kidney disease  
- Previous  
- Preeclampsia  
- None  
- Other, please state

8 How many weeks pregnant are you?
9 How is your pregnancy being cared for? Please tick all that apply

- I am attending the ante-natal day unit
- I have been admitted to the antenatal ward
- I am under routine midwife-led care
- I am being seen by my GP
- I am being seen in a consultant-led antenatal clinic

Health in current pregnancy

Do you currently have a diagnosis of any of the following:

10 following:

- Pre-eclampsia (high blood pressure and protein in urine)
- High blood pressure only
- Protein in urine only
- None
- Other

11 During this pregnancy have you suffered from any of the following:

- Sickness
- Back pain
- Urinary problems
- Swelling
- Hip or pelvic pain
- Bleeding
- None
12 **During this pregnancy have you experienced any of the following problems:**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td></td>
</tr>
<tr>
<td>Visual disturbance</td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td></td>
</tr>
<tr>
<td>Facial swelling</td>
<td></td>
</tr>
<tr>
<td>Hand and foot swelling</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

13 **Compared with one month ago, how would you rate your current health?**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent, better than usual</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>About the same as usual</td>
<td></td>
</tr>
<tr>
<td>A little worse</td>
<td></td>
</tr>
<tr>
<td>Much worse</td>
<td></td>
</tr>
<tr>
<td>The worst I have ever felt</td>
<td></td>
</tr>
</tbody>
</table>

14 **Compared with one month ago, how often have you felt unwell?**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost all of the time</td>
<td></td>
</tr>
<tr>
<td>A lot of the time</td>
<td></td>
</tr>
<tr>
<td>About half the time</td>
<td></td>
</tr>
<tr>
<td>Less than half the time</td>
<td></td>
</tr>
<tr>
<td>Rarely</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td></td>
</tr>
</tbody>
</table>
Please record how this pregnancy has affected the following aspects of your normal life.

15

a  **Ability to continue usual employment**

Not at all

A little disruption

Pregnancy has caused a lot of problems

Most of the time

I have had to stop working

Not applicable/would rather not answer

b  **Relationship with partner**

Our relationship has not changed

Our relationship is suffering

Pregnancy has caused some problems

Pregnancy has caused a lot of problems

Pregnancy has ended my relationship

Not applicable/would rather not answer
c  **Relationship with family**

- Our relationship has not changed
- Our relationship is suffering
- Pregnancy has caused some problems
- Pregnancy has caused a lot of problems
- Pregnancy has ended a relationship
- Not applicable/would rather not answer

d  **Relationship with existing children**

- Our relationship has not changed
- Our relationship is suffering
- Pregnancy has caused some problems
- Pregnancy has caused a lot of problems
- Pregnancy has ended a relationship
- Not applicable/would rather not answer

e  **Ability to enjoy being pregnant**

- No effect, I am enjoying pregnancy
- Enjoyment reduced slightly
- Enjoyment reduced moderately
- Enjoyment reduced greatly
- I am not enjoying this pregnancy
- I no longer want to be pregnant

f  **Ability to enjoy life**
No effect, I am enjoying life

Enjoyment reduced slightly

Enjoyment reduced moderately

Enjoyment reduced greatly

I am not enjoying my life currently

I am feeling extremely unhappy

g  **Ability to manage finances**

No effect, I have no financial concerns

Pregnancy has had a small impact

Pregnancy has had a moderate impact

Pregnancy has had a large impact

I am very worried about finances

I feel I cannot afford this pregnancy
h  **Ability to prepare for new baby**

- No effect, I feel prepared
- I have found it a bit harder to prepare
- I have found it somewhat harder to prepare
- I have found it a lot harder to prepare
- I have not been able to prepare at all
- I feel completely unprepared

16  **How has this pregnancy effected your mood**

a  **I feel anxious**

- None of the time
- Some of the time
- Half the time
- Most of the time
- All of the time
- I have anxiety attacks

b  **I feel sad**

- None of the time
- Some of the time
- Half the time
- Most of the time
- All of the time
- I am depressed
<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>None of the time</th>
<th>Some of the time</th>
<th>Half the time</th>
<th>Most of the time</th>
<th>All of the time</th>
<th>I never sleep well</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>I have problems sleeping.......</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>I feel tearful.......</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>I take medication for low mood.....</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>I use alcohol/other substances to improve mood.....</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Occasionally
Daily
Weekly
Monthly
When I'm not pregnant

**g  I would describe my diet as........**
Better than usual
Good-like usual
Fair-like usual
Poor-like usual
Worse than usual
Terrible

**Has being seen at hospital improved your anxiety?**
Not applicable
Not at all
Improved a little
Improved moderately
Improved greatly
I feel much better now

**i  My understanding of pre-eclampsia is.......**
Unchanged
A bit better
<table>
<thead>
<tr>
<th>Category</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somewhat better</td>
<td></td>
</tr>
<tr>
<td>Much better</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>
This pregnancy has made me less likely to want another

- Not at all
- Agree a little bit
- Somewhat agree
- Definitely agree
- Not for a long time
- I will never do it again

Any comments

*Appendix 2

Questions Included in Likert Scale

How has this pregnancy affected your ability to continue usual employment?

- Not at all
- A little disruption
- Pregnancy has caused a lot of problems
- Unable to work most of the time
- I have had to stop working
Not applicable/would rather not answer

I feel anxious...

None of the time

Some of the time

Half of the time

Most of the time

All of the time

I have anxiety attacks

I have problems sleeping...

None of the time

Some of the time

Half of the time

Most of the time

I never sleep well

How has this pregnancy affected your ability to enjoy life?

No effect, I am enjoying life

Enjoyment reduced slightly

Enjoyment reduced moderately

Enjoyment reduced greatly
I am not enjoying my life currently

I am feeling extremely unhappy

This pregnancy has made me less likely to want another...

Not at all

Agree a little bit

Somewhat agree

Definitely agree

Not for a long time

I will never do it again