Use of third trimester serum biomarkers and ultrasound paameters to predict the small for gestational age infant at delivery

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King's College London

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Abstract

Current techniques to identify growth-restricted fetuses, at risk of health complications, have limited accuracy. Placental insufficiency is a key pathological process in fetal growth restriction (FGR). I investigated the potential clinical benefit of placental biomarkers to identify pregnancies delivering small for gestational age (SGA) infants in pregnancies with suspected pre-eclampsia and in those with reduced symphysis-fundal height measurement using delivery of an SGA infant as a surrogate measure of FGR.

Suspected pre-eclampsia (PELICAN-PE study)

In a large multicentre prospective cohort study investigating diagnostic accuracy of placental growth factor (PlGF) in women with suspected pre-eclampsia, I assessed test performance of 47 biomarkers and ultrasound parameters to identify women delivering an SGA infant.

PlGF measurement outperformed all other biomarkers and currently used tests in predicting delivery of an SGA infant. Combinations of biomarkers added minimal value.

Reduced symphysis-fundal height measurement (PELICAN-FGR study)

I assessed the ability of PlGF and ultrasound parameters to predict delivery of an SGA infant in women with reduced symphysis-fundal height (current UK standard to identify pregnancies at risk of SGA) in a second multinational prospective cohort study. Test performance statistics were calculated for all parameters in isolation and combination.

Ultrasound parameters had modest test performance for predicting delivery of an SGA infant. PlGF performed no better. Incorporating PlGF with ultrasound parameters provided modest improvements.

In women presenting with suspected pre-eclampsia, PlGF measurement is a potentially useful adjunct to current practice in identifying those at risk of SGA. The findings of the PELICAN-FGR study cannot support the use of PlGF to risk stratify women referred with reduced symphysis-fundal height. The prevalence of FGR in the two studies differed, with a high number of normal pregnancies in those presenting with reduced symphysis-fundal height. The pathological process in normotensive versus hypertensive SGA may differ, potentially explaining these findings.

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Chapter 1: Introduction

1.1: Being born small-for-gestational-age (SGA): The scope of the problem

The small-for-gestational-age infant is defined as an being born with a birth weight below a pre-specified threshold, commonly the third or tenth birth weight centiles (Robson et al., 2013). Being born SGA is a global health problem with an estimated 18 million babies born SGA per annum, contributing to 60-80% of neonatal deaths (UNICEF, 2004). These infants are at increased risk of life-threatening complications compared to those born with birth weights appropriate-for gestational-age (AGA), even when born at term (Malin et al., 2014), likely secondary to the high incidence of fetal growth restriction (failure of a fetus to fulfill their growth potential) (FGR) amongst SGA infants.

These complications originate in the antenatal period and include stillbirth (Moraitis et al., 2014). As part of "The Lancet stillbirth series" Lawn et al. reported FGR (sometimes referred to as intrauterine growth restriction (IUGR)) and placental insufficiency as the highest attributable cause of stillbirth in high-income countries (including, Australia, Canada, The Netherlands, Norway, UK and USA), (present in 32% of antenatal and 26% of intrapartum stillbirths) and identified FGR as one of the five main targets to achieve a reduction in stillbirth globally (Lawn et al., 2011). FGR, secondary to placental insufficiency, has previously been reported in approximately 50% of stillbirth cases (Frøen et al., 2004).

Complications of being delivered SGA are not limited to the antenatal period but extend into adult life. These include perinatal and neurodevelopmental complications and long-term health complications, such as coronary heart disease, hypertension and type 2 diabetes mellitus. Maternal factors that predispose to SGA are discussed in detail in section 1.1.3.1.

To reduce the short and long-term health complications associated with being born SGA, it is paramount that pregnancies at risk are identified early in the antenatal period, allowing targeted monitoring and potentially early intervention. However, accurately identifying at-risk pregnancies, diagnosing FGR antenatally and optimising management of this high-risk group remains challenging. This largely relates to a lack of understanding of the pathological mechanisms underlying FGR and limited sensitivity of current screening tools and diagnostic tests to accurately identify those at risk. In clinical practice, SGA is the target of most screening tools currently employed, which partly explains the limited sensitivity of these tools as pregnancies complicated by SGA are a heterogenous group including those that are constitutionally small in addition to a proportion of pregnancies with FGR. Using SGA as a surrogate for FGR leads to the inevitable over investigation and possible intervention, including iatrogenic delivery and its ensuing complications, in constitutionally small pregnancies, which are not thought to be at increased risk of adverse outcome.

Within the Lancet stillbirth series, Flenady et al. highlighted '*the need for further research into the underlying mechanisms of fetal growth restriction facilitating early detection and effective management of women at increased risk'* (Flenady et al., 2011b) and Goldenberg et al. included; '*improving antenatal screening for risk factors for stillbirth, such as fetal growth restriction'*, as a high priority research theme in the concluding article of the series (Goldenberg et al., 2011).

There is no doubt of the importance of the risks associated with delivering a growth restricted infant on an individual and global scale and the impact of this on healthcare resources. Accurately predicting those at risk of delivering a growth restricted infant, enabling targeted intervention, has the potential to improve outcome and avoid some of the devastating consequences linked to this condition, whilst also avoiding intervention in the group of constitutionally small infants.

1.1.1 Clinical importance of identifying the SGA infant antenatally

Infants born SGA are at increased risk of short and long-term health complications.

1.1.1.1 Short term complications

1.1.1.1.1 Perinatal complications

Two North American population studies (including 12,317 and 19,759 pregnancies who delivered preterm) observed increased rates of respiratory distress syndrome, intraventricular haemorrhage and necrotising enterocolitis in those born SGA compared to those delivering AGA (McIntire et al., 1999, Bernstein et al., 2013). In term SGA infants, increased incidence of fetal acidosis (McIntire et al., 1999), seizures within the first week of life (Bukowski et al., 2003) and perinatal stroke have been reported (Wu et al., 2004), although generalisation and reproducibility of the findings of the latter two studies may be limited due to their small sample size and study design (single site nested case-control study). Polycythaemia, hyperbilirubinaemia and hypoglycaemia have also been cited as more common in growth-restricted infants, possibly secondary to chronic hypoxia and reduced hepatic glycogen stores (Mayer and Joseph, 2013).

1.1.1.1.2 Neurodevelopmental complications

SGA infants have been found to be at increased risk of cerebral palsy compared to those born AGA. This risk is reported as decreasing with advancing gestational age (Surveillance of Cerebral Palsy in Europe, 2000). A population based case-control study in Sweden compared rates of cerebral palsy in SGA infants to those born AGA. They found that the risk of cerebral palsy in those born SGA at term was 5-7 fold higher, where as in preterm

infants, incidence of cerebral palsy, was not more common with SGA (Jacobsson et al., 2008).

McCormick et al. reported poorer academic performance, lower intelligence, poor social interaction and behavioural problems amongst those born SGA in two cohorts of 8-10 year olds, with differing birth weights (McCormick et al., 1996). However they suggest that this relationship may occur due to the presence of risk factors for delivering an SGA infant rather than a direct effect from low birth weight. Pryor et al. and Walker et al. published similar findings with the addition of defects in short-term memory in infants born SGA, compared to those born AGA (McCormick et al., 1996, Schothorst and van Engeland, 1996, Pryor et al., 1995, Walker and Marlow, 2007). Strauss et al. analysed data from the National Collaborative Perinatal Project, a large multicentre cohort study, and concluded that there were significant differences in IQ at age seven between those born SGA and their AGA counterparts (Strauss and Dietz, 1998). However, when they compared the growth and development of 220 similar-sex term sibling pairs where one sibling was born SGA, they found no significant difference in IQ unless there was associated low head circumference. These findings suggest that genetic and environmental factors play a significant role in the differences observed in IQ when the whole cohort was analysed (Latal-Hajnal et al., 2003).

1.1.1.1.3 Stillbirth and neonatal death

Whilst acknowledging that stillbirth is uncommon in high income countries, a systematic review and meta analysis reported SGA as having the highest population attributable risk of all pregnancy specific disorders (23%) (Flenady et al., 2011a). A large cohort study of 92,218 singleton pregnancies (including 389 stillbirths) reported that if SGA (birth weight <10th customised centile) was not recognised antenatally, the risk of stillbirth increased five-fold, emphasising the importance of antenatal detection (Gardosi et al., 2013b). A recent multinational study evaluated the effects of preterm and SGA delivery on neonatal and postnatal mortality in a pooled analysis of data from low and middle-income countries. The relative risk of neonatal mortality in babies born SGA at term was 1.83 (CI 1.32-2.50) and 6.82 (CI 3.56-13.07) for those born preterm. In babies born preterm and SGA, the risk was much higher (15.42 (CI 9.11-26.12)) (Katz et al., 2013). Improved recognition of SGA would allow appropriate surveillance and timing of delivery. A large Swedish retrospective cohort study including 26,968 women (681 with SGA infants detected antenatally) concluded that instigating a structured antenatal surveillance program for pregnancies identified as SGA antenatally resulted in a lower risk of fetal adverse outcome (Lindqvist and Molin, 2005).

In 1992, Gardosi et al. proposed that use of customised growth centiles, as opposed to population derived centiles, improved detection of the SGA fetus (Gardosi et al., 1992). In 2009 they published a retrospective analysis on a large American database comprising 34,712 singleton pregnancies and

concluded that use of customised birth weight centiles more accurately identified those at most risk of adverse outcome, including stillbirth (Gardosi and Francis, 2009). However, in a population of women at high-risk of uteroplacental insufficiency, use of customised fetal weight limits only detected 68% of cases antenatally who subsequently delivered an SGA infant, highlighting the need for further improvement in identifying those at risk (De Jong et al., 2000). Customised birth weight centiles are discussed in more detail in section 1.1.2.1.

1.1.1.2 Long-term health complications

1.1.1.2.1 Complications in childhood

Two large cohort studies, in New Zealand and North America, have reported heights and weights of children who were born SGA, to be significantly less than those born AGA and this difference persisted into adulthood (Strauss and Dietz, 1998, Pryor et al., 1995).

1.1.1.2.2 Complications in adulthood

Multiple large cohort studies have reported increased incidence of coronary heart disease (Frankel et al., 1996, Leon et al., 1998, Bonamy et al., 2011), hypertension (Eriksson et al., 2000, Curhan et al., 1996, Huxley et al., 2000) and type 2 diabetes mellitus (Forsen et al., 2000, Lithell et al., 1996, Rich-

Edwards et al., 1999, Newsome et al., 2003) in adults who were born SGA, compared to those born AGA.

A possible explanation for these associations involves the concept of 'developmental plasticity', where the fetus adapts in response to the intrauterine environment and nutrition in early life (Barker, 2006). One such adaptation is insulin resistance, which is associated with the development of type 2 diabetes mellitus and hypertension in adult life. This is thought to occur secondary to persistence of a protective fetal metabolic adaptation to ensure adequate glucose supply to developing organs in a harsh intrauterine environment (Phillips, 1996).

Another possibility is the presence of a maternal genetic susceptibility to cardiovascular disease, which may contribute to delivery of an SGA infant, who may also inherit this trait. Women with a genetic predisposition to cardiovascular disease may have impaired haemodynamic changes in pregnancy, resulting in placental dysfunction and inadequate oxygen and nutrient supply to the developing fetus. Such women are at increased risk of vascular complications, including hypertensive disorders and pre-eclampsia, with the ensuing complications of preterm delivery and FGR. This offers some explanation why women who give birth to SGA infants have a higher risk of coronary heart disease themselves (Smith et al., 2000, Bonamy et al., 2011).

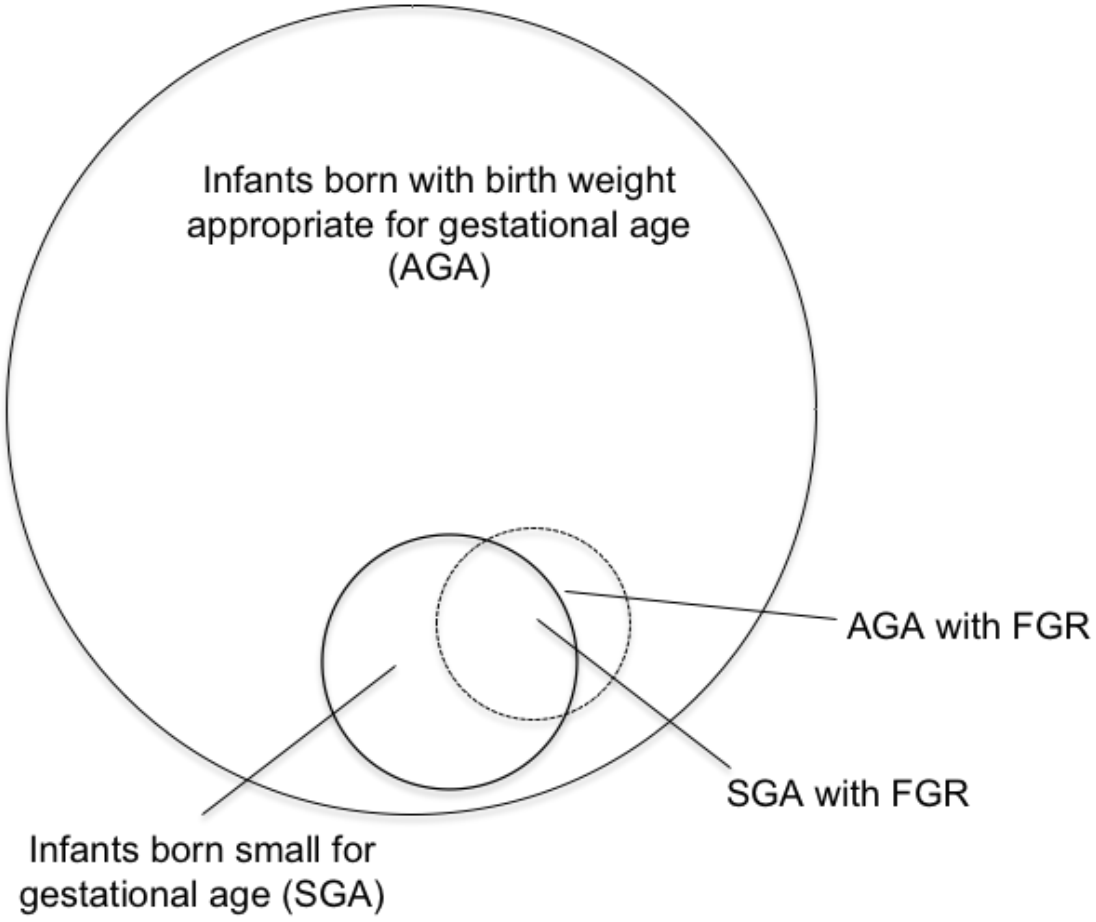
Possible mechanisms for these associations remain speculative and it remains unclear whether intervention would reduce incidence or improve outcome from these multifactorial conditions (Mayer and Joseph, 2013).

1.1.2: Defining the small for gestational age (SGA) infant

The SGA infant is defined as an infant born with a birth weight below a pre-specified threshold, commonly the third or tenth birth weight centiles (Robson et al., 2013). Other thresholds for defining SGA have been used, including the 2.5^{th}, 5^{th} and 20^{th} centiles, but the 10^{th} birth weight centile derived from appropriate healthy populations is the most frequently reported. This definition includes both constitutionally small infants and those with FGR. The latter refers to any fetus that fails to fulfill their growth potential, and represents a group at high risk of adverse outcome. Figure 1.1 illustrates the relationship between SGA and FGR.

Figure 1.1 Venn diagram displaying the relationship between SGA and FGR

The circle depicting FGR is dotted to represent the uncertainty of the proportion of SGA infants who have FGR.



FGR, fetal growth restriction

SGA infants are at increased risk of neonatal morbidity (Bernstein et al., 2013, McIntire et al., 1999) and mortality (Frøen et al., 2004, Lawn et al., 2005, Lackman et al., 2001), compared to their AGA counterparts, likely secondary to the high incidence of FGR within the SGA group. It is important to consider

which threshold is most appropriate for defining SGA, as this affects the proportion of infants within the group that are growth restricted.

If a threshold of the 10th centile from a healthy population is used to define SGA, it would be expected that 10% of normal pregnancies (i.e. constitutionally small infants) will be included in this group. These pregnancies are not at increased risk of adverse outcome (Alfirevic et al., 2010) but by inclusion in this definition of SGA they would be subject to extra surveillance which may lead to unnecessary anxiety and intervention. Using a lower threshold, such as the third centile from a healthy population will reduce the proportion of normal pregnancies included to 3%, but would potentially omit cases of FGR, included if a higher threshold is employed. Whilst use of any population based threshold will omit some cases of FGR it is proposed that those cases of FGR at most risk of adverse outcome are likely to be those with low birth weight, and therefore using a population based threshold such as the 10th centile offers the best probability to identify most cases at risk.

1.1.2.1 Customised birth weight centiles

Historically, SGA has been defined according to thresholds derived from healthy populations. In 1992, Gardosi et al. published data comparing use of customised growth centiles to population-based centiles for defining SGA. Customised charts incorporated data on maternal height, weight, ethnicity and parity, in addition to gestational age at delivery and infant sex, with the aim of

differentiating those SGA fetuses who are growth restricted from those who are constitutionally small. They concluded that 28% of babies identified as SGA using population based centiles, were within normal limits for that individual woman if customised centiles were used, whilst 24% of babies assigned as SGA using customised centiles were missed using conventional unadjusted centiles (Gardosi et al., 1992). Subsequent studies have shown that use of customised centiles improves identification of small babies at high-risk of adverse outcomes, including stillbirth (Odibo et al., 2011) and neonatal death (Clausson et al., 2001) and that a threshold of the 10th centile provides a high detection rate for SGA and its related perinatal complications (Gardosi and Francis, 2009, Clausson et al., 2001, Figueras et al., 2007, De Jong et al., 2000). However, there remains some controversy regarding widespread adoption of customised centiles into clinical practice. At earlier gestational ages, the customised centiles are calculated using Hadlock's proportionality formula, in contrast to conventional birth weight charts, which use the weights of live births. Hadlock's formula uses measurements of head circumference, abdominal circumference and femur length to estimate fetal weight (Hadlock et al., 1985). It has been proposed that this, rather than incorporation of maternal characteristics into the model, causes the improved detection of those most at risk (Hutcheon et al., 2008).

A further concern relates to the inclusion of ethnicity as a component of the customised fetal growth charts. The recently published Intergrowth 21st project aimed to investigate whether there were similarities in fetal growth across

geographically diverse areas when other factors affecting fetal growth were controlled for (e.g. nutritional intake, maternal health and level of antenatal care). They evaluated fetal growth and newborn size in eight geographically diverse urban areas and suggested that genetic variation has little effect on fetal growth (Villar et al., 2014). The World Health Organisation (WHO) Multicentre Growth Reference Study (MGRS) has previously reported very similar patterns of infant growth across six sites in Europe, Africa, India, The Middle East and North and South America, when there was little variation in environmental, health and nutritional status. This led to construction of International growth standards from birth to five years of age (WHO., 2006b, WHO., 2006a, WHO., 2006c). The authors of the Intergrowth 21st project suggest that previously reported differences in fetal growth in diverse populations are more likely due to socioeconomic and environmental factors rather than genetic variation. These data are currently being analysed to construct standardised International prenatal and neonatal growth standards, with the aim of integrating these with the standards derived from the WHO Multicentre Growth Reference Study.

The UK Royal College of Obstetrics and Gynaecology Guideline: The Investigation and Management of the SGA Fetus, published in 2011, prior to Intergrowth, suggests that use of customised fetal weight references may improve prediction of a SGA neonate and an adverse perinatal outcome (Robson et al., 2013).

1.1.3 Causes of fetal growth restriction

FGR is a multifactorial condition and risk factors can broadly be divided into, maternal, fetal and placental factors. Maternal factors predisposing to placental dysfunction account for the largest proportion of cases, with fetal and other placental factors playing a much smaller role.

1.1.3.1 Maternal factors

1.1.3.1.1 Pre-eclampsia and Placental dysfunction

Pre-eclampsia is a pregnancy specific condition which is classically defined as new onset of hypertension after 20 weeks' gestation with documented proteinuria (≥ 300 mg/day or urinary protein/creatinine ratio ≥ 30 mg/mmol) (Brown et al., 2001). The condition occurs when there is failure of adequate trophoblast invasion of the maternal spiral arteries leading to impaired placental blood flow and episodes of placental ischaemia, and oxidative stress (Huppertz, 2008, Kaufmann et al., 2003, Poston et al., 2011). Redman et al. suggest that proinflammatory mediators are released into the maternal circulation, triggering a widespread inflammatory response with alteration in concentrations of many acute phase proteins (Redman and Sargent, 2009). This combination of systemic inflammation and placental hypoxia leads to maternal endothelial dysfunction and the resulting non-specific clinical presentations of pre-eclampsia.

In addition to the mechanisms outlined above, increased vascular resistance and reduced placental perfusion area (likely secondary to microthrombi

deposition and placental infarction and abruption) lead to the frequent coexistence of FGR. The relationship between pre-eclampsia and FGR has been reported extensively, with severity of pre-eclampsia and gestation at onset correlating with presence and severity of FGR. Odegard et al. reported in a population based study, a 5% reduction in birth weight in pregnancies complicated by pre-eclampsia. This increased to a 12% reduction in birth weight with severe pre-eclampsia and birth weight was 23% lower than expected if there was early onset of disease (defined as delivery before 32 weeks' gestation). The risk of delivering an SGA infant was four times higher in cases complicated by pre-eclampsia compared to controls (Odegard et al., 2000). The relationship between late-onset pre-eclampsia and coexistence of FGR is less clear with a large Canadian retrospective cohort study including 97,270 pregnancies reporting that in women delivering beyond 37 weeks' gestation, infant birth weights were similar for women with pre-eclampsia compared to those who remained normotensive (Xiong et al., 2002). This supports the theory that abnormal uteroplacental perfusion plays a larger role in the pathogenesis of early onset pre-eclampsia compared to late onset disease.

Current NICE guidance on the Management of hypertensive disorders during pregnancy recommends that all women diagnosed with pre-eclampsia who are to be managed conservatively should be offered ultrasound scan to assess fetal growth, liquor volume and umbilical doppler measurement (National Institute for Health and Clinical Excellence, 2010). However, as discussed in section 1.1.2.1, sensitivity of ultrasonography to detect SGA and predict adverse

outcome is limited, even when customised centiles are used. There is a need for an accurate test to detect SGA antenatally, particularly in high-risk populations such as women with early onset pre-eclampsia, to identify women at increased risk of adverse perinatal outcome.

Several chronic maternal conditions including hypertensive and renal disease, diabetes mellitus, systemic lupus erythematous and antiphospholipid syndrome are associated with an increased risk of FGR and pre-eclampsia, mainly considered to be secondary to placental dysfunction (Howarth et al., 2007, Fink et al., 1998, Yasmeen et al., 2001, Yasuda et al., 1995). A large Canadian population based study, including 135,466 pregnancies, reported that women with any hypertensive disorder in pregnancy were 1.6 times more likely to deliver an SGA infant compared to those who remained normotensive (Allen et al., 2004).

It is hypothesised that procoagulant conditions, such as thrombophilias, lead to an increased risk of placental thrombosis with altered uteroplacental blood flow contributing to the development of FGR and in severe cases to fetal loss (Facco et al., 2009, Arias et al., 1998). Acquired thrombophilias such as anticardiolipin antibodies and lupus anticoagulant are associated with FGR and development of pregnancy complications such as pre-eclampsia and stillbirth (Yasuda et al., 1995), but evidence of association with inherited thrombophilias is limited and conflicting (Facco et al., 2009).

1.1.3.1.2 Other maternal factors

The association between smoking and FGR has been long established. A large international prospective cohort study, including 3513 nulliparous women, reported that smoking at 15 weeks' gestation was associated with a 30-40% increased risk of SGA for every 5 cigarettes smoked per day, irrespective of whether the pregnancy was complicated by hypertension (19.2% of women delivering an SGA infant were smokers compared to 10% in those delivering an AGA infant) (McCowan et al., 2010). McCowan et al. published that cessation of smoking prior to 15 weeks' gestation gave a risk of delivering an SGA infant comparable to a non-smoking population, highlighting the reversibility of this risk (McCowan et al., 2009). Smoking causes impaired uterine blood flow and a reduction in oxygen carrying capacity. The latter mechanism is also thought to contribute to the increased incidence of FGR in women who live at high altitude (Krampl, 2002), or have cyanotic heart disease (Patton et al., 1990), haemoglobinopathies or anaemia (Barfield et al., 2010).

A systematic review and meta analysis including 78 studies, involving over one million women, reported that singleton pregnancies born to underweight women were at higher risk of preterm delivery (both spontaneous and iatrogenic) and being born SGA compared to those of normal weight (Han et al., 2011). A paper included in this systematic review by Ronnenberg et al. proposed reduced fetal nutrition as the mechanism of action (Ronnenberg et al., 2003). Low weight gain during pregnancy has also been linked to development of FGR (Lang et al., 1996), although monitoring maternal weight during pregnancy is not

currently recommended (National Institute for Health and Clinical Excellence, 2008).

Fetal exposure to both prescription and recreational drugs can lead to FGR. Use of heroin or cocaine has been associated with delivery of an SGA infant in 50% and 27% of cases respectively (Naeye et al., 1973, Fulroth et al., 1989). This could be partially attributed to the increased risk of chronic placental abruption with cocaine use and the coexisting poor nutrition often observed with drug misuse. The later is supported by data published by Naeye et al. where severity of SGA correlated with degree of malnutrition (Naeye et al., 1973). Alcohol consumption in pregnancy has also been linked to FGR and a large prospective study including 31,604 pregnancies reported that this risk is dose dependent (Mills et al., 1984). However a recent systematic review and meta-analysis suggested that light to moderate alcohol intake had no effect on risk of delivering an SGA infant and that a dose dependent association was only established when daily intake exceeded 10g alcohol (one alcoholic drink) per day (Patra et al., 2011). Current national guidelines in the UK, issued prior to the systematic review discussed above, advise abstinence during pregnancy, perhaps related to the risks of alcohol, although there is little evidence that very low consumption (less than 1-2 international units of alcohol less than 1-2 times per week) affects fetal growth (Fraser, 2006).

There are numerous additional maternal factors, which have been linked with delivery of a SGA infant. These include previous delivery of an SGA infant

(estimated to increase risk more than two-fold) (Tejani, 1982, Bakketeig et al., 1993), low socioeconomic status (Wilcox et al., 1995), use of artificial reproductive techniques (independent of multiple gestation) (Jackson et al., 2004, Schieve et al., 2002), short inter-pregnancy interval (Zhu et al., 1999), maternal periodontal disease (Khader and Ta'ani, 2005) and extremes of maternal age (Lee et al., 1988),. Lee et al. conducted a population study including 184,567 women with singleton pregnancies, evaluating the effects of maternal age on incidence of low birth weight in women delivering at term. They concluded that women under 17 years of age and those over 35 years of age were more likely to deliver a SGA infant. They hypothesised that poor sociodemographic and prenatal care in women under 17 years of age and biologic ageing of maternal tissues and systems in those over 35 years of age may contribute to this finding (Lee et al., 1988). The findings of the About Teenage Eating (ATE) study support this hypothesis in teenagers. Baker et al. reported lower micronutrient intake (including iron, folate and vitamin D) in adolescents who delivered SGA infants compared to those delivering AGA (Baker et al., 2009). In the Screening for Pregnancy Endpoints (SCOPE) cohort (prospective multicentre cohort study including 3513 nulliparous women), increasing maternal age was identified as an independent risk factor for delivering an SGA infant. This group also reported a correlation between maternal dietary intake and risk of SGA, with a high fruit and vegetable intake being protective, whilst low fruit intake pre-pregnancy was identified as an independent risk factor (McCowan et al., 2010). The exact protective agents in fruit and vegetables have not been identified but McCowan et al. proposed that

micronutrients (including folate, ascorbate, carotenoids and magnesium) and dietary fibre and other phytochemicals may play a role. Alternatively they suggest that higher fruit and vegetable intake may merely reflect a healthier lifestyle (McCowan et al., 2010). Other groups have published on the contribution of micronutrient concentrations to delivering an SGA infant (Mistry and Williams, 2011). Mistry et al. measured concentrations of selenium, copper and zinc in 126 adolescent pregnant women recruited to the ATE study. Third trimester concentrations of selenium were lower in those delivering an SGA infant compared to their AGA counterparts, whereas differences in concentrations of zinc and copper were not significant (Mistry et al., 2014). Selenium is a cofactor for several important enzymes involved in antioxidant defence. The authors suggest that low maternal concentrations of selenium reduce this defence mechanism, leading to restriction of fetal growth. Whilst encouraging, large intervention trials would be needed to assess the impact of micronutrient supplementation on incidence of SGA prior to consideration of clinical adoption.

1.1.3.2 Fetal factors

Chromosomal defects are associated with FGR but the reported incidence of chromosomal abnormalities is this group varies between 5-20% (Sabogal, 2007). One study including 458 fetuses with suspected SGA in the second and third trimester, reported an incidence of chromosomal abnormality of 19% in cases of severe SGA (abdominal circumference (AC) and estimated fetal weight (EFW) were below the 5th centile) (Snijders et al., 1993). Triploidy was the

commonest abnormality in fetuses prior to 26 weeks' gestation with trisomy 18 (Edward's syndrome) after 26 weeks in this study (Snijders et al., 1993). Other aneuploidies and several genetic syndromes also have associations with delivery of an SGA infant.

Congenital malformations in the absence of genetic disorders are a rare cause of SGA (accounting for 1-2% of cases), but presence of multiple malformations has been reported to increase risk (Khoury et al., 1988).

Fetal infections have been reported in up to 5% of cases delivering an SGA infant (Hendrix and Berghella, 2008). Cytomegalovirus (CMV), toxoplasmosis, malaria and syphilis are the most commonly cited pathogens, whilst cases complicated by rubella infection, have decreased since widespread vaccination was adopted in developed countries (Hendrix and Berghella, 2008). However, a European multicentre prospective cohort study investigating the association between toxoplasmosis and subsequent delivery of a SGA infant found no causal relationship (Freeman et al., 2005).

A recent small case control study investigated the presence of congenital CMV in 19 pregnancies (nine uncomplicated pregnancies (controls), seven cases of idiopathic FGR and three cases of pre-eclampsia) and found evidence of congenital CMV infection with abnormal placental pathology in five cases of idiopathic FGR (three recurrent and two primary infections) (Pereira et al., 2014). Whilst acknowledging the very small sample size of this study, the

results suggest that subclinical antenatal infections could be responsible for a proportion of idiopathic FGR. Larger studies are warranted to substantiate these findings.

It has been reported that approximately 3% of cases of FGR are associated with multiple pregnancy, with approximately 15-30% of twin pregnancies at risk of FGR (Resnik, 2002). FGR in one or more fetuses is associated with neonatal adverse outcome (Hendrix and Berghella, 2008).

Whilst many fetal factors can contribute to delivery of an SGA infant, as a group fetal factors are causal in only a relatively small number of cases.

1.1.3.3 Placental factors

Placental abnormalities including placental abruption, infarction, and tumours (Zalel et al., 2002, Pham et al., 2006) have all been associated with increased risk of SGA likely secondary to reduced uteroplacental blood flow. A large American retrospective cohort study including 53,371 pregnancies reported SGA (birth weight <10th centile for gestational age) in 14.3% of pregnancies complicated by placental abruption compared to 8.1% in controls (Ananth et al., 1999). Cord malformations, such as velamentous insertion and single umbilical artery have also been linked to delivery of an SGA infant. A Finnish retrospective cohort study reported increased risk of low birth weight and SGA in a series of 216 cases of velamentous cord insertion, compared to normal

controls (Heinonen et al., 1996). They suggest that velamentous insertion may be a compensatory mechanism counteracting impaired early placentation. If the primary implantation site has poor vascularity, they propose that the placenta migrates to a better site leading to a velamentous cord insertion.

Placenta praevia and accreta have been quoted in review articles as increasing risk of delivering an SGA infant (Mayer and Joseph, 2013, Maulik, 2006), but a large retrospective cohort study including 59,149 women, (724 of whom had a placenta praevia) found no association (Harper et al., 2010).

1.1.4 FGR and placental pathology

Placental dysfunction, largely due to maternal factors, accounts for the greatest proportion of cases of FGR. Improved knowledge of placental pathology has facilitated identification of several pathological processes, which underpin the development of FGR. In 2008, Redline published a complex classification involving five chronic patterns of placental injury, which occur more frequently in the placentae from FGR infants (Redline, 2008). These included three categories affecting the maternal and fetal vasculature (maldevelopment, obstruction or loss of integrity) and two categories involving inflammatory processes (infectious and idiopathic). The idiopathic category included chronic histiocytic intervillositis and villitis of unknown aetiology (VUE). In VUE, a large maternal immune response is cited, predominately through activation of T lymphocytes, which infiltrate the villous stroma. The trigger for this process is

unknown but high grade VUE is associated with significant perinatal morbidity and mortality (Redline, 2005, Redline and O'Riordan, 2000).

Redline suggested that VUE is more common in normotensive term gestations with FGR whereas maternal vascular disorders are the most frequent finding in preterm deliveries and maternal hypertensive disease with FGR.

Redline's classification is not widely accepted. Vedmedovska et al. published a case series of placental pathology from 50 pregnancies complicated by placental disease (including FGR) and reported no differences in perivillous fibrin deposition between cases and controls (Vedmedovska et al., 2011). Perivillous fibrin deposition was classified by Redline as a maternal vasculature maldevelopment, observed in the placentae of women with pre-eclampsia. Huppertz et al. have proposed a different model for understanding differing placental pathologies for pre-eclampsia and FGR, involving dysregulation of trophoblast differentiation. Timing of dysregulation influences development of pre-eclampsia or FGR or coexistence of these conditions; it is proposed that early dysregulation of trophoblast development results in a combination of pre-eclampsia and FGR whilst later dysregulation of syncytiotrophoblast leads to pre-eclampsia and dysregulation of cytotrophoblast development results in isolated FGR (Huppertz, 2011).

Both Redlines and Huppertzs models, propose that different pathological processes occur in pre-eclampsia and FGR uncomplicated by hypertension.

This may be relevant when considering potential diagnostic tools for FGR, as concentrations of markers may be altered in normotensive FGR compared to FGR with hypertension and different markers may be more useful in these two distinct groups. The theory of differing pathological mechanisms in women with pre-eclampsia compared to those remaining normotensive but delivering an SGA infant is supported by the findings of Pecks et al. (Pecks et al., 2012). This group evaluated the oxidative state of low-density lipoproteins (LDL) in FGR and pre-eclampsia. Several authors have previously reported an increased maternal plasma oxidised LDL concentration in pre-eclampsia (Kim et al., 2007, Uzun et al., 2005). Pecks et al. proposed that oxidised LDL impairs trophoblast invasion affecting remodeling of the spiral arteries contributing to poor placentation and the sequelae of pre-eclampsia, and assumed a similar pattern would be evident in those pregnancies complicated by delivering an SGA infant. However the authors reported an inverse relationship with low concentrations of oxidised LDL in pregnancies delivering an SGA infant (Pecks et al., 2012).

1.1.5 Summary

Being born SGA is a global health problem, associated with significant neonatal morbidity and mortality with complications extending into adult life. Identifying pregnancies at risk remains challenging but integration of maternal and fetal factors into assessment of growth may improve detection and outcome.

FGR is a multifactorial condition, but placental dysfunction secondary to 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.

1.2 Current clinical practice for identifying the SGA infant in a low-risk population

Antenatal identification of infants who are subsequently delivered SGA remains challenging. Currently in the UK, healthcare professionals routinely conduct abdominal palpation and measurement of symphysis-fundal height from 24 weeks' gestation to assess fetal size and wellbeing and therefore reduced symphysis fundal height measurement was chosen as the inclusion criteria for the PELICAN FGR study. Several studies have demonstrated that abdominal palpation has limited accuracy (sensitivity 16-50%, specificity 45-95%) for detecting the SGA fetus, with Kean et al. publishing a false positive rate of 51% from their cohort of 2060 women (Bais et al., 2004, Kean and Liu, 1996, Hall et al., 1980, Rosenberg et al., 1982). This high false positive rate results in unnecessary follow up, incurring significant costs to the health service whilst also causing increased anxiety for women. Despite this, abdominal palpation

remains part of routine clinical assessment as it provides information regarding fetal lie and possible abnormalities in amniotic fluid volume (e.g. oligo- or polyhydramnios).

1.2.1 Symphysis fundal height (SFH) measurement

Symphysis-fundal height measurement is easily performed in the community setting as it does not require expensive equipment, but the test performance of this technique is low (sensitivity 27% and specificity 88%) (Persson et al., 1986). Use of customised symphysis-fundal height charts, which adjust for maternal characteristics including maternal height, weight, parity and ethnic group, may improve detection of a SGA neonate whilst also reducing intervention. However, antenatal detection rates for SGA $<10^{th}$ birth weight centile with use of customised fundal height charts have been reported as only 48% (Gardosi and Francis, 1999). Similar findings have been reported in an Australian study where only approximately half of all cases of SGA (44/87) were being detected antenatally with customised symphysis-fundal height charts (Roex et al., 2012). Despite these results, implementation of customised charts in conjunction with accredited training has been associated with a reduction in stillbirth rates in areas of high uptake but these findings are yet to be substantiated in a randomised control trial (Gardosi et al., 2013a). A Cochrane systematic review identified only one randomised control trial of 1639 women, comparing symphysis-fundal height measurement to abdominal palpation. The review concluded that whilst this small study reported that symphysis-fundal

height measurement was less accurate than abdominal palpation at identifying women who subsequently delivered an SGA infant (28% and 48% sensitivity respectively) and adoption of this technique had no significant effect on perinatal outcome, symphysis-fundal height measurement should not be discarded as a screening tool without a much larger trial verifying these findings (Neilson, 2000).

1.2.2 Screening by routine ultrasound assessment

In women with high body mass index (BMI), large fibroids, multiple pregnancy, abnormal fetal lie or polyhydramnios, symphysis-fundal height measurement is even less accurate and an alternative technique, such as serial ultrasound assessment should be considered. Current UK national guidance does not advocate screening all women using routine ultrasound to detect SGA due to reported low sensitivities (21-54%) (Ben-Haroush et al., 2007, Secher et al., 1987, Souka et al., 2012, David et al., 1996, Lindqvist and Molin, 2005) with no evidence of improved perinatal outcome (Bricker et al., 2008). However, the preliminary findings of a recent prospective cohort study in an unselected nulliparous population including 4006 women compared routine sonography in the third trimester to current practice of selective sonography. Improved detection of SGA was reported with routine sonography at 28 and 36 weeks' gestation (sensitivity to detect SGA 57% for routine sonography and 20% for selective sonography) (Sovio et al., 2014). Whilst this study suggests that adoption of routine third trimester ultrasound screening may improve detection

of SGA, test sensitivity is limited, with nearly half of cases still remaining undetected in this study population. Ultrasound scanning requires expensive equipment and highly trained operators; there is therefore a need to evaluate its introduction as a screening approach through a randomised controlled trial prior to widespread adoption of routine third trimester scanning. This should consider the impact on adverse perinatal outcomes and cost implications to the health service.

1.2.3 Doppler measurement

A systematic review investigating the ability of second trimester uterine artery Doppler measurement to predict delivery of an SGA infant concluded that in low-risk women, an increased pulsatility index alone or with diastolic notching best predicted delivery of an SGA infant. However the authors commented that for a test to be clinically useful, it should ideally have a high positive likelihood ratio (>10) and low negative likelihood ratio (<0.10) and whilst increased pulsatility index and bilateral notching are the most promising indices to reach these parameters, their inclusion into a low-risk screening program would incur considerable cost, limiting use in a low resource setting. Inclusion of the test in settings where routine anomaly scanning is undertaken in the second trimester should be achievable, but this would lead to the inevitable identification of false positive cases. This could cause unnecessary anxiety in those who test positive but do not have the condition and more concerning, as this test has a negative likelihood ratio of 0.89, a considerable number of cases would not be detected,

which would be unacceptable for a stand-alone “rule out” screening tool (Cnossen et al., 2008). Whilst highly specific for predicting delivery of an SGA infant, the pooled sensitivity of increased pulsatility index and bilateral notching (12%) is lower than other proposed or currently used screening tools (routine ultrasound scan 21-54%, symphysis-fundal height measurement 27%). In isolation Doppler studies are unlikely to be of benefit but in combination with other parameters test performance could be improved. A further systematic review evaluating the effects of routine second trimester uterine artery Doppler measurement on pregnancy outcome reported no improvement in maternal or neonatal outcome, but only two studies were included in this review (Stampalija et al., 2010). At present, there is insufficient conclusive evidence to support the routine screening of a low-risk population using this technique. Similar conclusions are reported in a systematic review assessing the effects of routine umbilical artery measurement on pregnancy outcome in low-risk populations and therefore routine screening with this technique is not currently recommended (Alfirevic et al., 2010).

In high-risk populations, second trimester uterine artery Doppler measurement (specifically increased resistance index >90th gestation specific centile) has been shown to have a moderate prediction for delivery of a severely SGA infant (sensitivity 82% (CI 76–87%), specificity 92% (CI 92–93%), positive likelihood ratio (LR) 10.9 (CI 10.4–11.4%), negative LR 0.20 (CI 14–26%)) (Cnossen et al., 2008). Current UK guidance therefore recommends this technique for screening high-risk women (Robson et al., 2013).

1.2.4 Summary

Current clinical practice in the UK to identify pregnancies at risk of delivering an SGA infant in a low risk population relies on techniques with limited test performance. Whilst other tests, such as routine third trimester screening with ultrasound assessment or second trimester uterine artery Doppler assessment show promise as screening tools in the low risk population, they are not currently recommended either due to limited test performance, cost implications or lack of data regarding effect on adverse perinatal outcome. At present, there is a need for a cost effective screening tool, which will accurately identify those at risk of delivering an SGA infant.

1.3 Diagnosing the SGA fetus

UK national guidelines suggest that any woman with a symphysis-fundal height measurement below the 10th centile or serial measurements that show diminished or static growth should be referred for ultrasound assessment of fetal size. Abdominal circumference (AC) and/ or estimated fetal weight (EFW) are routinely measured; the 10th centile has been quoted as the optimal threshold for predicting delivery of an SGA infant and adverse outcome (customised EFW for prediction of SGA in a high-risk population sensitivity 68%, specificity 89%, positive predictive value (PPV) 72%, negative predictive value (NPV) 86%, AC for prediction of SGA sensitivity 48-64%, false positive rate 38-55%) (Chang et al., 1992, De Jong et al., 2000). However, a recent large multicentre prospective observational study reported that EFW less than the 3rd centile was more strongly associated with adverse perinatal outcome (Unterscheider et al., 2013). Measurement of EFW, in addition to AC, has the benefit of allowing use of customised EFW centiles, which may improve detection of those pregnancies at greatest risk of adverse outcome (discussed in section 1.1.2.1).

However, despite the reported improvement in identifying SGA infants using customised centiles, a large proportion are still not detected antenatally. DeJong et al. published a series of 215 high-risk pregnancies (defined as presence of pre-existing hypertension, age over 35 years, smoker, previous

history of FGR or hypertensive disorder of pregnancy) and reported that despite use of customised EFW centiles, 32% of those who delivered an SGA infant were not detected antenatally (De Jong et al., 2000). Gardosi et al. conducted a cohort study in the West Midlands between 2009 and 2011, to investigate maternal and fetal risk factors for stillbirth. Of the 389 cases of stillbirth, 195 were SGA (with birth weight <10th customised centile for gestational age) but 82% of these cases were not detected antenatally, despite use of customised EFW centiles. In pregnancies where SGA was detected antenatally the risk of stillbirth was 9.7 compared to 19.8 in those where SGA was not known, emphasising the importance of antenatal detection (Gardosi et al., 2013b). On further investigation, it was reported that adherence to guidelines on use of customised EFW charts and levels of training varied between recruiting centres, which may explain why this study reported detection rates below those previously noted (Gardosi et al., 2013b).

In addition to assessing fetal size, the amniotic fluid volume is routinely assessed. This is usually reported as a single deepest vertical pocket measurement or amniotic fluid index (AFI). The latter is calculated by the sum of the deepest vertical liquor pool depth in each of the four quadrants. Whilst low AFI has been correlated with delivery of an SGA infant (Hashimoto et al., 2013), it has low predictive accuracy (sensitivity 14-45% and specificity 57-97%) and is a poor predictor of adverse perinatal outcome (Chauhan et al., 2008, Niknafs and Sibbald, 2013, Magann et al., 2011).

AFI is one of five factors (AFI, fetal breathing, movements, tone and cardiotocograph) assessed in the calculation of biophysical profile (BPP). BPP has a maximum score of 10 (each factor scored out of two) and low scores have been associated with perinatal mortality (Manning, 2002). However, its clinical implementation, even in high-risk pregnancies, has not been shown to improve perinatal outcome, or reduce perinatal death, limiting its utility as a surveillance tool in SGA (Lalor et al., 2008).

1.3.1 Summary

Current practice to diagnose the SGA fetus relies on techniques with limited diagnostic capability. From the current literature, customised estimated fetal weight measurement appears to perform best but still misses a large proportion of cases. Given the extent and severity of the morbidity associated with being born SGA, and the limitations of current diagnostic tools to identify whose at risk, there is a need for a cost effective, accurate test which could improve detection. Whilst the pathophysiology of FGR is multifactorial, placental dysfunction plays a key role. With improved knowledge of the pathophysiology of placental disease, multiple biomarkers have been identified which reflect placental function. These could provide a useful adjunct to current techniques in identifying at-risk pregnancies.

1.4 Biomarkers in placental disease

A biomarker has been defined as “any substance, structure or process that can be measured in bio specimens and may be associated with health-related outcomes” (Gallo et al., 2011). Biomarkers relating to specific cellular and molecular events can inform of early biological mechanisms often prior to clinical manifestation of disease. Altered concentrations of biomarkers could therefore be utilised to identify those at particular risk of specific diseases and would be especially useful where current diagnostic tools are limited, such as FGR.

In conditions where the aetiology is multifactorial, the level of understanding of the complex pathological processes implicated may dictate which biomarkers are selected for prediction. If a final common pathway from the differing aetiologies has been identified, then measurement of a single downstream biomarker may be appropriate. Alternatively measurement of multiple biomarkers chosen to reflect the numerous biological processes associated with that particular condition maybe more accurate. The causes of placental dysfunction and subsequent FGR are numerous, utilising multiple biological pathways. This section will focus on proposed biomarkers of placental disease, subdivided according to the biological process with which they are associated. Table 1.1 summarises the main biomarkers discussed in the following sections.

Table 1.1: Biomarkers and mechanism of action

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓ in placental disease
Angiogenic factors			
PlGF	Placental Growth Factor	Angiogenic marker produced by trophoblastic tissues.	↓
VEGF-C	Vascular endothelial growth factor C	Angiogenic marker produced by trophoblastic tissues.	↓
sFlt-1	Soluble fms-like tyrosine kinase-1	Also known as sVEGFR1. Binds VEGF reducing plasma concentrations.	↑
Endoglin	Endoglin	Anti-angiogenic cell surface glycoprotein. ↓ TGFβ binding ↓ nitric oxide signaling leading to ↓ angiogenesis.	↑
Angiogenin	Angiogenin	Potent angiogenic factor which interacts with endothelial cells facilitating migration, invasion, proliferation and formation of tubular structures	↑
C-Met	Tyrosine kinase	Proto-oncogene encoding a protein, hepatocyte growth factor receptor which binds HGF and promotes angiogenesis	↓

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
Endothelial function/damage			
Arginase-1	Arginase 1	Enzymes which compete with nitric oxide synthase (NOS) for l-arginine, ↓ NO formation and ↑ superoxide formation by NOS	↑
Endothelin	Endothelin	Potent vasoconstricting peptide produced by the endothelium. Modulates blood pressure.	↑
NGAL	Neutrophil gelatinase-associated lipocalin	Renal factor. Protein released post ischaemic damage or sepsis	↑
HIF	Hypoxia inducible factor 1-alpha inhibitor	Inhibits transcription factor HIF-1alpha, which mediates cellular responses to hypoxia, preventing tissue repair.	↑
PODXL	Podocalyxin	Renal marker expressed in glomerular podocytes and vascular endothelium, remains patency of the filtration slit. Correlates to eGFR.	↑

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
Cell invasion/ adhesion			
ADAM 9	Disintegrin and metalloproteinase domain-containing protein 9	Modulates cell-cell interactions possibly affecting trophoblast invasion and spiral artery formation. Role in angiogenesis. Marker in renal and prostate cancers	↑
CPA-4	Carboxypeptidase A4	Metallocarboxypeptidase, which cleaves angiotensin-1, a potent vasoconstrictor. Low concentrations in normal tissue	↓
ESAM-1	Endothelial Cell-selective adhesion molecule	Junctional type cellular adhesion molecule expressed by vascular endothelium. Regulates angiogenesis and endothelial permeability.	↓
ICAM-1	Intercellular adhesion molecule 1	SIgnalling protein involved in immune activation.	↑
VCAM	Vascular cell adhesion molecule	SIgnalling protein involved in immune activation.	↑
Kunitz-2 (HAI-2)	Kunitz-type protease inhibitor 2	Trans-membrane serine proteases inhibitor inhibits clotting factors and hepatic growth factor activation.	↓
MMP-9	Matrix metalloproteinase-9	Expressed by cytotrophoblast and aids trophoblast invasion and remodeling of spiral arteries	↑

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
TIMP-1	Metalloproteinase Inhibitor 1	Inhibits matrix metalloproteases, therefore inhibiting trophoblast invasion. Interacts with MMP-12→ increasing plasminogen→angiostatin which inhibits angiogenesis	↑
Cell apoptosis			
Caspase	Caspase	Cysteine protease involved in apoptosis. Expressed in syncytiotrophoblast cytoplasm. Key role in cell digestion. Linked to increased apoptosis in placental disease.	↑
FAS	Tumor necrosis factor receptor superfamily member 6	Chemokine that when bound activates cascade of caspases mediating apoptosis	↑
FasL	Tumor necrosis factor ligand superfamily member 6	Chemokine that when bound causes apoptosis	↑
TNFR1A	Tumor necrosis factor receptor superfamily member 1A	TNF a binds to this receptor stimulating IL-1 activation and pyrexia and cell death.	↓

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
Markers of inflammation			
CRP	C reactive protein	Non-specific inflammatory marker raised in the acute phase immune response. CRP activates the complement system	↑
CXCL10	CXC motif chemokine 10	Immune activator released by endothelial cells.	↑
Elafin	Elafin	Elastase-specific protease inhibitor involved in inflammation.	↑
IL-1ra	Interleukin 1 receptor antagonist	Competitive inhibitor of IL-1, which activates inflammatory response with release of prostaglandins.	↓
MIF	Macrophage migration inhibitory factor	Pro-inflammatory cytokine	↓
PCT	Procalcitonin	Precursor of calcitonin. Involved in calcium homeostasis (↓ plasma [calcium]) and raised in inflammation.	↑
ST2	Interleukin-1 receptor-like 1	Receptor for IL33 detected in liver, kidney, pancreas, prostate, spleen, small intestine and placenta, (particularly in the syncytiotrophoblast). Activation produces modulatory cytokines.	↑
TGFβ-R2	Transforming growth factor beta-receptor 2	Receptor for TGFβ a multifunctional protein controlling proliferation, differentiation and other functions in many cell types.	↓

Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
Markers of coagulation			
PAI-1 and -2	Plasminogen activator inhibitor 1 and 2	Produced by trophoblasts, inhibits fibrinolysis. PAI-1 is pro-angiogenic and modulates coagulation, cell adhesion & migration.	PAI-2 ↓
Pentraxin-3	Pentraxin-related protein PTX3	Involved in the activation of the complement system. Role in innate resistance to pathogens.	↑
Metabolic markers			
PAPP-A	Pregnancy specific plasma protein A	Metalloprotease produced by the syncytiotrophoblast, which cleaves IGFBP-4, increasing IGF-1, which is anti-apoptotic and promotes fetal and placental growth.	↓
IGF-1	Insulin growth factor 1	IGF-1 enhances substrate uptake and suppresses catabolism in fetal tissues.	↓
Leptin	Leptin	Protein product of Ob gene. Released by the placenta and stimulates growth and inhibits apoptosis. Produced in response to hypoxia as a possible defense response to sustain growth.	↑

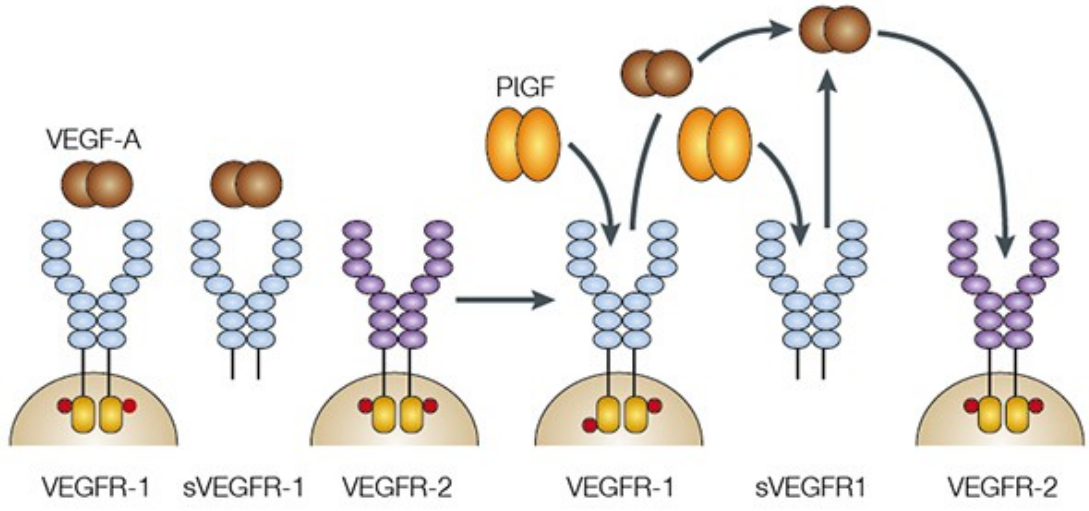
Biomarkers	Biomarker full name	Mechanism of action	↑ or ↓
Renal and cardiovascular biomarkers			
ANP	Natriuretic peptide A	Cardiac hormone causing vasodilatation. Released by the atria in response to stretch	↑
BNP	Natriuretic peptide B	Cardiac hormone causing vasodilatation, and inhibition of renin and aldosterone. Synthesised in ventricle in response to volume expansion and pressure overload.	↑
Nephrin	Nephrin	Renal marker essential for normal glomerular function and cardiovascular development.	↑
Cystatin	Cystatin	Renal marker. Also inhibits cysteine proteases possibly reducing trophoblast invasion.	↑

1.4.1 Biomarkers relating to angiogenesis

Establishing a successful healthy pregnancy is dependent on adequate trophoblast invasion of the maternal spiral arteries, forming a low resistance feto-maternal circulation. Angiogenesis is the process of new blood vessel formation and is essential in facilitating this process. Placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), members of the platelet-derived growth factor family of cysteine knot growth factors, are potent angiogenic factors (Clark et al., 1998a, Clark et al., 1998b, Maglione et al., 1991), which share 53% identity (Iyer et al., 2001, Maglione et al., 1991). PlGF is a 149 amino acid long protein, encoded by a single gene, the PlGF gene, on chromosome 14 and is expressed by placental trophoblastic tissue (Maglione et al., 1993, Clark et al., 1998b, Maglione et al., 1991). There are four isotopes of PlGF; PlGF-1 and PlGF-3 are non-heparin binding isoforms, whilst PlGF-2 and PlGF-4 have additional heparin binding domains (Hauser and Weich, 1993, Yang et al., 2003). PlGF binds directly to VEGFR-1, a tyrosine kinase receptor (also known as Flt-1), activating a number of pro-angiogenic genes (Schoenfeld et al., 2004, Autiero et al., 2003b).

VEGF is a protein, key for the regulation of angiogenesis. VEGF binds to the same tyrosine kinase receptor as PlGF, but also to VEGFR-2, (also known as Flk-1), for which it has higher affinity (Bates, 2011). Binding results in a transient calcium influx, which triggers a rapid increase in permeability (Bates et al., 2002). The overall affects increase vascular permeability, angiogenesis and vasodilatation (Bates and Harper, 2002).

Figure 1.2: Molecular mechanisms of placental growth factor and vascular endothelial growth factor. Reproduced from (Fischer et al., 2008)



In addition to direct angiogenic effects, PlGF also displaces VEGF-A from VEGFR-1 (Flt-1), allowing it to bind to VEGFR-2, which indirectly enhances angiogenesis (Park et al., 1994). PlGF also up regulates the expression of several other angiogenic factors (Roy et al., 2005, Marcellini et al., 2006).

Reduced maternal concentrations of PlGF and VEGF have been correlated with delivery of an SGA infant in numerous studies prior to onset of disease (Tjoa et al., 2001, Bersinger and Odegard, 2004, Thadhani et al., 2004, Espinoza et al., 2007, Stepan et al., 2007, Diab et al., 2008, Erez et al., 2008, Poon et al., 2008b, Asvold et al., 2011, Vandenberghe et al., 2011, Benton et al., 2012, Chappell et al., 2013, Bersinger and Odegard, 2005). Both factors bind to soluble VEGF receptor-1 (sVEGFR-1) (also known as soluble fms-like tyrosine kinase-1 (s-Flt-1)), which prevents interaction of PlGF and VEGF with membrane bound Flt-1 in vascular tissues, leading to endothelial dysfunction

(Autiero et al., 2003a). VEGF has higher affinity for s-Flt-1 than Flk-1 and therefore if low concentrations of PlGF are expressed then VEGF preferentially binds to sFlt-1 and Flt-1, which induces only weak tyrosine kinase activity, reducing angiogenic activity. Three studies measuring s-Flt-1 in the second trimester have correlated increased maternal s-Flt-1 concentrations with delivery of an SGA infant (Stepan et al., 2007, Diab et al., 2008, Asvold et al., 2011). The first two studies published test performance statistics (sensitivity 64-89% and specificity 54-62%) to detect SGA but were limited by very small case numbers (n=9, n=11) and part of the inclusion criteria for both studies was abnormal second trimester uterine artery Doppler measurement, a risk factor for delivery of an SGA infant.

Further biomarkers involved in angiogenesis include soluble endoglin (s-Eng), angiogenin and C-met. S-Eng is an anti-angiogenic soluble TGF-ß co-receptor, which inhibits TGF-ß binding, leading to reduced nitric oxide signaling, with associated reduction in angiogenesis (Venkatesha et al., 2006). Three small case-control studies have reported increased maternal concentrations of S-Eng in cases delivering an SGA infant (Erez et al., 2008, Asvold et al., 2011, Romero et al., 2008). Two of these studies compared S-Eng concentrations between the first two trimesters whilst the third investigated concentrations throughout pregnancy. None of the studies commented on whether concentration of S-Eng correlated with severity of disease. Angiogenin is a potent angiogenic factor, which interacts with endothelial cells facilitating migration, invasion, proliferation and formation of tubular structures. Raised maternal concentrations of angiogenin have been correlated with pre-eclampsia and co-existing SGA but there were no cases of normotensive SGA in this

study (n=91) (Shaarawy et al., 2005). In contrast, Yamashiro et al. found no difference in maternal or fetal plasma concentrations of angiogenin at delivery between SGA and AGA pregnancies (Yamashiro et al., 2000). Both studies were case-control design and limited by small sample sizes (n=91 and n=61 respectively). Larger prospective studies would be necessary to further evaluate a possible relationship between angiogenin concentrations and delivery of an SGA infant. C-met, also known as hepatocyte growth factor receptor (HGFR), binds to HGF and promotes angiogenesis. Zeng et al. published the results of a small prospective case-control study (n=44) evaluating the ability of soluble C-met measured in the second and third trimester to predict pre-eclampsia. When sampled between 25-30 weeks' gestation, the ROC area under the curve (AUC) for C-met to predict development of pre-eclampsia was 0.95 (CI 0.9-1.0) (Zeng et al., 2009). However, they did not include delivery of an SGA infant as an endpoint and to date there are no published data regarding the ability of C-met concentrations to predict this outcome.

Conde-Agudelo et al. conducted a systematic review and meta analysis of biomarkers for predicting intrauterine growth restriction (IUGR, which they defined as failure of the fetus to achieve its optimal growth potential) and reported that the overall predictive accuracy of angiogenic biomarkers for IUGR was minimal (Conde-Agudelo et al., 2013). The review included 53 studies, evaluating 37 biomarkers, including thirteen studies reporting on PlGF, one on VEGF, three on s-Flt-1 and two on s-Eng. The overall summary ROC curve of PlGF to predict FGR gave an AUC of 0.66 (95% CI 0.44-0.87). However, there were multiple limitations to this review. The ROC curve calculation of PlGF to predict FGR did not stratify for gestational age at sampling and the only attempt

made by the authors to further subdivide according to gestational age split these data according to sampling before or after 20 weeks' gestation. The ROC area did improve if sampling occurred after 20 weeks, but only two of the five studies in this calculation included women sampled in the third trimester, where a predictive test would be particularly helpful to aid decisions regarding timing of delivery. All of these studies were case-control design with the exception of one prospective cohort study (Espinoza et al., 2007) where the primary outcome was pre-eclampsia rather than delivery of an SGA infant. Whilst the authors acknowledged the difficulty in defining IUGR, there was great heterogeneity in definitions of FGR which included; delivery of an infant with birth weight $<5^{th}$ or $<10^{th}$ centiles with or without additional clinical or pathological evidence of FGR and IUGR requiring delivery before 34 weeks' gestation. Many of the studies in this review used delivery of an SGA infant (I.e. birth weight $<5^{th}$ or $<10^{th}$ centiles for gestational age) as a surrogate for FGR with the inherent problem of including constitutionally small infants within this definition. Only six of the 53 studies used an appropriate reference standard that included birth weight centile and additional clinical or pathological evidence of FGR. Only three studies fulfilled all five criteria of methodological quality for inclusion in the analysis and thresholds for determining an abnormal result differed between studies when the same biomarker was being evaluated.

A small case-control study (n=88), included in the systematic review and fulfilling all five criteria for methodological quality, defined SGA according to birth weight, additional clinical data (second trimester uterine artery Doppler notching, or absent/reversed umbilical artery end diastolic flow or oligohydramnios) and placental pathology. This group reported much more encouraging test performance than the pooled ROC AUC published in the

meta-analysis by Conde-Agudelo et al. (sensitivity 100% (CI 66–100%), specificity 86% (CI 42–100%)) (Benton et al., 2012). This is supported by the findings of a recent large, prospective, multicentre observational study in women presenting with suspected pre-eclampsia (n=625). In this study, low PlGF concentrations <5th centile predicted delivery of an infant with birth weight <3rd centile in those sampled before 35 weeks' gestation, with high sensitivity (90%; CI 82 to 95%) and negative predictive values (91%; CI 85 to 96%) (Chappell et al., 2013). In addition to the limitations of the systematic review and meta-analysis by Conde-Agudelo et al. outlined above, other explanations for the improved performance seen in these two studies include the use of a PlGF assay that measured the PlGF-1 isoform with minimal cross-reactivity for PlGF-2, with proven higher sensitivity and specificity than assays used in the other studies (Benton et al., 2011), and both studies sampling high-risk populations. Further investigation is required in the general antenatal population, prior to considering routine incorporation of PlGF into clinical practice.

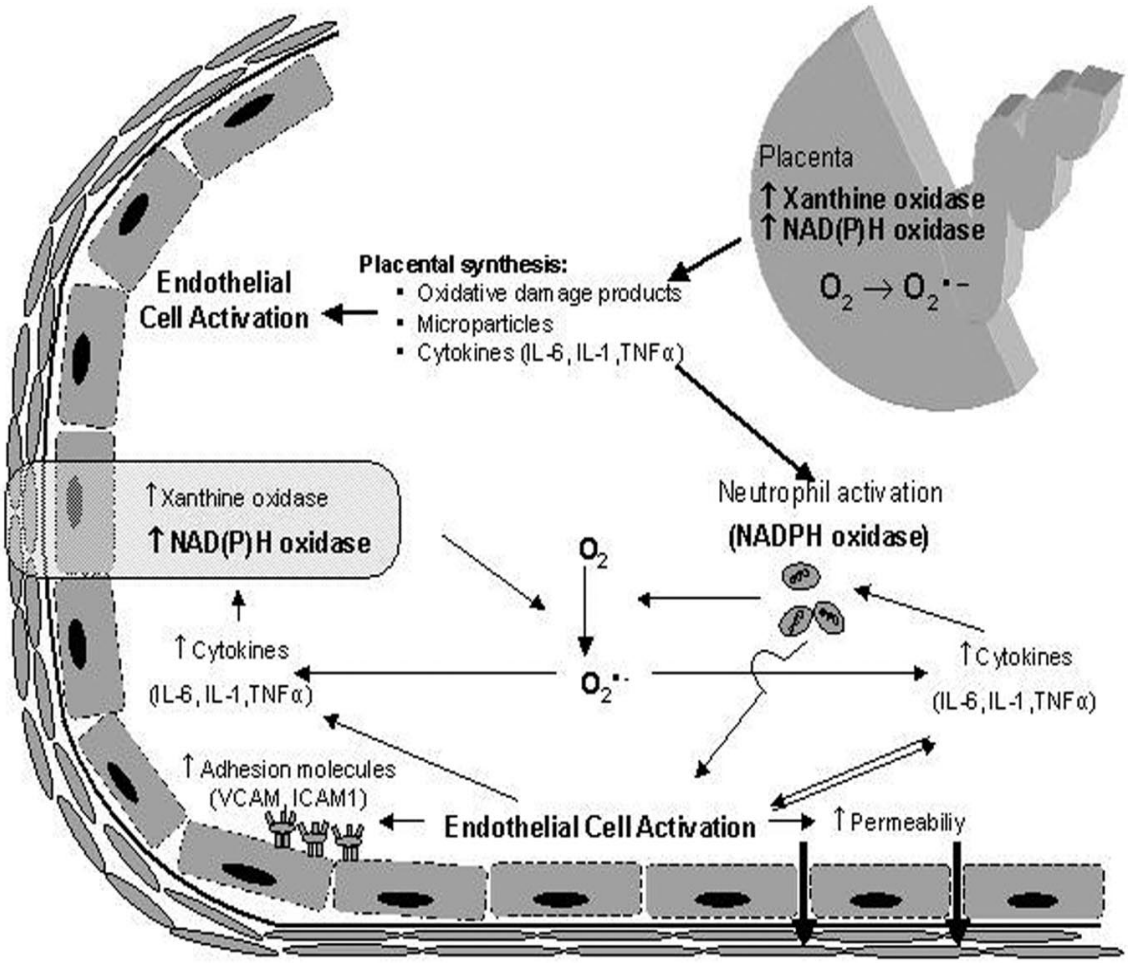
1.4.2 Biomarkers relating to endothelial function/ oxidative stress/ inflammation and coagulation

The multiple causes of poor uteroplacental blood flow outlined in sections 1.1.3.1 and 1.1.3.3 (maternal and placental factors), lead to a chronic hypoxic state in the placenta and intermittent flow with reperfusion results in widespread damage and systemic oxidative stress (a disturbance in the balance between reactive oxygen species production and clearance). Nevo et al. and Yinon et al. suggest that in *in vivo* and *in vitro* models, hypoxia up-regulates anti-angiogenic

markers including s-Flt (Nevo et al., 2006) and S-Eng (Yinon et al., 2008). Up-regulation of s-Flt is mediated by Hypoxia Inducible Factor (HIF-1), which has been proposed as a possible biomarker to identify pregnancies at risk of pre-eclampsia, but was not found to be a good predictor of those with SGA (Rolfo et al., 2010, Rajakumar et al., 2007).

It is proposed that oxidative stress up-regulates xanthine oxidase and NAD(P)H oxidase in the placenta producing superoxides and oxygen free radicals, which cause localised cell injury. Associated release of cytokines (e.g. IL-1, IL-6 and TNF-α) activate neutrophils and have direct effects on endothelial cell activation (Raijmakers et al., 2004).

Figure 1.3: Summary of pathways involving placental oxidative stress leading to maternal endothelial dysfunction. Reproduced from (Raijmakers et al., 2004)



Several biomarkers released by the endothelium have been proposed as potential diagnostic tools for SGA and pre-eclampsia. These include endothelin, arginase, Neutrophil gelatinase-associated lipocalin (NGAL) and podocalyxin. Endothelin is a potent vasoconstrictor and it is suggested that increased release contributes to hypertension observed in pre-eclampsia. Raised maternal concentrations are reported prior to onset of pre-eclampsia, but association with SGA has not been established (Shaarawy and Abdel-Magid, 2000). Arginase

competes with nitric oxide synthase (NOS) for L-arginine and reduces nitric oxide formation with increased superoxide formation by NOS (Sankaralingam et al., 2009). Associations have been made with pre-eclampsia, but not reported for SGA. NGAL is a protein released post ischaemic damage and sepsis; elevated plasma concentrations have been correlated with the presence and severity of pre-eclampsia, but not with birth weight (Kim et al., 2013). A recent small case-control study evaluated the changes in NGAL urinary concentrations in normal pregnancy and pre-eclampsia and reported that whilst the NGAL/creatinine ratio was lower in pre-eclampsia than in healthy pregnancies, urinary NGAL was not a valuable early marker for pre-eclampsia (Odum et al., 2014).

Podocalyxin is a renal marker expressed in podocytes and vascular endothelium, which is important in maintaining glomerular filtration slit patency. Concentrations are raised in several cancers (Nielsen and McNagny, 2009). Given the association between hypertensive disorders, renal disease and placental dysfunction, podocalyxin may be a useful marker in placental disorders although as association with delivery of an SGA infant is yet to be reported.

Endothelial cell activation leads to increased expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM) and a further increase in cytokine release, establishing a positive feedback loop, further increasing xanthine and NAD(P)H oxidase release (Raijmakers et al., 2004). This cycle is key to the ensuing endothelial dysfunction reported in placental disease.

1.4.2.1 Markers involved with cell adhesion

A range of markers involved in cell adhesion have been proposed as potential targets for prediction of pre-eclampsia and delivery of an SGA infant. In addition to ICAM and VCAM, discussed above, data have been published on matrix metalloproteinase 9 (MMP9) regarding its suitability as a marker of placental dysfunction. MMP9 is expressed by the cytotrophoblast and aids trophoblast invasion and remodeling of the spiral arteries (Rahimi et al., 2013). HIgher concentrations measured in the first trimester have been reported with placental disease, specifically pre-eclampsia compared to controls (Poon et al., 2009), but this has not been substantiated in later trimesters and association with SGA is less well established (Myers et al., 2005). High concentrations of an MMP-9 variant have been reported in cases of severe pre-eclampsia, but SGA was not assessed independently in the study (Rahimi et al., 2013). Carboxypeptidase A4 (CPA-4) is a metallocarboxypeptidase, which cleaves angiotensin-I, a potent vasoconstrictor (Wang et al., 2006, Lipscomb et al., 1970). Low concentrations are found in healthy tissue but currently there is no published data relating concentrations to FGR. The ADAM (disintegrin and metalloprotease) family of proteins is involved with cell-to-cell interactions and potentially affect trophoblast invasion and spiral artery formation. There are at least 33 identified family members and there is most detail in the literature regarding the ability of ADAM-12 to predict placental disease. In a retrospective case-control study, Spencer et al. measured ADAM-12 in the first and second trimesters of pregnancy and reported reduced first trimester serum concentrations in those women who developed pre-eclampsia, but elevated concentrations in the second trimester compared to gestation matched controls (Spencer et al.,

2008). Subsequent to this, Poon et al. published the findings of a case-control study evaluating first trimester ADAM-12 concentrations to predict adverse pregnancy outcome, including delivery of an SGA infant. They reported that measurement of first trimester ADAM-12 did not provide useful prediction of SGA bringing into question the clinical utility of this biomarker to determine this endpoint (Poon et al., 2008a).

1.4.2.2 Markers involved with cell apoptosis

Apoptosis is the natural process of programmed cell death, ensuring control of cell numbers within a multicellular organism. If excessive, this process results in tissue atrophy. Concentrations of several markers involved in cell apoptosis have been linked to development of placental disease. Caspase is a serine protease expressed in the syncytiotrophoblast cytoplasm that increases cell apoptosis. Several isoforms have been identified and one small case control study (n=20) reported elevated serum concentrations of caspase-3 in women with pre-eclampsia (Park et al., 2008), Activation of tumour necrosis factor receptor superfamily member 6 (FAS) by tumour necrosis factor ligand superfamily member 6 (FasL), triggers a cascade of caspase release, resulting in cell death. Two small case-control studies (n= 20-38) have reported increased maternal concentrations of FAS and FasL in women with pre-eclampsia and severe FGR (Kuntz et al., 2001, Laskowska et al., 2006), but larger studies are needed to verify these results.

1.4.2.3 Markers of inflammation

Normal pregnancy induces a systemic inflammatory response and the processes outlined above exaggerate this effect. Multiple molecules, which have been identified as key to this process (including those listed in Table 1.1) are therefore altered in placental disease and are attractive potential diagnostic tools as their concentrations change early in the disease process. However, due to the multitude of activators of this process, they are often non-specific making them less suited to clinical application in identifying those at risk of delivering an SGA infant.

1.4.2.4 Markers of coagulation

The exaggerated inflammatory response observed in placental dysfunction is generated by the systemic inflammatory network, which involves not only activation of immune cells, but also the clotting and complement systems and metabolic changes. Alterations in concentrations of modulators of coagulation, such as low plasminogen activator inhibitor 1 and 2 (PAI-1 and 2), have been reported in pre-eclampsia and SGA but this study only included 17 women who delivered an SGA infant (Chappell et al., 2002). Pentraxin-3 is involved in activation of the complement system and plays a role in innate resistance to pathogens. Maternal concentrations have been shown to be elevated at time of diagnosis of pre-eclampsia and SGA and in one small study (n= 23) concentrations correlated to severity, but further evidence from larger cohorts are required to verify these findings (Rovere-Querini et al., 2006, Cozzi et al., 2012).

1.4.2.5 Metabolic markers

The insulin like growth factor (IGF) system is the main regulator of fetal growth. Factors include IGF-I and II and their binding proteins (IGFBP1-6). The IGFs are small polypeptides, which have structural similarity to proinsulin. They are mainly bound to IGFBPs in extracellular tissues, and activity is restricted to the unbound fraction. They are produced by maternal and fetal tissues. Fetal IGF-1 production is mainly regulated by fetal insulin production. IGF-1 enhances substrate uptake and suppresses catabolism in fetal tissues. Fetal serum IGF-1 concentrations have been correlated with birth weight, with lower fetal concentrations in SGA fetuses (Maulik et al., 2006). A prospective cohort study including 153 women evaluated IGF-1 concentrations in maternal serum and reported a significantly lower concentration in those women who delivered a preterm SGA infant (median [IQR]: 134 [99–181] µg/L, P <0.05) compared with AGA pregnancies (200 [181–221] µg/L), but this association was not significant in women delivering at term (Chiesa et al., 2008). A review of the endocrine regulation of fetal growth summarised conflicting data regarding altered maternal serum concentrations of IGF-1 in cases of FGR. They concluded that at present there are no clear data supporting measurement of maternal serum IGF-1 as a screening tool for FGR (Murphy et al., 2006).

IGF-II modulates early embryonic growth and overexpression leads to excessive growth. Unlike IGF-1, there has been little association with SGA. In addition to the insulin growth factors, other metabolic markers have been proposed. Dessi et al. have recently published a review article evaluating the ability of myo-inositol to predict FGR (Dessi and Fanos, 2013). Myo-inositol is a stereoisomer of inositol, a naturally occurring sugar alcohol. This group

proposed that in the growth restricted state, insulin concentrations are reduced as an adaptive mechanism allowing preservation of fetal energy stores with a consequent increased excretion of inositol into the extracellular compartment and increased concentrations of metabolites in maternal plasma. This review article included six small case-control studies (n=8-56) with four reporting a statistically significant increase in concentrations of myo-inositol in the plasma of cases complicated by FGR. However, only two of these studies were in humans and given the small numbers included, larger cohort studies are needed to further investigate these findings.

Pregnancy specific plasma protein A (PAPP-A) is a metalloprotease produced by the syncytiotrophoblast, which cleaves IGFBP-4, increasing the free fraction of IGF-1 and thus promoting 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. Low maternal concentrations have been reported in both pre-eclampsia and SGA (Cowans and Spencer, 2007, Smith et al., 2002, Vandenberghe et al., 2011, Bersinger and Odegard, 2004) but a systematic review and meta analysis assessing the ability of serum Down's syndrome screening markers to predict pre-eclampsia and SGA concluded that in isolation, PAPP-A had low predictive accuracy for delivery of an SGA infant. This review included 10 studies investigating SGA (seven where measurement was in first trimester), and concluded that the most accurate predictor for determining a birth weight <10th centile was PAPP-A <1st centile (even then with a modest positive LR of 3.50 (2.53-4.82)) (Morris et al., 2008). Another ADAM-like metalloprotease has been identified, PAPP-A2, but an accurate immunoassay for this marker has not been available and reference

ranges in pregnancy had not been established. Kløverpris et al. have recently evaluated an immunoassay for PAPP-A2 and have published normal ranges in pregnancy (Kloverpris et al., 2013). Assessment of the performance of PAPP-A2 performance as a marker of placental disease is now awaited.

In addition to the IGF family, increased expression of leptin, a placental protein that stimulates growth and inhibits apoptosis, has been reported in pre-eclampsia, however, differences in maternal concentrations between pregnancies delivering an SGA infant and those delivering an AGA infant were not statistically significant (Chappell et al., 2002). In addition, leptin concentrations are affected by BMI making clinical application more challenging, and this itself could be a potential confounder.

1.4.3 Renal and cardiovascular biomarkers

Given the association of hypertensive and renal disorders with placental dysfunction, established markers of these disease processes may be useful in identifying those at risk of placental disease. Raised maternal concentrations of the cardiac markers natriuretic peptides A and B (ANP and BNP) have been observed in pre-eclampsia (Szabo et al., 2011, Ringholm et al., 2011). BNP is synthesized in the ventricle in response to volume expansion and pressure overload and causes vasodilatation and inhibition of renin and aldosterone. ANP is released by the atria in response to stretch and also results in vasodilatation. However, to date there are no published data to support an association with delivery of an SGA infant.

Several markers of renal function have been linked to placental dysfunction. Cystatin is a low molecular weight protein, which is filtered from the bloodstream by the glomerulus. Any deterioration in glomerular filtration rate leads to increased plasma concentrations of cystatin. Cystatin is also an inhibitor of cysteine proteinases and may therefore affect regulation of trophoblast invasion by proteinases. Raised maternal cystatin concentrations have been correlated with subsequent development of pre-eclampsia (Thilaganathan et al., 2009, Thilaganathan et al., 2010, Saleh et al., 2010), however, an association with SGA is yet to be established. Nephrin is a transmembrane protein that is a structural component of glomerular filtration slits. It is essential for normal glomerular function and cardiovascular development. Nephrin concentrations are closely correlated to glomerular filtration rate (Zheng et al., 2011) and it is proposed that high urinary concentrations could be observed in pre-eclampsia. However, there is no current evidence to link this marker to placental disease.

1.4.4 Summary

To date, the ability of an isolated biomarker to accurately identify pregnancies at risk of delivering an SGA infant has been debated but from the current literature the angiogenic group of factors, specifically PlGF, offers the most promise.

1.5 Combinations of clinical parameters and biomarkers for determining the SGA infant

Whilst none of the parameters discussed so far have, in isolation, been recommended for use in clinical practice to identify the pregnancy at risk of SGA, a combined approach may offer improved detection. Several studies have evaluated the utility of first trimester biomarker panels for determining delivery of an SGA infant (Pihl et al., 2008, Poon et al., 2009), but none have reported sufficient test performance to warrant recommendation for use in clinical practice. The findings of a systematic review evaluating the predictive capabilities of combinations of serum biomarkers measured in the late first and early second trimesters (as part of screening for chromosomal disorders) to determine subsequent delivery of an SGA infant corroborate this as they report low test performance (Hui et al., 2012). However, this review was hampered by the heterogeneity of studies included; the authors concluded that large cohort studies, with standardised screening test parameters and outcomes, should be undertaken to better evaluate these combinations (Hui et al., 2012).

Other studies have incorporated maternal characteristics and/ or ultrasound parameters (Karagiannis et al., 2011, Poon et al., 2008b, Poon et al., 2013, Papastefanou et al., 2012). However the most promising combinations of markers and clinical characteristics proposed had only modest predictive ability in determining delivery of an SGA infant; e.g. for a combination of mean arterial pressure, maternal characteristics, uterine artery pulsatility index, PAPP-A and PlGF, sensitivity was 55.5% for preterm SGA <37 weeks' gestation for 10% false positive rate (Poon et al., 2013). Whilst detection rates of SGA are

improved in these studies, compared to those combining biomarkers alone, combinations include ultrasound measurements requiring highly skilled operators, which may limit acceptability for women and preclude adoption in a low resource setting.

1.6 Who and when to screen for SGA

Given the morbidity and mortality associated with being born SGA, identifying those at risk and allowing appropriate follow up and timing of delivery may improve outcome. However, deciding the most appropriate population to screen for SGA is problematic. A population-based approach would be costly and may lead to false identification of cases, resulting in increased anxiety and potentially unnecessary follow-up and intervention in this group. Consideration of the economic implications of adopting a population based approach is also important as screening a large population has significant financial implications for detection of a condition that whilst common, has poor outcome in only a small subgroup. Given the multifactorial nature of SGA, it is unlikely that a low cost test with sufficient sensitivity and specificity to identify those at increased risk of FGR within a large, low-risk population will be identified.

An alternative approach is to screen a more defined population already identified as being at increased risk (e.g. previous history of delivery of an SGA infant or reduced symphysis fundal height measurement). Whilst the latter is currently utilised to identify those within the antenatal population who warrant further investigation for SGA, its low sensitivity and specificity to detect the condition lead to true cases being missed. However, this is likely to be a more cost effective option than a population based screening program.

Timing of screening is also challenging. Assessment early in pregnancy has the benefit of identifying women at risk early, allowing appropriate follow-up, but the further the time interval from assessment to development of the condition generally leads to a reduction in any test performance. Ideally assessment of risk should be late enough to minimise false positive and false negative results, but early enough to identify cases with sufficient time to plan appropriate follow-up and timing and place of delivery. To date, most studies assessing biomarker panels in predicting subsequent delivery of an SGA infant have involved sampling in the first and second trimesters of pregnancy, where test performance was insufficient to recommend incorporation into clinical practice. It is possible that sampling in the third trimester, closer to time of delivery maybe an appropriate alternative.

1.7 Chapter Summary

Whilst numerous biomarkers have been proposed as potential tools for identifying those at risk of placental disease, sufficient test performance for adoption into clinical algorithms to identify pregnancies at risk of SGA has not yet been established. Of all the mechanistic groups discussed, there are most published data on the angiogenic factors. The data relating to PlGF and prediction of delivery of an SGA infant in a high-risk population are promising, but further evaluation is required before recommendations can be made for widespread deployment.

Whilst acknowledging its limitations (as discussed in section 1.4.1), a recent systematic review concluded that at present there is no individual clinically useful biomarker for predicting SGA in women with singleton pregnancies (Conde-Agudelo et al., 2013). They give the multifactorial nature of SGA as a possible explanation for this finding. It is suggested that combining measurement of multiple biomarkers, covering a variety of pathological processes contributing to delivery of an SGA infant may provide a more promising test. However, from the current data summarised in section 1.5, the ideal combination providing sufficient test performance for clinical practice, whilst also maintaining clinical acceptability, is still elusive. This may be partially related to the timing of sampling as most studies reported on samples taken in the first and second trimesters, where changes in these markers may be too subtle to be detected by current assays or alterations in concentrations may occur at later gestations. There is a paucity of evidence assessing measurement of third trimester biomarker panels, covering a wide range of pathological processes, to determine delivery of an SGA infant. Considering this is the most frequent time of presentation with suspected SGA and is closer to time of delivery, it seems logical to evaluate tests within this timeframe.

Given the limitations of current parameters for identifying the SGA infant, and the current evidence summarised above, there is an acute need for improved detection, which has led to the formation of the hypotheses below.

1.8 Aims

1. To assess the ability of third trimester biomarkers and ultrasound parameters to determine delivery of a SGA infant in women with suspected pre-eclampsia

2. To assess the diagnostic accuracy of PlGF and ultrasound parameters to predict the SGA infant in women presenting with suspected SGA.

1.9 Hypotheses

In this work, I collected data from two cohorts (the first with suspected pre-eclampsia and the second with suspected SGA fetuses) to test the following hypotheses:

1. A combination of biomarkers, identified from 47 biomarkers, selected for their biological plausibility, can accurately identify women at risk of delivering an SGA infant from a cohort presenting with signs and symptoms, but not proven pre-eclampsia (suspected pre-eclampsia).

2. PlGF can more accurately determine delivery of an SGA infant in women presenting with suspected SGA (i.e. reduced symphysis fundal height measurement, the current clinical tool for referral for further investigation in the UK) compared to current ultrasound parameters.

Chapter 2: Biomarkers and ultrasound parameters to determine placental dysfunction

2.1 Introduction

Whilst numerous individual biomarkers have been proposed as potential diagnostic tools to identify pregnancies at risk of delivering an SGA infant, there is currently insufficient evidence to recommend incorporating any of these tests into routine clinical practice (Conde-Agudelo et al., 2013). It is suggested that given the multifactorial nature of SGA, a combination of biomarkers, which reflect a variety of pathological pathways key to development of SGA, may provide a more promising approach. A test early in pregnancy for distant prediction of delivering an SGA infant would allow appropriate follow up and planning of delivery. However, numerous studies measuring biomarker panels in the late first and early second trimesters of pregnancy have failed to identify women at risk of delivering an SGA infant with sufficient accuracy. This may be due to changes in these factors occurring beyond the first and second trimesters, alterations being below the level of detection for the selected biomarker assays at the time of testing, or lack of discrimination between subsequent cases and those with normal pregnancy outcome when measured many weeks prior to manifestation of the growth restriction.

Measuring biomarker panels at later gestations may offer more accurate prediction, as testing closer to the onset of any condition generally yields improved test performance; variation in certain biomarker concentrations between those who deliver an appropriate for gestational age infant and those whose pregnancies are complicated by SGA may become more marked at later gestations, while providing clinical information at a time that influences immediate management. Biomarker measurement in the late second and third

trimesters of pregnancy to identify those at greatest risk of delivering an SGA infant may be especially useful in high-risk pregnancies, such as those with hypertensive disorders, as this could provide additional information to aid decision making regarding level of monitoring and timing of delivery.

At present there are few published data regarding performance of individual biomarkers or panels measured at later gestations to predict delivery of an SGA infant.

We identified a large group of biomarkers with either a biological role in placental function, known association with pre-eclampsia or involvement with placental disease. These biomarkers were measured in women with suspected pre-eclampsia, a group at high-risk of placental dysfunction with the aim of developing a biomarker panel capable of accurately identifying those at risk of delivering an SGA infant.

2.1.1 Involvement with the study

The primary aim of the PELICAN-PE study was to assess the diagnostic accuracy of placental growth factor (PlGF) to determine need for delivery for confirmed pre-eclampsia in women presenting with suspected pre-eclampsia. I acted as study coordinator for the PELICAN-PE study from August 2011, until study completion (February 2012). This involved liaising with all study sites and conducting regular site visits, providing database training for new members of staff and coordinating study teleconferences to provide updates. I received training in use of the PlGF Triage meter and accompanied the study monitor on

site visits and assisted in assessing quality control of the meters at enrolling sites. In addition to my role as study coordinator, I also recruited over 100 women from the day assessment unit, antenatal ward and delivery suite at St Thomas' Hospital, London and coordinated and inputted study data. I was a member of a panel of expert adjudicators who reviewed all final maternal diagnoses to assign final adjudicated diagnoses, adjudicating for sites other than where I enrolled women. I was involved in the statistical analysis plan for the study and contributed to the first manuscript (as a co-author) arising from the results, published in the peer-reviewed journal, Circulation, in November 2013 (published manuscript in appendix).

In addition to the primary aim of the PELICAN-PE study I also assessed the ability of a large panel of biomarkers to determine pre-eclampsia and delivery of an SGA infant in women presenting with suspected pre-eclampsia.

2.2 Methods

Data presented in this chapter are taken from the PELICAN-PE study. Women were recruited from seven consultant-led maternity units in the United Kingdom and Ireland, between January 2011 and February 2012. Ethical approval was granted by East London Research Ethics Committee (ref. 10/H0701/117).

Women presenting with signs or symptoms of suspected pre-eclampsia, between 20^{+0} and 40^{+6} weeks' gestation with a singleton or twin pregnancy and aged ≥16 years were eligible for inclusion in the study. Written informed consent

was obtained from participants. A study-specific database was created and baseline demographic and pregnancy-specific information were entered. At study enrolment, blood was drawn into ethylenediamine tetra-acetic acid and samples were labelled, and transported to the laboratory where they were spun at 3000 rpm for 10 minutes. Plasma was extracted and stored at -80^{o}C until analysis. All cases were adjudicated by a panel of experts to assign a final maternal diagnosis using definitions defined below in 2.2.1. All diagnoses were assigned without knowledge of any biomarker values.

2.2.1 Definitions

Definitions were pre-defined by the PELICAN study co-investigator group in collaboration with a neonatologist to reflect adverse perinatal outcomes relevant to pre-eclampsia, particularly that requiring preterm delivery. A decision was made to include the consequences of prematurity rather than preterm delivery itself.

Definitions for maternal hypertensive disorders of pregnancy were taken from the American College of Obstetricians and Gynecologist's practice bulletin available at the time of study design and adjudication (ACOG Practice Bulletin, 2002) and are as follows:

Mild gestational hypertension:

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

Severe gestational hypertension:

Systolic BP ≥ 160 mmHg or DBP ≥ 110 mmHg on two occasions at least 4 hours to one week apart presenting after gestational week 20 without proteinuria or markers of severe pre-eclampsia.

Chronic hypertension:

Documented presence of chronic non-gestational hypertension (systolic BP >140 mm Hg and/or DBP > 90 mm Hg) prior to this pregnancy,

or

On anti-hypertensive medication prior to 20^{+0} weeks' gestation, or at 6 weeks post partum.

Proteinuria*:*

Any of the following*:

1) 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);
2) Protein: Creatinine ratio ≥ 30 (mg/mmol).
3) Urinary protein 1+ on dipstick on two occasions at least 4 hours apart.
4) Urinary protein ≥2+ on dipstick on one occasion.

*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 mmHg or DBP ≥ 110 mmHg 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 upper limit of normal for AST and/or ALT);
- Thrombocytopenia (platelet count <100,000/mm^3);
- Fetal growth restriction (fetal weight <10^{th} percentile for gestational age).

Superimposed pre-eclampsia (Traditional definition):

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

Superimposed pre-eclampsia (Atypical):

- Chronic hypertension plus abnormal laboratory test (low platelets or elevated liver enzymes).
- Chronic proteinuria plus gestational hypertension.

Atypical pre-eclampsia:

In the absence of proteinuria:

Gestational hypertension plus any of the following:

- Haemolysis (elevated total bilirubin >1.2 mg/dl);
- Thrombocytopenia (platelet count <100,000/mm^3);
- Elevated liver function tests (2X upper limit of normal for AST and/or ALT).
- Fetal growth restriction (birth weight <10% percentile)

In the absence of hypertension:

Gestational proteinuria plus any of the following:

- Haemolysis (elevated total bilirubin >1.2 mg/dl);
- Thrombocytopenia (platelet count <100,000/mm^3);
- Elevated liver function tests (2X upper limit of normal for AST and/or ALT).
- Fetal growth restriction (birth weight <10% percentile)

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.

HELLP syndrome:

Gestational hypertension and/or gestational proteinuria plus elevated liver enzymes (2X upper limit of normal), elevated LDH (2X upper limit of normal), thrombocytopenia (platelet count <100,000/mm^3) and evidence of Haemolysis (elevated total bilirubin >1.2 mg/dl).

Delivery of a small for gestational age infant was defined as birth weight <3^{rd} and <10^{th} customised centile, calculated using the Gestation Related Optimal Weight (GROW) method (Gardosi et al., 1992).

Adverse perinatal outcome was 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.

Adverse maternal outcome was 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 $<50\times10^{9}$/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.

2.2.2 Biomarker selection and measurement

A detailed literature review was undertaken, in consultation with medical experts, to identify potential biomarkers for assessment. The 47 biomarkers selected had either a known association with pre-eclampsia and/or a biologically plausible role in placentation, or a role in the pathophysiology of placental disease e.g. angiogenesis, inflammation, coagulation. A full list of the 57 biomarker assays with abbreviations, units and assay information are displayed in Tables 2.1 and 2.2.

Table 2.1: List of Biomarker abbreviations and units

Biomarker	Biomarker full name	Units
PlGF	Placental Growth Factor	pg/ml
ADAM 9	Disintegrin and metalloproteinase domain-containing protein 9	pg/ml
Angiogenin	Angiogenin	µg/ml
ANP	Natriuretic peptide A	ng/ml
Arginase-1 10a	Arginase 1	ng/ml
Arginase-2 11a	Arginase 2	ng/ml
BNP	Brain natriuretic peptide	ng/ml
Caspase	Caspase	ng/ml
CCL23	MIP3, C motif chemokine 23	ng/ml
C-Met-109a	Tyrosine kinase	ng/ml
C-Met-111a	Tyrosine kinase	ng/ml
CPA-4	Carboxypeptidase A4	ng/ml
CRP	C reactive protein	µg/ml
CXCL10	CXC motif chemokine 10	ng/ml
Cystatin	Cystatin	ng/ml
Elafin-131	Elafin	ng/ml
Elafin-132	Elafin	ng/ml
Endoglin	Endoglin	ng/ml
Endothelin	Endothelin	pg/ml
Ephrin	Ephrin	pg/ml
ESAM-1	Endothelial Cell-selective adhesion molecule	ng/ml
FAS	Tumor necrosis factor receptor superfamily member 6	ng/ml
FasL	Tumor necrosis factor ligand superfamily member 6	ng/ml
HIF	Hypoxia inducible factor 1-alpha inhibitor	ng/ml
HbF	Haemoglobin Fetal	ng/ml
ICAM-1	Intercellular adhesion molecule 1	ng/ml
IL-1ra	Interleukin 1 receptor antagonist	pg/ml
Kunitz-2 (HAI-2) 34a	Kunitz-type protease inhibitor 2	ng/ml
Kunitz-2 (HAI-2) 35b	Kunitz-type protease inhibitor 2	ng/ml

Biomarker	Biomarker full name	Units
Kunitz-2 (HAI-2) 40b	Kunitz-type protease inhibitor 2	ng/ml
Leptin	Leptin	ng/ml
Leptin receptor	Leptin receptor	ng/ml
MIF-49a	Macrophage migration inhibitory factor	ng/ml
MIF-49b	Macrophage migration inhibitory factor	ng/ml
MMP-9	Matrix metalloproteinase-9	ng/ml
Nephrin-100a	Nephrin	ng/ml
Nephrin-101a	Nephrin	ng/ml
NGAL-MT	Neutrophil gelatinase-associated lipocalin	ng/ml
PAI-1 (active)	Plasminogen activator inhibitor 1	ng/ml
PAI-2	Plasminogen activator inhibitor 2	ng/ml
PAPP-A	Pregnancy specific plasma protein A	ng/ml
PCT-95a	Procalcitonin	pg/ml
PCT-99b	Procalcitonin	pg/ml
Pentraxin-3-64a	Pentraxin-related protein PTX3	ng/ml
Pentraxin-3-67a	Pentraxin-related protein PTX3	ng/ml
Periostin	Periostin	ng/ml
PODXL-74b	Podocalyxin	ng/ml
sFlt-1	Soluble fms-like tyrosine kinase-1	ng/ml
ST2-116b	Interleukin-1 receptor-like 1	ng/ml
ST2-75b	Interleukin-1 receptor-like 1	ng/ml
TGFβ	Transforming growth factor beta-1	ng/ml
TIMP-1	Metelloproteinase Inhibitor 1	ng/ml
TNFR1A	Tumor necrosis factor receptor superfamily member 1A	ng/ml
VEGF-C	Vascular endothelial growth factor C	ng/ml
Visfatin-82a	Visfatin	ng/ml
WAP4C-HE4-85b	WAP four disulfide core domain protein 2	ng/ml
WAP4C-HE4-91a	WAP four disulfide core domain protein 2	ng/ml

Table 2.2: Biomarker assay information

Biomarker	Low Cutoff	High Cutoff	Assay Coefficient Variable	Assay format
PlGF	12	3000	13	Sandwich, Luminex
ADAM-9	38.07	7913.74	11	Sandwich, Luminex
Angiogenin	0.14	61.24	7	Competitive, Luminex
ANP	0.048	71.93	13	Sandwich, Luminex
Arginase-1 10a	0.035	30.50	9	Sandwich, Luminex
Arginase-2 11a	1.318	378.14	15	Sandwich, Luminex
BNP	0.007	5.83	18	Sandwich, Luminex
Caspase	0.292	114.29	13	Sandwich, Luminex
CCL23	0.009	3.52	7	Sandwich, Luminex
C-Met 109a	7.999	453.54	11	Sandwich, Luminex
C-Met 111a	77.147	1035.48	7	Sandwich, Luminex
CPA-4	0.119	19.14	5	Sandwich, Luminex
CRP	0.07871	141.96	5	Competitve, Luminex
CXCL10	0.006	5.81	10	Sandwich, Luminex
Cystatin	165.009	9072.87	21	Competitive, Microtitre
Elafin-131	28.670	42668.61	10	Competitve, Luminex
Elafin-132	21.159	42668.61	5	Competitve, Luminex
Endoglin	1.981	654.84	18	Sandwich, Microtitre
Endothelin-1	0.704	901.9	13	Sandwich, Luminex
Ephrin	43.71	4009.97	20	Sandwich, Luminex
ESAM-1	1.073	32.77	9	Sandwich, Luminex
FAS	0.115	152.86	22	Sandwich, Luminex

Biomarker	Low Cutoff	High Cutoff	Assay Coefficient Variable	Assay format
FasL	0.156	30.20	11	Sandwich, Luminex
FIH	0.003	18.50	6	Sandwich, Luminex
HbF	0.848	386.32	18	Sandwich, Microtitre
ICAM-1	106.275	30231.73	6	Competitive, Luminex
IL-1ra	0.477	1434.20	9	Sandwich, Luminex
Kunitz-2-34a	0.016	10.17	19	Sandwich, Luminex
Kunitz-2-35b	0.140	57.15	13	Sandwich, Luminex
Kunitz-2-40b	0.159	57.38	7	Sandwich, Luminex
Leptin-43a	5.509	148.56	11	Sandwich, Luminex
Leptin-46b	2.244	1079.61	9	Sandwich, Luminex
MIF-49a	3.912	25.99	10	Sandwich, Luminex
MIF-49b	0.414	70.85	9	Sandwich, Luminex
MMP-9	4.542	202.82	5	Sandwich, Luminex
Nephrin-100a	0.517	19.98	25	Sandwich, Luminex
Nephrin-101a	0.094	19.74	22	Sandwich, Luminex
NGAL-MT	0.625	2924.00	22	Sandwich, Microtitre
PAI-1-52b	0.194	103.48	12	Sandwich, Luminex
PAI-2	0.047	77.90	5	Sandwich, Luminex
PAPP-A	0.189	812.10	7	Sandwich, Luminex
PCT-95a	12.22	9165.34	14	Sandwich, Luminex
PCT-99b	9.55	3982.50	14	Sandwich, Luminex
Pentraxin-3-64a	0.221	91.01	10	Sandwich, Luminex
Pentraxin-3-67a	0.940	59.41	16	Sandwich, Luminex
Periostin	0.538	107.38	7	Sandwich, Luminex

Biomarker	Low Cutoff	High Cutoff	Assay Coefficient Variable	Assay format
Podocalyxin	0.075	20.79	15	Sandwich, Luminex
sFlt-1	0.006	27.86	10	Sandwich, Luminex
ST2-116b	0.038	21.25	14	Sandwich, Luminex
ST2-75b	0.075	44.63	6	Sandwich, Luminex
TGF	0.040	63.98	9	Sandwich, Luminex
TIMP-1	9.127	1917.45	5	Competitive, Luminex
TNFR-1A	0.230	31.02	18	Sandwich, Luminex
VEGF-C	0.527	74.07	5	Sandwich, Luminex
Visfatin	2.535	1738.71	13	Sandwich, Luminex
WAP4C-HE4-85b	0.129	89.71	11	Sandwich, Luminex
WAP4C-HE4-91a	1.516	54.27	6	Sandwich, Luminex

All samples were analysed for PlGF at the recruiting sites by laboratory staff, trained in measurement of plasma PlGF using the Triage® PlGF Test (Alere, San Diego, CA). The test utilises a quantitative fluorescence immunoassay, measuring free PlGF. A fluorescently labelled murine monoclonal antibody against PlGF is fixed within the test device. When the plasma sample is added to the test device, free PlGF binds to the fixed antibody and the triage meter quantifies the concentration of plasma PlGF by measuring the degree of fluorescence. The lower level of detection of the assay is 12 pg/ml. The cost of the Alere Triage meter is £2,500 and each PlGF test is priced at £40.

The additional 56 biomarker assays were measured at a single central laboratory (Alere, San Diego, CA). Stored samples were thawed to room temperature prior to the assays being performed. All immunoassays were either antibody sandwich assays or competitive assays (using biotinylated antigen), using either Luminex or ELISA technology. For all Luminex assays, the primary antibody (mouse-derived recombinant Fab fragment) was conjugated to magnetic Luminex beads, which were added to 384-well assay plates. The plates were placed on a magnetic ring stand to avoid the beads being removed during washing. For Luminex sandwich assays the plasma sample was then added and incubated for one hour and then washed. A biotinylated secondary antibody was then added and incubated prior to a further wash to remove any unbound detection antibody. Streptavidin-conjugated phycoerythrin (which binds to biotin on the detection antibody) was then added and washed prior to reading using a Luminex 200 reader.

For Luminex competitive assays the initial steps were as above but the plasma sample was mixed with a biotinylated antigen prior to transfer to the plate

containing the primary antibody. After addition and incubation, washing removed any unbound biotinylated antigen. Streptavidin-labelled phycoerythrin was then added, which binds to biotin on the detection antigen. The degree of fluorescence was then measured using a Luminex 200 reader.

The micro-titre ELISA assays used a streptavidin-coated plate and biotin or fluorescein conjugated recombinant Fab fragments. The ELISA sandwich assay used a biotin-conjugated recombinant Fab as the capture antibody and a fluorescein-conjugated recombinant Fab as the detection antibody. Capture antibody was coated on the plate, incubated, washed and sample added. After sample incubation, the plate was washed and then incubated with detection antibody. Following washing, the plate was incubated with anti-fluorescein antibody conjugated to alkaline phosphatase, washed, fluorescent substrate added and then read using a Tecan infinite F200 reader.

The ELISA competitive assay used a biotin-conjugated antigen as the capture and a fluorescein-conjugated recombinant Fab as the detection antibody. The plate was coated with capture antigen, incubated and washed. Addition of sample was immediately followed by addition of the detection antibody and incubated. The final steps were the same as the ELISA sandwich.

All assays were performed in 384-well microtitre plates using a Perkin-Elmer Minitrak robotic liquid handling system for all liquid handling steps. For all sandwich assays, one concentration in each set of calibrators included neutralizing antibody for correction of endogenous antigen present in the plasma pool. Each assay used an eight-point dose curve prepared

gravimetrically in EDTA plasma or buffer. Plasma 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.

All results were concealed until a final adjudicated diagnosis had been made and laboratory staff were masked to the clinical diagnosis. Results were not revealed until all participants had delivered.

Table 2.3 contains individual biomarker median concentrations displayed according to birth weight within the study group.

Table 2.3: Individual median biomarker concentrations (quartiles) in women presenting before 35 weeks' gestation

Biomarkers	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n = 130)	Women with infant ≥ 10th centile (n = 144)
ADAM 9 *	89.6% below limit of detection	91.5% below limit of detection	84.0% below limit of detection
Angiogenin (µg/ml)	11.2 (7.71 to 19.3)	11.2 (7.56 to 18.7)	9.44 (6.56 to 15.1)
ANP (ng/ml)	1.28 (0.52 to 3.39)	1.06 (0.53 to 3.04)	0.83 (0.42 to 2.27)
Arginase 1 (ng/ml)	0.66 (0.43 to 1.09)	0.65 (0.43 to 1.10)	0.70 (0.39 to 1.10)
Arginase 2 (ng/ml)	15.6 (8.70 to 20.1)	13.9 (8.70 to 19.2)	10.2 (6.63 to 14.1)
BNP (pg/ml)	0.15 (0.10 to 0.22)	0.14 (0.08 to 0.19)	0.09 (0.06 to 0.14)
CCL23 (ng/ml)	0.22 (0.17 to 0.33)	0.23 (0.17 to 0.33)	0.28 (0.21 to 0.35)
CRP (µg/ml)	17.9 (8.98 to 33.0)	15.7 (7.53 to 32.5)	12.6 (6.52 to 23.3)
CPA-4 (ng/ml)	2.41 (1.84 to 2.80)	2.45 (1.85 to 2.99)	2.82 (2.15 to 3.51)
Caspase (ng/ml)	4.02 (1.90 to 8.48)	4.08 (1.81 to 8.29)	3.10 (1.45 to 6.08)
CXCL10 (ng/ml)	0.23 (0.15 to 0.33)	0.23 (0.16 to 0.32)	0.22 (0.16 to 0.30)
Cystatin C (ng/ml)	3175 (2234 to 5271)	3158 (2230 to 5309)	2789 (1873 to 3880)
C-Met 109a (ng/ml)	123 (92.7 to 147)	126 (94.2 to 152)	136 (103 to 171)
C-Met 111a (ng/ml)	356 (291 to 426)	373 (294 to 441)	398 (341 to 494)

Biomarkers	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n = 130)	Women with infant ≥ 10th centile (n = 144)
Elafin 131 (ng/ml)	146 (98.1 to 191)	133 (96.0 to 180)	128 (87.8 to 165)
Elafin 132 (ng/ml)	59.18 (41.13 to 121.35)	61.40 (40.54 to 112.69)	65.51 (44.99 to 111.07)
Endoglin (ng/ml)	134 (67.5 to 243)	126 (55.5 to 216)	33.6 (16.5 to 104)
ESAM-1 (ng/ml)	4.97 (3.99 to 6.11)	4.97 (4.18 to 6.15)	5.29 (4.49 to 6.37)
Endothelin-1 (pg/ml)	1.42 (0.91 to 2.40)	1.42 (0.87 to 2.40)	1.61 (1.04 to 2.53)
Ephrin (pg/ml) *	94.8% below limit of detection	95.6% below limit of detection	97.2% below limit of detection
HbF (ng/ml)	50.9 (26.0 to 90.9)	50.7 (25.6 to 88.7)	46.0 (23.1 to 72.7)
HIF (ng/ml)	0.18 (0.08 to 0.41)	0.19 (0.07 to 0.40)	0.13 (0.06 to 0.30)
ICAM-1 (ng/ml)	679 (538 to 932)	665 (517 to 914)	609 (478 to 828)
IL1RA (pg/ml)	19.7 (13.4 to 30.8)	19.0 (13.1 to 31.1)	23.8 (16.6 to 34.5)
ST2 116 (ng/ml)	1.43 (0.76 to 2.43)	1.21 (0.68 to 2.18)	0.78 (0.53 to 1.70)
ST2 75b (ng/ml)	6.74 (4.81 to 12.4)	6.39 (4.49 to 10.2)	5.86 (3.17 to 9.15)
Kunitz 2 34a (ng/ml)	0.42 (0.30 to 0.55)	0.42 (0.30 to 0.54)	0.41 (0.29 to 0.56)
Kunitz 2 35b (ng/ml)	0.25 (0.12 to 0.39)	0.26 (0.12 to 0.40)	0.29 (0.15 to 0.46)
Kunitz 2 40b (ng/ml)	0.14 (0.14 to 0.31)	0.15 (0.14 to 0.31)	0.24 (0.14 to 0.46)
Leptin 43a (ng/ml)	17.5 (14.1 to 22.9)	17.2 (13.7 to 22.7)	17.3 (10.3 to 25.8)

Biomarkers	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n = 130)	Women with infant ≥ 10th centile (n = 144)
Leptin 46b (ng/ml)	138 (104 to 178)	140 (105 to 182)	154 (113 to 199)
MIF 49a (ng/ml)	10.7 (8.97 to 13.1)	10.5 (8.96 to 13.1)	11.3 (9.76 to 12.9)
MIF 49b (ng/ml)	8.95 (5.73 to 14.0)	8.78 (5.72 to 13.7)	7.88 (5.31 to 11.6)
MMP-9 (ng/ml)	40.3 (30.3 to 54.6)	39.7 (29.5 to 55.7)	41.6 (31.5 to 58.4)
TIMP-1 (ng/ml)	132 (94.9 to 187)	126 (94.7 to 180)	110 (83.6 to 155)
Nephrin 100a (ng/ml) *	72.9% below limit of detection	74.6% below limit of detection	84.7% below limit of detection
Nephrin 101a (ng/ml)	0.42 (0.26 to 0.73)	0.38 (0.26 to 0.66)	0.30 (0.16 to 0.49)
NGAL (ng/ml)	48.24 (35.0 to 75.6)	46.2 (34.2 to 71.0)	38.7 (24.4 to 56.1)
PAPP-A (ng/ml)	90.7 (40.6 to 154)	90.0 (40.6 to 156)	135 (67.4 to 224)
Pentraxin 3 64a (ng/ml) *	77.1% below limit of detection	77.7% below limit of detection	72.9% below limit of detection
Pentraxin 3 67a (ng/ml)	3.32 (1.71 to 5.10)	2.97 (1.68 to 5.03)	1.97 (0.90 to 3.35)
Periostin (ng/ml)	9.16 (6.86 to 11.3)	9.07 (6.78 to 11.1)	8.53 (6.29 to 10.7)
PlGF (pg/ml)	11.6 (5.01 to 33.1)	16.7 (6.11 to 58.2)	195 (33.2 to 494)
PAI-1 (ng/ml)	0.50 (0.24 to 0.81)	0.45 (0.24 to 0.78)	0.46 (0.26 to 0.73)
PAI-2 (ng/ml)	9.18 (7.39 to 11.9)	9.93 (7.63 to 12.3)	11.9 (9.42 to 14.3)

Biomarkers	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n = 130)	Women with infant ≥ 10th centile (n = 144)
Podocalyxin (ng/ml)	0.16 (0.09 to 0.29)	0.14 (0.07 to 0.28)	0.10 (0.07 to 0.19)
PCT 95a (pg/ml)	76.0 (44.6 to 128)	67.1 (41.2 to 122)	45.8 (27.5 to 72.1)
PCT 99b (pg/ml)	10.6 (5.63 to 28.9)	11.5 (5.63 to 28.5)	11.4 (5.63 to 21.6)
TGFBeta-2 (ng/ml)	1.77 (1.32 to 2.18)	1.81 (1.35 to 2.30)	1.99 (1.62 to 2.43)
FasL (ng/ml) *	72.9% below limit of detection	74.6% below limit of detection	68.1% below limit of detection
TNFR-1A (ng/ml)	7.29 (5.01 to 11.6)	7.67 (5.48 to 11.5)	7.50 (5.92 to 10.6)
FAS (ng/ml)	2.7 (2.1 to 3.5)	2.71 (2.10 to 3.53)	2.72 (2.01 to 3.66)
VEGF-C (ng/ml)	15.2 (12.9 to 18.3)	15.1 (12.9 to 18.0)	14.1 (12.1 to 16.6)
sFlt-1 (ng/ml)	3.60 (1.54 to 5.82)	2.74 (1.33 to 5.38)	0.95 (0.50 to 2.49)
Visfatin (ng/ml)	2.37 (1.62 to 3.32)	2.35 (1.61 to 3.29)	1.93 (1.22 to 3.16)
WAP4C HE4 85b (ng/ml)	1.86 (1.15 to 2.65)	1.65 (1.12 to 2.63)	1.40 (0.94 to 1.90)
WAP4C HE4 91a (ng/ml)	13.8 (12.0 to 17.1)	13.9 (12.2 to 16.8)	13.3 (11.1 to 15.7)

***** Meaningful quartiles cannot be calculated as the concentrations in most samples were below the lower limit of assay detection.

2.2.3 Statistical analysis

I undertook the statistical analysis with the medical statistician having devised the statistical plan with the study Chief investigator and Principal Investigator at St Thomas' Hospital.

The first part of this analysis evaluated the ability of 47 biomarkers to determine subsequent delivery of an SGA infant. Logged values of all 57 biomarker assays were used as standard distributional checks showed high levels of skewness, which were consistent with underlying log normal distributions. Prior to disclosure of pregnancy outcomes, factor analysis of biomarker data was undertaken on the whole data set, reducing the 57 biomarker assays to a smaller number of highly correlated groups, solely on the basis of the correlations between the biomarkers and without reference to outcome. Consideration of scree plots and Eigen-values (> two) identified the most important factors (Costello and Osborne, 2005) which were rotated (orthogonal varimax method) so that each factor related strongly (correlation >0.6) to a small number of biomarkers only. Scores were calculated on each factor for each subject.

The principal outcome for this analysis was delivery of an SGA infant (defined as birth weight less than the 3^{rd} or 10^{th} customised centile). The factor scores were entered into a multiple logistic regression model for prediction of subsequent SGA. The best performing factors (and their biomarkers) were investigated further by using stepwise logistic regression (a parametric method) to determine which of the biomarkers within these factors, appeared to provide additional information beyond that derived from PlGF (known to be a good

predictor of need for delivery for pre-eclampsia) (Chappell et al., 2013). Prediction scores were extracted for the best combinations.

Some biomarkers, with high uniqueness scores, were not strongly associated with any factor. To investigate whether any of these biomarkers had predictive power in addition to that provided by PlGF and biomarkers identified earlier, stepwise logistic regression was undertaken. To avoid excluding a biomarker that may be of potential value, a standard multiple-testing correction to p-values, such as Bonferroni was not used. 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 (or having high uniqueness and not appearing in any factor), being a significant predictor in logistic regression both alone and after allowing for PlGF, having a ROC area for the combined score significantly greater than PlGF alone, being a useful predictor for both definitions of SGA. 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.

The analysis was initially conducted on samples from women enrolled prior to 35 weeks' gestation as maternal plasma PlGF concentrations decline towards the end of the third trimester reducing test performance beyond 35 weeks' gestation. This was then repeated in women enrolled between 35^{+0} and 36^{+6} weeks' gestation.

The second part of this analysis compared the best performing biomarker(s) to currently utilised ultrasound parameters. To allow this comparison data was restricted to women who had an ultrasound within 14 days of enrolment (when blood was drawn for biomarker analysis). The sensitivity, specificity and predictive values were calculated for three ultrasound parameters (estimated fetal weight (EFW) or abdominal circumference (AC) $<10^{th}$ centile, absent or reversed end diastolic flow in the umbilical artery (AREDF) and oligohydramnios (defined as amniotic fluid index $<5^{th}$ centile)) and the best performing biomarker to allow comparison. The performance of these parameters was assessed in isolation and combination, to determine both delivery of an SGA infant and adverse perinatal outcome.

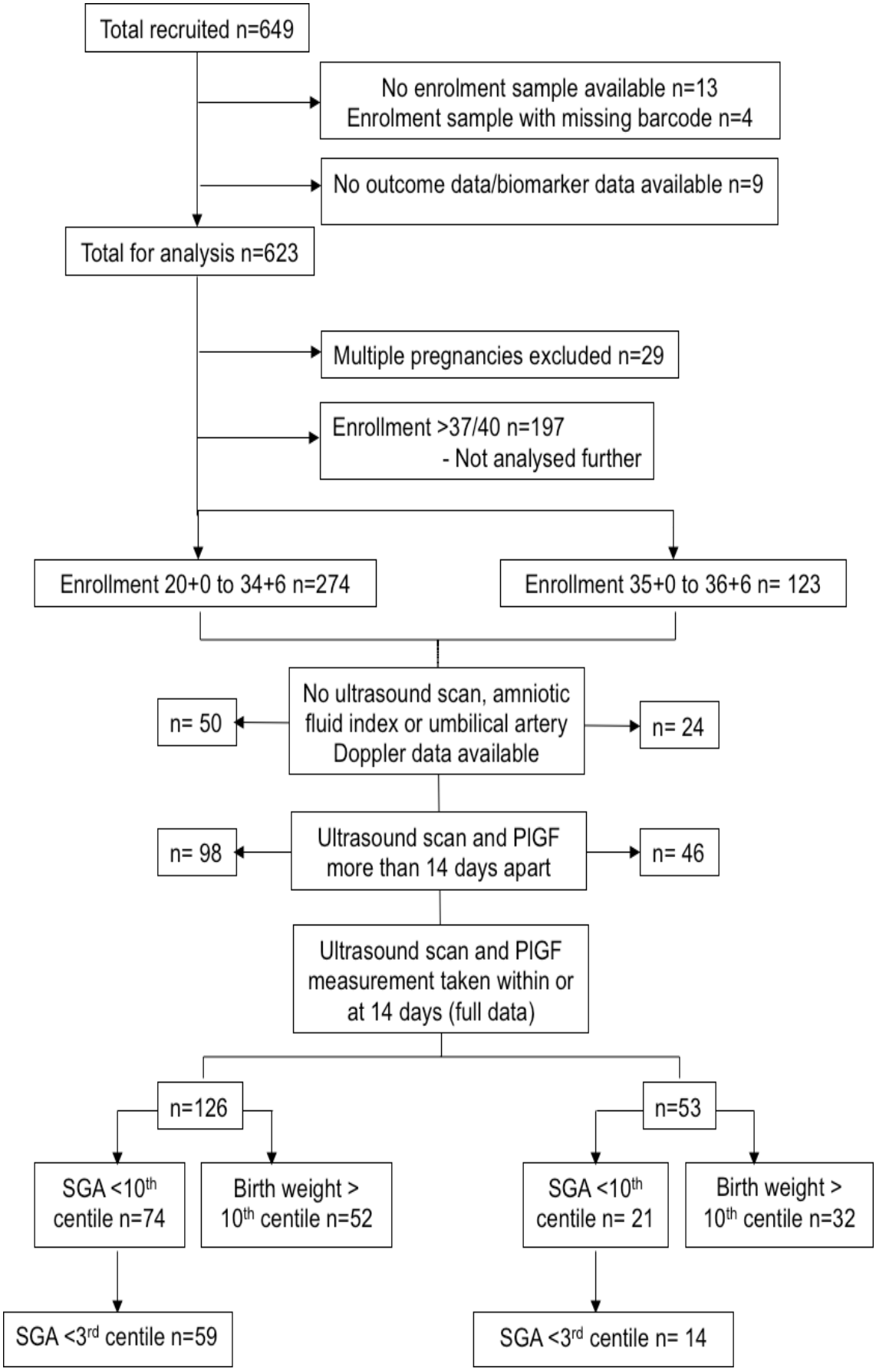
Statistical analysis was carried out in the statistical package Stata (version 11.2), College Station Texas, USA. Formal significance was taken at $p<0.05$. The study is reported in accordance with STROBE guidelines.

2.3 Results

649 women presenting with suspected pre-eclampsia between 20^{+0} and 40^{+6} weeks' gestation were recruited across seven sites between January 2011 and February 2012. 17 of these women did not have a valid enrolment plasma sample and there was no outcome data available on a further nine women, excluding them from any further analysis. Of the remaining 623 women, 29 had multi-fetal pregnancies and were excluded from this analysis. 197 women were recruited over 37 weeks' gestation and the samples from these women were not analysed further. This decision followed the findings of the HYPITAT study, and publication of National Institute of Clinical Excellence (NICE) guidance on the management of hypertensive disorders during pregnancy. The HYPITAT study concluded that induction of labour in women presenting with gestational hypertension or mild pre-eclampsia beyond 37 weeks' gestation improved maternal outcome (Koopmans et al., 2009). Subsequent NICE guidance recommended induction of labour in women presenting with pre-eclampsia beyond 37 weeks' gestation (National Institute for Health and Clinical Excellence, 2010) and therefore investigating the utility of biomarkers to determine placental dysfunction beyond 37 weeks' gestation was not justified as this would not alter clinical management. With particular reference to PlGF, maternal plasma concentrations decline after 32 weeks' gestation with convergence of concentrations between normal and pathological pregnancies with advancing gestation (Knudsen et al., 2012) and therefore the clinical utility of this biomarker beyond 37 weeks' gestation is likely to be less predictive of outcome even if women remained pregnant.

274 women were recruited prior to 35 weeks' gestation and 126 of these women had an ultrasound scan with all parameters recorded within or at 14 days of enrolment (where PlGF sample was taken). Of these women, 74 delivered an SGA infant <10th customised centile and 59 were below the 3rd centile. 123 women were recruited between 35^{+0} and 36^{+6} weeks' gestation and 53 of these women had an ultrasound scan and PlGF measured within or at 14 days. Of these women, 21 delivered an SGA infant <10th centile and 14 had a birth weight below the 3rd centile (Figure 2.1).

Figure 2.1: Flow diagram of study participants



2.3.1 Women presenting prior to 35 weeks' gestation (n = 274)

All descriptive Tables are separated according to subsequent infant birth weight. Characteristics of participants at booking and enrolment are displayed in Tables 2.4 and 2.5 respectively. There is little difference between groups at booking and enrolment except the presence of new onset proteinuria and hypertension, which occurred more frequently at enrolment in women who subsequently delivered an SGA infant. A likely explanation for this finding is the known association between pre-eclampsia and SGA. Table 2.6 details delivery and maternal and neonatal outcomes; delivery by planned caesarean section was higher in women with an SGA infant (62% in pregnancies complicated by SGA <10th centile) compared to those delivering an infant ≥ 10th centile (32%). The median gestation at delivery was much lower in the cases complicated by SGA, likely secondary to increased iatrogenic delivery in this group (34.4 weeks, versus 38.1 weeks' gestation in women delivering an infant with a birth weight ≥10th centile). There were six stillbirths and two neonatal deaths, and with the exception of one case, all had a birth weight <3rd customised centile. In all cases of stillbirth the PlGF concentration at enrolment was <100 pg/ml and predated ultrasound abnormalities by 7 to 39 days and stillbirth by 10 to 53 days. The prevalence of adverse perinatal outcomes (definition excluded SGA) was much higher in the SGA groups compared to those delivering AGA infants, emphasising the importance of identifying those at risk (39%, 32% and 13% for SGA <3rd centile, SGA <10th centile and birth weight ≥10th centile respectively). The most common adverse perinatal outcomes in cases with birth weight <3rd customised centile at delivery were respiratory distress syndrome (21/39), followed by admission to NICU for >48 hrs at term (6/39) and retinopathy of

prematurity (5/39). There were four cases each of bronchopulmonary dysplasia and necrotising enterocolitis and one case of intraventricular haemorrhage within this group.

Table 2.4: Characteristics of participants at booking (grouped by subsequent infant birth weight) under 35 weeks' gestation. Values given are median (quartiles) or n (%) as appropriate.

Characteristics	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n=130)	Women with infant ≥ 10th centile (n=144)
Age (years)	31.9 (27.2 to 36.2)	31.9 (27.4 to 36.4)	31.7 (26.3 to 35.6)
BMI (kg/m^2)	26.8 (24.1 to 31.2)	28.0 (23.9 to 32.8)	29.3 (24.7 to 34.9)
White ethnicity	63 (65.6)	87 (66.9)	92 (63.9)
Highest systolic BP (mmHg)	120 (110 to 130)	121 (110 to 130)	120 (110 to 130)
Highest diastolic BP (mmHg)	74 (65 to 81)	74 (65 to 81)	75 (68 to 82)
Smoker at booking	17 (18.5)	24 (19.2)	29 (20.4)
Quit smoking during pregnancy	10 (10.9)	14 (11.2)	19 (13.4)
Previous medical history:			
Previous preeclampsia requiring delivery <34/40 †	15 (15.8)	18 (14.0)	12 (8.6)
Chronic hypertension	11 (11.5)	21 (16.2)	23 (16.0)
SLE or APS	5 (5.2)	6 (4.6)	6 (4.2)
Pre-existing diabetes mellitus	2 (2.1)	2 (1.5)	4 (2.8)
Renal disease	6 (6.3)	9 (6.9)	10 (6.9)

SLE or APS, Systemic lupus erythematosus or Antiphospholipid syndrome

BP, Blood Pressure

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 2.5: Characteristics of participants at study enrolment under 35 weeks' gestation. Values given are median (quartiles) or n (%) as appropriate.

Characteristics	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n=130)	Women with infant ≥ 10th centile (n=144)
Gestational age at sampling (weeks)	31.0 (27.6 to 33.0)	31.0 (27.6 to 33.1)	31.1 (28.0 to 33.6)
Signs or symptoms of suspected pre-eclampsia			
New onset hypertension †	60 (63)	80 (62)	65 (45)
Worsening of underlying hypertension	16 (17)	24 (19)	32 (22)
New onset of dipstick proteinuria	58 (60)	79 (61)	71 (49)
Dipstick proteinuria:			
Not done	18 (19)	24 (19)	12 (8)
Negative	22 (23)	34 (26)	67 (47)
Proteinuria (≥ +1)	56 (58)	72 (55)	65 (45)
Headaches	24 (25)	32 (25)	49 (34)
Suspected SGA (customised birth weight centiles) †	40 (42)	40 (31)	1 (1)
Highest systolic BP (mmHg) †	147 (137 to 160)	148 (138 to 160)	141 (128 to 156)
Highest diastolic BP (mmHg) †	94 (83 to 100)	94 (83 to 100)	90 (80 to 100)
Epigastric/ right upper quadrant pain	4 (4)	6 (5)	12 (8)
Laboratory tests at time of enrolment			
Alanine transaminase (U/L)	15 (11 to 19)	16 (12 to 20)	13 (10 to 19)
Creatinine (μmol/L)	55 (46 to 64)	55 (46 to 64)	50 (41 to 60)
Uric acid (μmol/L) †	310 (217 to 359)	290 (195 to 355)	241 (184 to 287)
Platelet count ($x10^9$/L)	230 (199 to 275)	230 (191 to 275)	236 (202 to 266)

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 2.6: Characteristics of delivery, maternal and neonatal outcome for women presenting before 35 weeks' gestation. Values given are median (quartiles) or n (%) as appropriate.

Characteristics	Women with SGA infant <3rd centile (n = 96)	Women with SGA infant <10th centile (n = 130)	Women with infant ≥ 10th centile (n = 144)
Onset of labour			
Spontaneous †	3 (3)	7 (5)	32 (23)
Induced	29 (30)	42 (33)	64 (45)
Pre labour caesarean section †	64 (67)	80 (62)	46 (32)
Mode of delivery			
Spontaneous †	15 (16)	25 (20)	45 (31)
Assisted vaginal	5 (5)	8 (6)	21 (15)
Caesarean section †	75 (79)	95 (74)	78 (54)
Adverse maternal outcome*	44 (46)	61 (47)	56 (39)
Gestation at delivery (weeks) †	33.8 (30.8 to 36.1)	34.4 (31.4 to 37.3)	38.1 (36 to 39.4)
Fetal death †	5 (5)	5 (4)	1 (1)
Neonatal death	2 (2)	2 (2)	0 (0)
Birth weight (g) †	1537 (1043 to 1910)	1660 (1200 to 2310)	3128 (2698 to 3545)
SGA <10th birth weight centile	96 (100)	130 (100)	0 (0)
SGA <3rd birth weight centile	96 (100)	96 (74)	0 (0)
SGA <1st birth weight centile	68 (71)	68 (53)	0 (0)
Adverse perinatal outcome** †	37 (39)	41 (32)	19 (13)
Maternal diagnosis			
No maternal disease	0	1 (0.8)	21 (15)
Gestational hypertension	1 (1)	1 (0.8)	25 (17)
Chronic hypertension	4 (4)	12 (9)	16 (11)
Pre-eclampsia	86 (90)	106 (81)	59 (41)
HELLP syndrome	1 (1)	1 (0.8)	1 (0.7)
Other diagnosis	4 (4)	9 (7)	22 (16)

*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 $<50\times10^9$/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.

**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.

† indicates p value <0.05 for comparison of women with SGA infant $<3^{rd}$ centile (the main outcome) to women with infant ≥ 10^{th} centile

Factor analysis of all 57 biomarkers was performed as described in section 2.2.3. The results of the factor analysis showing the five largest factors are displayed in Table 2.7. Factors three and four had the highest odds ratios in women recruited before 35 weeks' gestation. The biomarkers within factors 3 and 4 were investigated further by using stepwise logistic regression to determine which if any of these biomarkers provided additional information beyond that derived from PlGF. Prediction scores were calculated to identify the best combinations of biomarkers for further analysis. PlGF was consistently the strongest predictor and did not need to be forced into the model.

Table 2.7: Odds ratios derived from multiple logistic regression analysis of the 5 factors in women presenting before 35 weeks' gestation (Odds ratios are for a change of 1 SD in the factor score).

Factor	Biomarkers contained in factor	Women with SGA infant <3rd centile Odds Ratio (95% CI)	Women with SGA infant <10th centile Odds Ratio (95% CI)
1	ANP, Arginase-1, CCL23, CPA-4, ESAM-1, FAS, Kunitz-2, TGFBeta-1, TNFR-1A, WAP4C-HE4-85b, WAP4C-HE4-91a	0.85 (0.64 to 1.13)	0.89 (0.68 to 1.16)
2	ADAM-9, Ephrin, FasL, Kunitz 35b, Kunitz 40b, Nephrin, PAI-1, Pentraxin-3-64a	0.79 (0.57 to 1.1)	0.78 (0.58 to 1.03)
3	Arginase-2, BNP, Nephrin, PCT-95a, Pentraxin 3-67a, Podocalyxin	1.67 (1.23 to 2.28)	1.57 (1.18 to 2.07)
4	PlGF, Endoglin, sFlt-1	2.85 (2.13 to 3.82)	2.38 (1.84 to 3.08)
5	Angiogenin, Caspase, FIH, ICAM-1, MIF, TIMP-1	1.04 (0.76 to 1.41)	0.99 (0.74 to 1.31)

A summary of the best performing individual biomarkers and combinations derived from logistic regression to determine SGA <3rd and <10th birth weight centiles are displayed in Table 2.8. PlGF outperformed all other individual biomarkers and biomarker ratios in determining delivery of an SGA infant, with a ROC area of 0.83 (95% CI 0.78 to 0.88) for SGA <3rd centile and 0.79 (95% CI 0.73 to 0.84) for SGA <10th centile (Figure 2.2). Biomarker combinations incorporating PlGF added little to the test performance of PlGF in isolation, with a ROC area of 0.84 (95% CI 0.79 to 0.89) for the most promising combination to predict SGA <3rd centile and 0.80 (95% CI 0.74 to 0.85) for SGA <10th centile (Figure 2.3). Table 2.9 contains ROC areas for all 57 biomarker assays measured in determining SGA <3rd and <10th birth weight centiles. To allow comparison of individual biomarker test performance in women enrolled prior to 35 weeks' gestation with those women enrolled between 35^{+0} and 36^{+6} weeks' gestation (discussed in section 2.3.2), this data has been displayed together in Table 2.9.

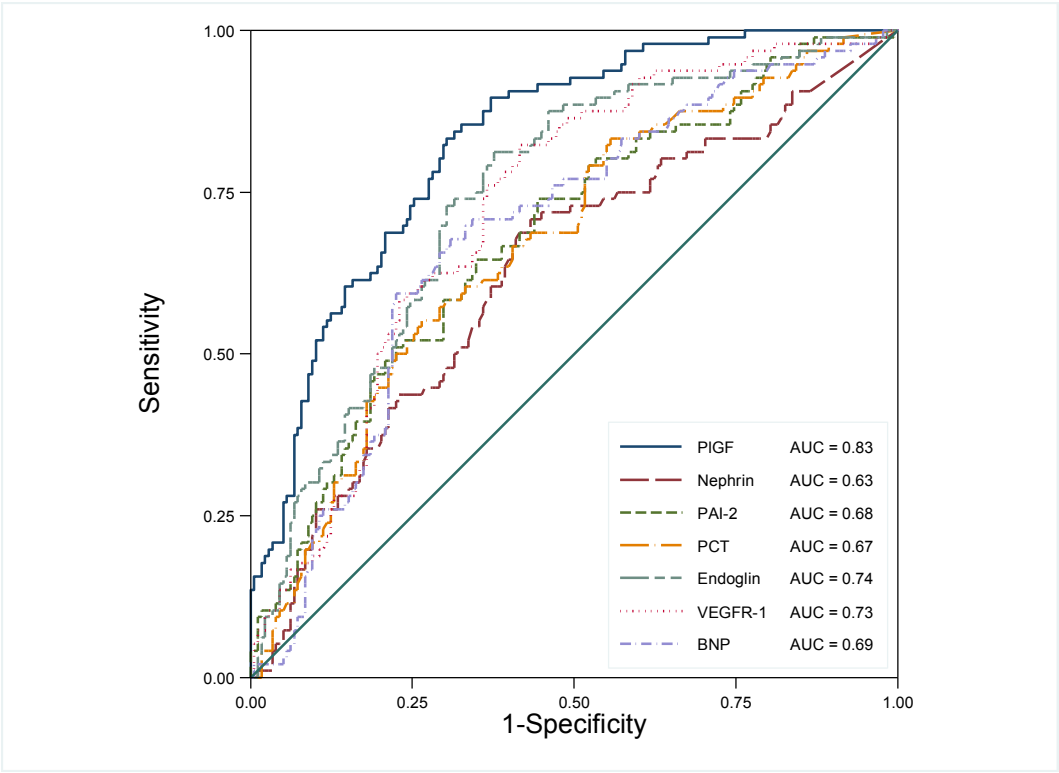
Table 2.8: Test performance statistics for individual biomarkers and combinations (derived from logistic regression) to predict SGA <3rd centile and <10th centile in women presenting before 35 weeks' gestation (ROC areas with 95 confidence intervals).

Biomarkers or combinations	SGA <3rd centile	SGA <10th centile
Nephrin	0.63 (0.56 to 0.70)	0.62 (0.55 to 0.69)
[CPA-4]	0.63 (0.57 to 0.70)	0.62 (0.55 to 0.68)
PCT 95a	0.67 (0.61 to 0.74)	0.64 (0.57 to 0.71)
[PAI-2]	0.68 (0.62 to 0.75)	0.66 (0.59 to 0.72)
BNP	0.69 (0.62 to 0.75)	0.64 (0.58 to 0.71)
sFlt-1	0.73 (0.67 to 0.79)	0.69 (0.63 to 0.76)
Endoglin	0.74 (0.68 to 0.80)	0.73 (0.67 to 0.79)
[PlGF]	0.83 (0.78 to 0.88)	0.79 (0.73 to 0.84)
[PlGF/s-Flt ratio]	0.80 (0.75 to 0.85)	0.77 (0.71 to 0.82)
[PlGF/Endoglin ratio]	0.82 (0.77 to 0.86)	0.78 (0.73 to 0.83)
[PlGF], [CPA-4]	0.83 (0.78 to 0.88)	0.79 (0.74 to 0.84)
[PlGF], Nephrin	0.84 (0.79 to 0.88)	0.80 (0.74 to 0.85)
[PlGF], Nephrin, [CPA-4]	0.84 (0.79 to 0.89)	0.80 (0.74 to 0.85)

[ ] low concentration of biomarker/ratio correlated to disease

Figure 2.2 ROC areas of individual biomarkers measured under 35 weeks' gestation to determine:

SGA < 3rd centile



SGA <10th centile

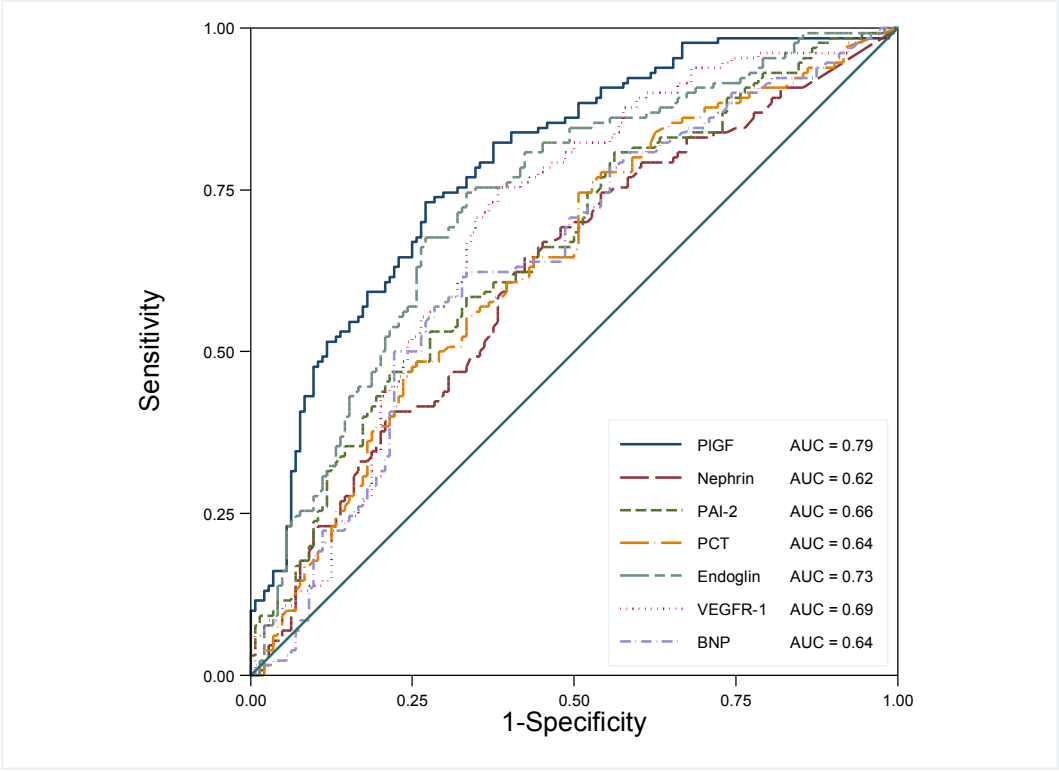
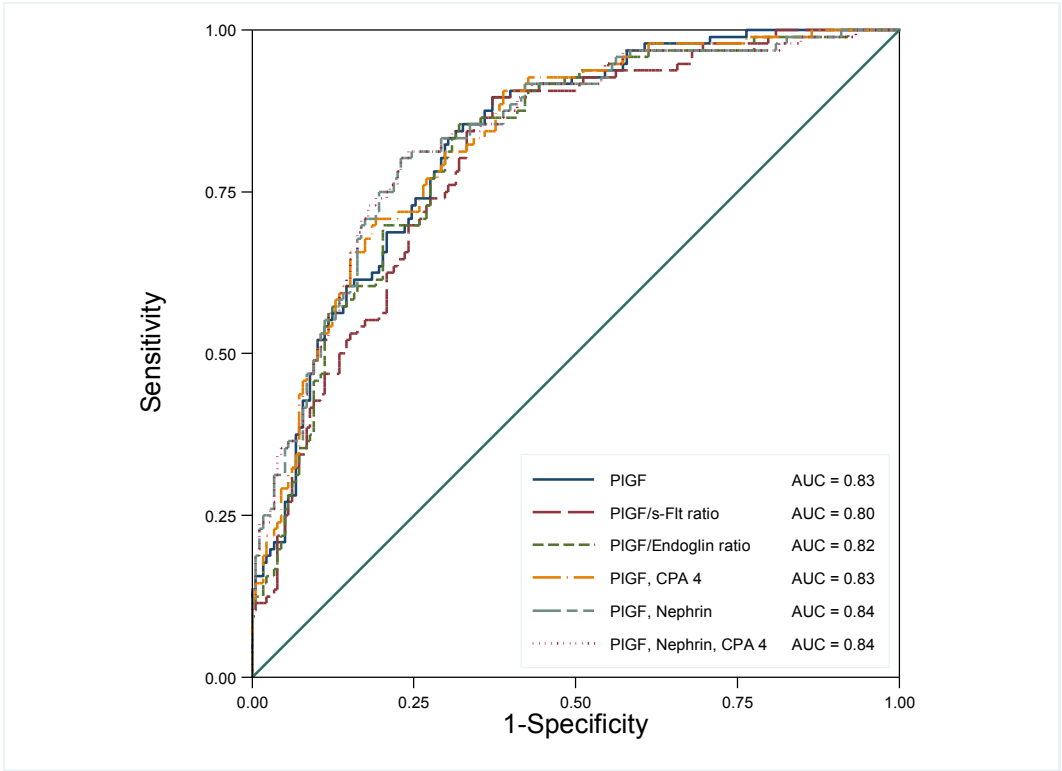


Figure 2.3 ROC areas of biomarker combinations measured under 35 weeks' gestation to determine:

SGA < 3rd centile



SGA <10th centile

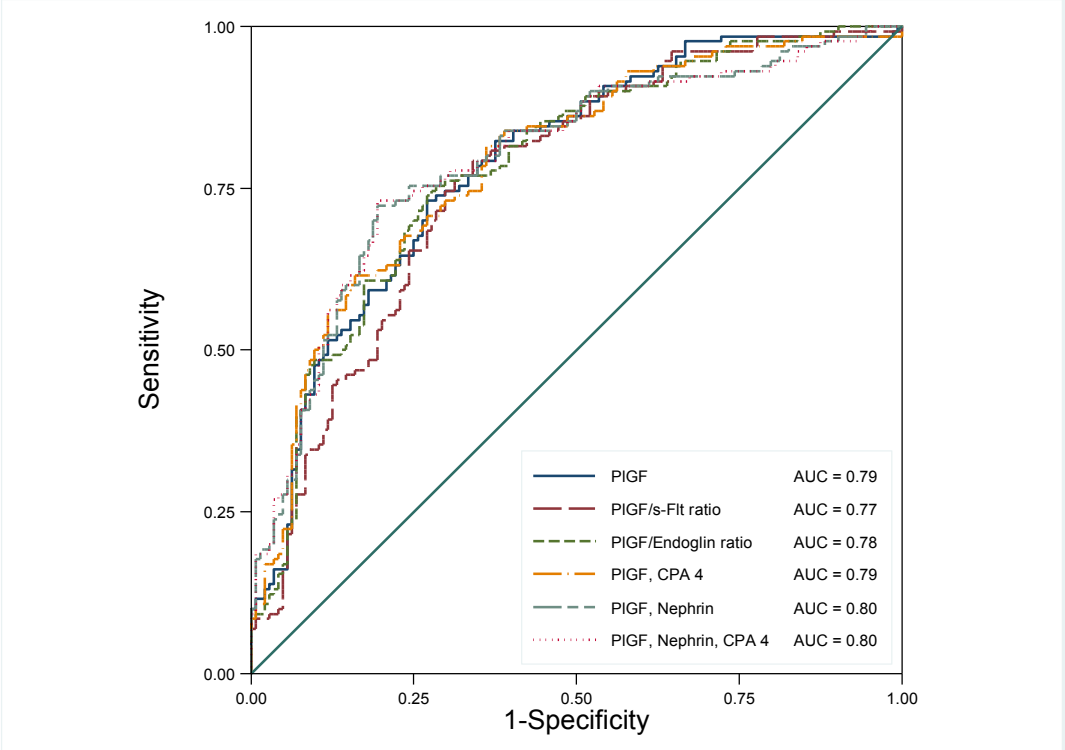


Table 2.9: Individual biomarker test performance (ROC areas with 95 confidence intervals) under 35 weeks' gestation and 35^{+0} to 36^{+6} weeks' gestation.

	< 35 weeks' gestation		35^{+0} - 36^{+6} weeks' gestation	
	(<3rd centile)	(<10th centile)	(<3rd centile)	(<10th centile)
[PlGF]	0.83 (0.78 to 0.88)	0.79 (0.73 to 0.84)	0.69 (0.57 to 0.81)	0.74 (0.64 to 0.83)
ADAM-9	0.52 (0.48 to 0.56)	0.54 (0.50 to 0.58)	0.55 (0.49 to 0.62)	0.56 (0.50 to 0.62)
Angiogenin	0.59 (0.51 to 0.66)	0.58 (0.51 to 0.64)	0.61 (0.49 to 0.73)	0.57 (0.46 to 0.68)
ANP	0.56 (0.49 to 0.64)	0.56 (0.49 to 0.63)	0.49 (0.35 to 0.63)	0.54 (0.42 to 0.65)
Arginase 1	0.51 (0.44 to 0.58)	0.51 (0.44 to 0.58)	0.61 (0.48 to 0.73)	0.54 (0.42 to 0.65)
[Arginase 2]	0.64 (0.56 to 0.71)	0.63 (0.57 to 0.70)	0.67 (0.55 to 0.79)	0.64 (0.54 to 0.75)
BNP	0.69 (0.62 to 0.75)	0.64 (0.58 to 0.71)	0.65 (0.54 to 0.77)	0.68 (0.58 to 0.78)
Caspase	0.56 (0.49 to 0.63)	0.56 (0.49 to 0.62)	0.58 (0.46 to 0.70)	0.52 (0.42 to 0.63)
[CCL23]	0.57 (0.50 to 0.64)	0.58 (0.51 to 0.65)	0.52 (0.40 to 0.65)	0.54 (0.43 to 0.65)
[CPA-4]	0.63 (0.57 to 0.70)	0.62 (0.55 to 0.68)	0.72 (0.61 to 0.83)	0.72 (0.63 to 0.82)
CRP	0.58 (0.51 to 0.65)	0.55 (0.48 to 0.62)	0.68 (0.57 to 0.80)	0.59 (0.48 to 0.70)
CXCL10	0.51 (0.44 to 0.59)	0.53 (0.46 to 0.59)	0.46 (0.32 to 0.61)	0.51 (0.40 to 0.63)
Cystatin C	0.58 (0.51 to 0.65)	0.59 (0.52 to 0.66)	0.53 (0.39 to 0.66)	0.54 (0.44 to 0.65)
[C-Met 109a]	0.59 (0.52 to 0.66)	0.58 (0.51 to 0.64)	0.64 (0.51 to 0.76)	0.64 (0.54 to 0.74)

	< 35 weeks' gestation		35^{+0} -36^{+6} weeks' gestation	
	(<3rd centile)	**(<10th centile)**	**(<3rd centile)**	**(<10th centile)**
[C-Met 111a]	0.61 (0.54 to 0.68)	0.59 (0.52 to 0.65)	0.64 (0.52 to 0.76)	0.64 (0.54 to 0.74)
Elafin 131	0.57 (0.50 to 0.64)	0.54 (0.47 to 0.61)	0.64 (0.52 to 0.76)	0.55 (0.44 to 0.66)
Elafin 132	0.48 (0.41 to 0.55)	0.47 (0.41 to 0.54)	0.60 (0.49 to 0.72)	0.52 (0.42 to 0.63)
Endoglin	0.74 (0.68 to 0.80)	0.73 (0.67 to 0.79)	0.58 (0.46 to 0.70)	0.65 (0.55 to 0.76)
[Endothelin-1]	0.53 (0.45 to 0.60)	0.54 (0.48 to 0.61)	0.57 (0.45 to 0.70)	0.55 (0.45 to 0.66)
[Ephrin]	0.49 (0.46 to 0.51)	0.49 (0.47 to 0.51)	0.51 (0.50 to 0.52)	0.51 (0.49 to 0.52)
[ESAM-1]	0.55 (0.48 to 0.63)	0.55 (0.48 to 0.62)	0.67 (0.55 to 0.79)	0.66 (0.55 to 0.76)
[FAS]	0.53 (0.45 to 0.60)	0.49 (0.42 to 0.56)	0.53 (0.40 to 0.65)	0.55 (0.45 to 0.66)
[FasL]	0.52 (0.46 to 0.58)	0.54 (0.48 to 0.59)	0.52 (0.41 to 0.62)	0.54 (0.45 to 0.62)
HIF	0.55 (0.48 to 0.62)	0.56 (0.49 to 0.62)	0.56 (0.43 to 0.68)	0.51 (0.40 to 0.62)
[HbF]	0.44 (0.37 to 0.51)	0.45 (0.38 to 0.52)	0.55 (0.41 to 0.69)	0.49 (0.38 to 0.61)
ICAM-1	0.57 (0.50 to 0.64)	0.55 (0.48 to 0.62)	0.67 (0.56 to 0.78)	0.60 (0.49 to 0.71)
[IL-1ra]	0.58 (0.51 to 0.65)	0.59 (0.53 to 0.66)	0.62 (0.50 to 0.75)	0.62 (0.51 to 0.73)
Kunitz 2 34a	0.52 (0.45 to 0.59)	0.51 (0.44 to 0.58)	0.36 (0.24 to 0.48)	0.40 (0.30 to 0.51)
[Kunitz 2 35b]	0.57 (0.50 to 0.64)	0.56 (0.49 to 0.63)	0.74 (0.63 to 0.85)	0.73 (0.65 to 0.82)

	< 35 weeks' gestation		35^{+0} -36^{+6} weeks' gestation	
	(<3rd centile)	**(<10th centile)**	**(<3rd centile)**	**(<10th centile)**
[Kunitz 2 40b]	0.58 (0.51 to 0.65)	0.60 (0.53 to 0.66)	0.69 (0.57 to 0.81)	0.68 (0.58 to 0.78)
[Leptin 43a]	0.47 (0.40 to 0.54)	0.49 (0.42 to 0.56)	0.50 (0.37 to 0.63)	0.49 (0.38 to 0.60)
[Leptin 46b]	0.59 (0.52 to 0.66)	0.56 (0.49 to 0.63)	0.65 (0.52 to 0.78)	0.62 (0.51 to 0.73)
[MIF-49a]	0.54 (0.47 to 0.62)	0.58 (0.51 to 0.65)	0.57 (0.45 to 0.70)	0.57 (0.46 to 0.68)
[MIF-49b]	0.55 (0.47 to 0.62)	0.54 (0.47 to 0.61)	0.48 (0.36 to 0.60)	0.49 (0.38 to 0.59)
[MMP-9]	0.52 (0.44 to 0.59)	0.53 (0.46 to 0.60)	0.57 (0.45 to 0.69)	0.56 (0.45 to 0.67)
Nephrin 100a	0.55 (0.50 to 0.60)	0.55 (0.50 to 0.60)	0.53 (0.44 to 0.62)	0.52 (0.45 to 0.59)
Nephrin 101a	0.63 (0.56 to 0.70)	0.62 (0.55 to 0.69)	0.67 (0.55 to 0.80)	0.63 (0.52 to 0.74)
NGAL	0.62 (0.55 to 0.69)	0.60 (0.54 to 0.67)	0.46 (0.33 to 0.58)	0.49 (0.39 to 0.60)
PAI-1 (active)	0.53 (0.46 to 0.60)	0.50 (0.43 to 0.57)	0.49 (0.36 to 0.62)	0.48 (0.37 to 0.59)
[PAI-2]	0.68 (0.62 to 0.75)	0.66 (0.59 to 0.72)	0.67 (0.56 to 0.79)	0.65 (0.55 to 0.75)
[PAPP-A]	0.60 (0.53 to 0.67)	0.64 (0.57 to 0.70)	0.62 (0.50 to 0.74)	0.66 (0.56 to 0.77)
PCT 95a	0.67 (0.61 to 0.74)	0.64 (0.57 to 0.71)	0.61 (0.48 to 0.73)	0.59 (0.48 to 0.69)
PCT 99b	0.51 (0.44 to 0.58)	0.52 (0.45 to 0.59)	0.38 (0.26 to 0.51)	0.45 (0.34 to 0.55)
Pentraxin-3 64a	0.49 (0.44 to 0.55)	0.48 (0.43 to 0.53)	0.46 (0.36 to 0.57)	0.45 (0.36 to 0.54)

	< 35 weeks' gestation		35^{+0} -36^{+6} weeks' gestation	
	(<3rd centile)	**(<10th centile)**	**(<3rd centile)**	**(<10th centile)**
Pentraxin- 3	0.64	0.63	0.59	0.61
67a	(0.57 to 0.71)	(0.56 to 0.69)	(0.46 to 0.71)	(0.50 to 0.71)
Periostin	0.56	0.54	0.50	0.57
	(0.48 to 0.63)	(0.47 to 0.61)	(0.37 to 0.63)	(0.46 to 0.68)
Podocalyxin	0.62	0.59	0.65	0.62
	(0.55 to 0.69)	(0.53 to 0.66)	(0.53 to 0.76)	(0.52 to 0.72)
sFlt-1	0.73	0.69	0.57	0.61
	(0.66 to 0.79)	(0.63 to 0.76)	(0.45 to 0.70)	(0.51 to 0.71)
ST2-116b	0.62	0.60	0.55	0.61
	(0.55 to 0.69)	(0.53 to 0.66)	(0.43 to 0.67)	(0.51 to 0.72)
ST2 -75b	0.59	0.57	0.49	0.55
	(0.52 to 0.66)	(0.51 to 0.64)	(0.35 to 0.62)	(0.44 to 0.66)
[TGFβ-1]	0.60	0.59	0.66	0.64
	(0.53 to 0.68)	(0.52 to 0.66)	(0.55 to 0.77)	(0.54 to 0.74)
TIMP-1	0.60	0.56	0.56	0.52
	(0.53 to 0.67)	(0.49 to 0.63)	(0.45 to 0.68)	(0.41 to 0.63)
[TNFR-1A]	0.54	0.51	0.60	0.57
	(0.46 to 0.62)	(0.44 to 0.58)	(0.47 to 0.73)	(0.47 to 0.68)
VEGF-C	0.57	0.58	0.42	0.46
	(0.50 to 0.65)	(0.51 to 0.64)	(0.29 to 0.56)	(0.35 to 0.58)
Visfatin	0.56	0.56	0.46	0.49
	(0.49 to 0.63)	(0.49 to 0.62)	(0.35 to 0.57)	(0.39 to 0.60)
WAP4C	0.62	0.60	0.52	0.55
HE4 85b	(0.55 to 0.69)	(0.53 to 0.66)	(0.39 to 0.66)	(0.45 to 0.66)
WAP4C	0.55	0.57	0.40	0.47
HE4 91a	(0.48 to 0.63)	(0.51 to 0.64)	(0.27 to 0.53)	(0.35 to 0.58)

In addition to analysing the test performance of multiple biomarkers, the ability of currently utilised ultrasound parameters to determine delivery of an SGA infant was assessed and compared to low PlGF. Tables 2.10 and 2.11 summarise the sensitivity, specificity, positive and negative predictive values and positive and negative likelihood ratios of low maternal plasma PlGF (<100 pg/ml) and currently measured ultrasound parameters (including EFW/AC <10th centile, oligohydramnios (AFI <5cm) and AREDF) to determine delivery of an SGA infant <3rd and <10th customised centiles respectively. EFW/AC <10th centile had the best performance of currently used ultrasound parameters, with a sensitivity of 71.2% (95% CI 57.9 to 82.2%) and NPV 78.5% (95% CI 67.8 to 86.9%) to determine SGA <3rd centile. However PlGF outperformed all currently measured ultrasound parameters with a sensitivity of 93.2% (95% CI 83.5 to 98.1%) and NPV 89.7% (95% CI 75.8 to 97.1%) to determine SGA <3rd centile (Table 2.10). When PlGF was combined with currently used ultrasound parameters, sensitivity improved to 96.6% (95% CI 88.3 to 99.6%) with NPV 94.3% (95% CI 80.8 to 99.3%).

In predicting adverse perinatal outcome (excluding SGA in this definition) PlGF had higher sensitivity (89.7%) and negative predictive value (89.7%) than all other ultrasound measurements (Table 2.12). If PlGF was combined with fetal ultrasound parameters (AC or EFW <10th centile) there was a marginal rise in test sensitivity to 92.3% (95% CI 79.1 to 98.4%).

Table 2.10: Test performance statistics (with 95% confidence intervals) for individual indicators and in combination to predict small for gestational age (SGA) <3rd customised birth weight centile in women presenting before 35 weeks' gestation (n=126)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
AC or EFW <10th centile	71.2 (57.9 to 82.2)	92.5 (83.4 to 97.5)	89.4 (76.9 to 96.5)	78.5 (67.8 to 86.9)	9.5 (4.0 to 22.5)	0.31 (0.21 to 0.47)
Oligohydramnios	18.6 (9.7 to 30.9)	98.5 (92.0 to 100)	91.7 (61.5 to 99.8)	57.9 (48.3 to 67.1)	12.5 (1.7 to 93.9)	0.83 (0.73 to 0.94)
AREDF	20.3 (11.0 to 32.8)	98.5 (92 to 100)	92.3 (64 to 99.8)	58.4 (48.8 to 67.6)	13.6 (1.8 to 102)	0.81 (0.71 to 0.92)
PlGF <100 pg/ml	93.2 (83.5 to 98.1)	52.2 (39.7 to 64.6)	63.2 (52.2 to 73.3)	89.7 (75.8 to 97.1)	2.0 (1.5 to 2.5)	0.13 (0.05 to 0.34)
Combinations						
AC or EFW <10th centile or oligo or AREDF	72.9 (59.7 to 83.6)	91.0 (81.5 to 96.6)	87.8 (75.2 to 95.4)	79.2 (68.5 to 87.6)	8.1 (3.7 to 17.7)	0.30 (0.19 to 0.46)
AC or EFW <10th centile or PlGF <100 pg/ml	96.6 (88.3 to 99.6)	49.3 (36.8 to 61.8)	62.6 (51.9 to 72.6)	94.3 (80.8 to 99.3)	1.90 (1.5 to 2.4)	0.07 (0.02 to 0.3)

AC or EFW, Abdominal Circumference or Estimated Fetal Weight, Oligohydramnios defined as amniotic fluid index <5th centile for gestational age, AREDF, Absent or Reversed End Diastolic Flow in umbilical artery

Table 2.11: Test performance statistics (with 95% confidence intervals) for individual indicators and in combination to predict small for gestational age (SGA) <10th customised birth weight centile in women presenting before 35 weeks' gestation (n=126)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
AC or EFW <10th centile	58.1 (46.1 to 69.5)	92.3 (81.5 to 97.9)	91.5 (79.6 to 97.6)	60.8 (49.1 to 71.6)	7.6 (2.9 to19.8)	0.5 (0.3 to 0.6)
Oligohydramnios	16.2 (8.7 to 26.6)	100 (93.2 to 100)	100 (73.5 to100)	45.6 (36.3 to 55.2)	0	0.8 (0.8 to 0.9)
AREDF	16.2 (8.7 to 26.6)	98.1 (89.7 to 100)	92.3 (64.0 to 99.8)	45.1 (35.8 to 54.8)	8.4 (1.1 to 62.9)	0.9 (0.8 to 1.0)
PlGF <100 pg/ml	83.8 (73.4 to 91.3)	51.9 (37.6 to 66.0)	71.3 (60.6 to 80.5)	69.2 (52.4 to 83.0)	1.7 (1.3 to 2.4)	0.3 (0.2 to 0.6)
Combinations						
AC or EFW <10th centile or oligo or AREDF	60.8 (48.8 to 72.0)	92.3 (81.5 to 97.9)	91.8 (80.4 to 97.7)	62.3 (50.6 to 73.1)	7.9 (3.0 to 20.6)	0.42 (0.32 to 0.57)
AC or EFW <10th centile or PlGF <100 pg/ml	87.8 (78.2 to 94.3)	50.0 (35.8 to 64.2)	71.4 (61.0 to 80.4)	74.3 (56.7 to 87.5)	1.8 (1.3 to 2.3)	0.24 (0.12 to 0.48)

AC or EFW, Abdominal Circumference or Estimated Fetal Weight, Oligohydramnios defined as amniotic fluid index <5th centile for gestational age, AREDF, Absent or Reversed End Diastolic Flow in umbilical artery

Table 2.12: Test performance statistics (with 95% confidence intervals) for individual indicators and in combination to predict adverse perinatal outcome in women presenting before 35 weeks' gestation (n=126)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
AC or EFW <10th centile	48.7 (32.4 to 65.2)	67.8 (56.9 to 77.4)	40.4 (26.4 to 55.7)	74.7 (63.6 to 83.8)
Oligohydramnios	12.8 (4.3 to 27.4)	92.0 (84.1 to 96.7)	41.7 (15.2 to 72.3)	70.2 (60.9 to 78.4)
AREDF	12.8 (4.3 to 27.4)	90.8 (82.7 to 95.9)	38.5 (13.9 to 68.4)	69.9 (60.6 to 78.2)
PlGF <100 pg/ml	89.7 (75.8 to 97.1)	40.2 (29.9 to 51.3)	40.2 (29.9 to 51.3)	89.7 (75.8 to 97.1)
Combinations				
AC or EFW <10th centile or oligohydramnios or AREDF	53.8 (37.2 to 69.9)	67.8 (56.9 to 77.4)	42.9 (28.8 to 57.8)	76.6 (65.6 to 85.5)
AC or EFW <10th centile or PlGF <100 pg/ml	92.3 (79.1 to 98.4)	36.8 (26.7 to 47.8)	39.6 (29.5 to 50.4)	91.4 (76.9 to 98.2)

AC or EFW, Abdominal Circumference or Estimated Fetal Weight

Oligohydramnios defined as amniotic fluid index <5th centile for gestational age

ARDEF, Absent or Reversed End Diastolic Flow in umbilical artery

2.3.2 Women presenting between 35^{+0} and 36^{+6} weeks' gestation (n = 123)

Of 123 women presenting between 35^{+0} and 36^{+6} weeks' gestation, 39 (31.7%) delivered an SGA infant <10^{th} customised centile for birth weight. Patterns in demographics at booking and enrolment were similar to those seen in women presenting prior to 35 weeks' gestation (Table 2.13).

Table 2.14 summarises data for delivery and maternal and neonatal outcomes. In contrast to women recruited prior to 35 weeks' gestation, onset and mode of delivery and maternal adverse outcome did not vary greatly with infant birth weight. However, delivery by caesarean section was very high in the whole cohort, at approximately 50%. This is nearly twice that of the current national average and may reflect that pregnancies recruited to this study were high risk. There were no fetal of neonatal deaths in this group and whilst prevalence of adverse perinatal outcomes was higher in pregnancies complicated by delivery of an SGA infant compared to those delivering an AGA infant, this was much lower than in women recruited prior to 35 weeks' gestation (12% in women recruited between 35^{+0} and 36^{+6} weeks and delivering an SGA infant <3^{rd} centile compared to 39% in those recruited prior to 35 weeks' gestation who delivered an SGA infant <3^{rd} birth weight centile).

Table 2.13: Characteristics of participants recruited between 35^{+0} to 36^{+6} weeks' gestation at booking and enrolment (grouped by subsequent infant birth weight). Values given are median (quartiles) or n (%) as appropriate.

Characteristics	Women with SGA infant <3rd centile (n = 25)	Women with SGA infant <10th centile (n=39)	Women with infant ≥ 10th centile (n=84)
At booking:			
Age (years)	32.8 (25.2 to 36.1)	29.6 (25.1 to 35.3)	32.4 (28.0 to 35.0)
BMI (kg/m^2)	28.6 (25.6 to 30.5)	28.0 (24.3 to 30.5)	28.8 (24.3 to 32.9)
White ethnicity	16 (64)	23 (59)	53(63)
Highest first trimester systolic BP (mmHg)	110 (104 to 122)	116 (104 to 122)	120 (110 to 128)
Highest first trimester diastolic BP (mmHg)	70 (60 to 75)	70 (62 to 78)	76 (67 to 80)
Smoker at booking	6/25 (24)	8/39 (21)	11/80 (14)
Quit smoking during pregnancy	4/25 (16)	4/39 (10)	8/80 (10)
Previous pre-eclampsia requiring delivery <34/40	3 (12)	4 (10)	2 (2)
Chronic hypertension	2 (8)	3 (7.7)	6 (7)

Characteristics	Women with SGA infant <3rd centile (n = 25)	Women with SGA infant <10th centile (n=39)	Women with infant ≥ 10th centile (n=84)
At enrolment:			
Gestational age at sampling (weeks)	35.7 (35.4 to 36.3)	36 (35.4 to 36.4)	36.1 (35.4 to 36.4)
New onset hypertension †	20 (80)	31 (79.5)	51 (60.7)
Worsening of underlying hypertension	1 (4)	3 (7.7)	15 (17.9)
New onset of dipstick proteinuria	18 (72)	28 (71.8)	49 (58.3)
Suspected SGA (customised birth weight centiles) †	8 (32)	8 (20.5)	1 (1.2)
Highest systolic BP (mmHg)	140 (130 to 151)	145 (131 to 153)	143 (132 to 152)
Highest diastolic BP (mmHg)	90 (82 to 98)	92 (82 to 99)	94 (87 to 99)

BP, Blood Pressure

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 2.14: Characteristics of delivery and maternal and neonatal outcome for women recruited between 35^{+0} and 36^{+6} weeks' gestation. Values given are median (quartiles) or n (%) as appropriate.

Characteristics	**Women with SGA infant <3rd centile (n = 25)**	**Women with SGA infant <10th centile (n=39)**	**Women with infant ≥ 10th centile (n=84)**
Onset of labour			
Spontaneous	4 (16)	6 (15.4)	17 (20.2)
Induced	16 (64)	23 (59)	47 (56)
Pre labour caesarean section	5 (20)	10 (25.6)	19 (22.6)
Mode of delivery			
Spontaneous	9 (36)	13 (33.3)	37 (44)
Assisted vaginal	3 (12)	4 (10.3)	7 (8.3)
Caesarean section	12 (48)	21 (53.8)	40 (47.6)
Adverse maternal outcome*	6 (24)	11 (28.2)	26 (31)
Gestation at delivery (weeks)	37.3 (36.7 to 37.9)	37.1 (36.4 to 37.9)	37.7 (37 to 39.4)
Fetal death	0 (0)	0 (0)	0 (0)
Neonatal death	0 (0)	0 (0)	0 (0)
Birth weight (g) †	2170 (2030 to 2340)	2250 (2055 to 2480)	3240 (2925 to 3525)
SGA <10th birth weight centile	25 (100)	39 (100)	0 (0)
SGA <3rd birth weight centile	25 (100)	25 (64.1)	0 (0)
SGA <1st birth weight centile	11 (44)	11 (28.2)	0 (0)
Adverse perinatal outcome**	3 (12)	4 (10.3)	5 (6)
Maternal diagnosis			
No maternal disease	0	0	5 (6)
Gestational hypertension	0	0	14 (17)
Chronic hypertension	2 (8)	4 (10)	5 (6)
Pre-eclampsia	20 (80)	31 (80)	38 (45)
HELLP syndrome	0	0	0
Other diagnosis	3 (12)	4 (10)	22 (26)

*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 $<50\times10^9$/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.

**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.

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

As described in section 2.3.1, factor analysis of all 57 biomarkers was performed and the results showing the five largest factors in women enrolled between 35^{+0} and 36^{+6} weeks' gestation is displayed in Table 2.15. Factors three and four had the highest odds ratios and the biomarkers within these factors were investigated further as described in section 2.3.1.

Table 2.15: Odds ratios derived from multiple logistic regression analysis of the 5 factors in women presenting between 35^{+0} and 36^{+6} weeks' gestation (Odds ratios are for a change of 1 SD in the factor score).

Factor	Biomarkers contained in factor	Women with SGA infant <3rd centile Odds Ratio (95% CI)	Women with SGA infant <10th centile Odds Ratio (95% CI)
1	ANP, Arginase-1, CCL23, CPA-4, ESAM-1, FAS, Kunitz-2, TGFBeta-1, TNFR-1A, WAP4C-HE4-85b, WAP4C-HE4-91a	0.46 (0.26 to 0.78)	0.42 (0.25 to 0.72)
2	ADAM-9, Ephrin, FasL, Kunitz 35b, Kunitz 40b, Nephrin, PAI-1, Pentraxin-3-64a	0.97 (0.36 to 2.56)	0.54 (0.21 to 1.41)
3	Arginase-2, BNP, Nephrin, PCT-95a, Pentraxin 3-67a, Podocalyxin	1.80 (1.03 to 3.16)	1.73 (1.02 to 2.94)
4	PlGF, Endoglin, sFlt-1	3.54 (1.52 to 8.26)	6.89 (2.88 to 16.4)
5	Angiogenin, Caspase, FIH, ICAM-1, MIF, TIMP-1	1.07 (0.61 to 1.90)	0.85 (0.50 to 1.45)

Table 2.16 summarises the best performing individual biomarkers and combinations in determining delivery of an SGA infant <3rd and <10th customised centiles. In determining SGA <10th centile PlGF outperforms all other biomarkers measured with a ROC area of 0.74 (0.64 to 0.83) (Figure 2.4). Combinations of biomarkers derived from logistic regression improved test performance to determine SGA <10th centile, with PlGF, Nephrin and Carboxypeptidase A4 (CPA-4) producing a ROC area of 0.81 (0.73 to 0.90) (Figure 2.5). In determining SGA <3rd centile Kunitz 2 had the best individual test performance (ROC area 0.74 (0.63 to 0.85)) (Figure 2.4) with combinations of PlGF, Nephrin and CPA-4 improving test performance to a ROC area of 0.77 (0.66 to 0.88) (Figure 2.5). The test performance of all individual biomarkers are displayed in Table 2.9.

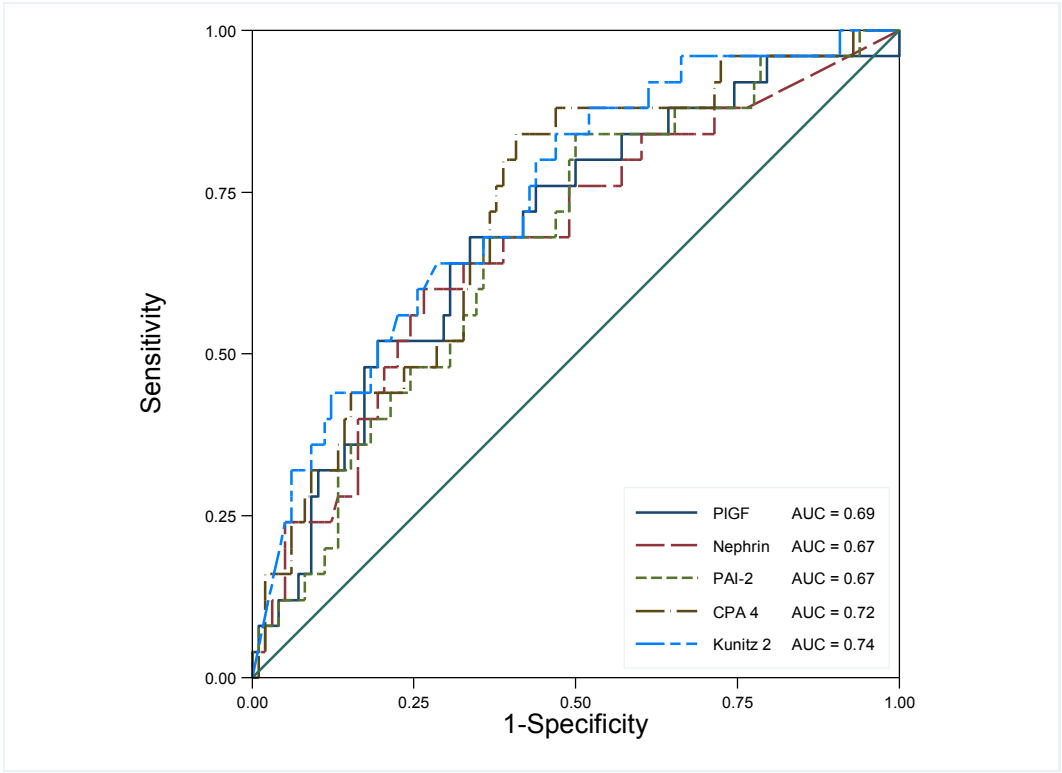
Table 2.16: ROC areas (with 95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to predict small for gestational age (SGA) <3rd and <10th customised birth weight centiles in women presenting between 35+0 and 36+6 weeks' gestation.

Biomarkers or combinations	SGA <3rd centile	SGA <10th centile
Nephrin	0.67 (0.55 to 0.80)	0.63 (0.52 to 0.74)
[PAI-2]	0.67 (0.56 to 0.79)	0.65 (0.55 to 0.75)
[PlGF]	0.69 (0.57 to 0.81)	0.74 (0.64 to 0.83)
[CPA-4]	0.72 (0.61 to 0.83)	0.72 (0.63 to 0.82)
Kunitz 2 35b	0.74 (0.63 to 0.85)	0.73 (0.65 to 0.82)
[PlGF/s-Flt ratio]	0.66 (0.54 to 0.78)	0.70 (0.60 to 0.80)
[PlGF/Endoglin ratio]	0.66 (0.54 to 0.78)	0.73 (0.63 to 0.82)
[PlGF], Nephrin	0.73 (0.62 to 0.84)	0.76 (0.67 to 0.85)
[PlGF], [CPA-4]	0.77 (0.67 to 0.88)	0.81 (0.73 to 0.90)
[PlGF], Nephrin, [CPA-4]	0.77 (0.66 to 0.88)	0.81 (0.73 to 0.90)

[ ] low concentrations of biomarker/ ratio correlated to severe disease

Figure 2.4: ROC areas of individual biomarkers measured between 35^{+0} and 36^{+6} weeks' gestation to determine:

SGA < 3rd centile



SGA <10th centile

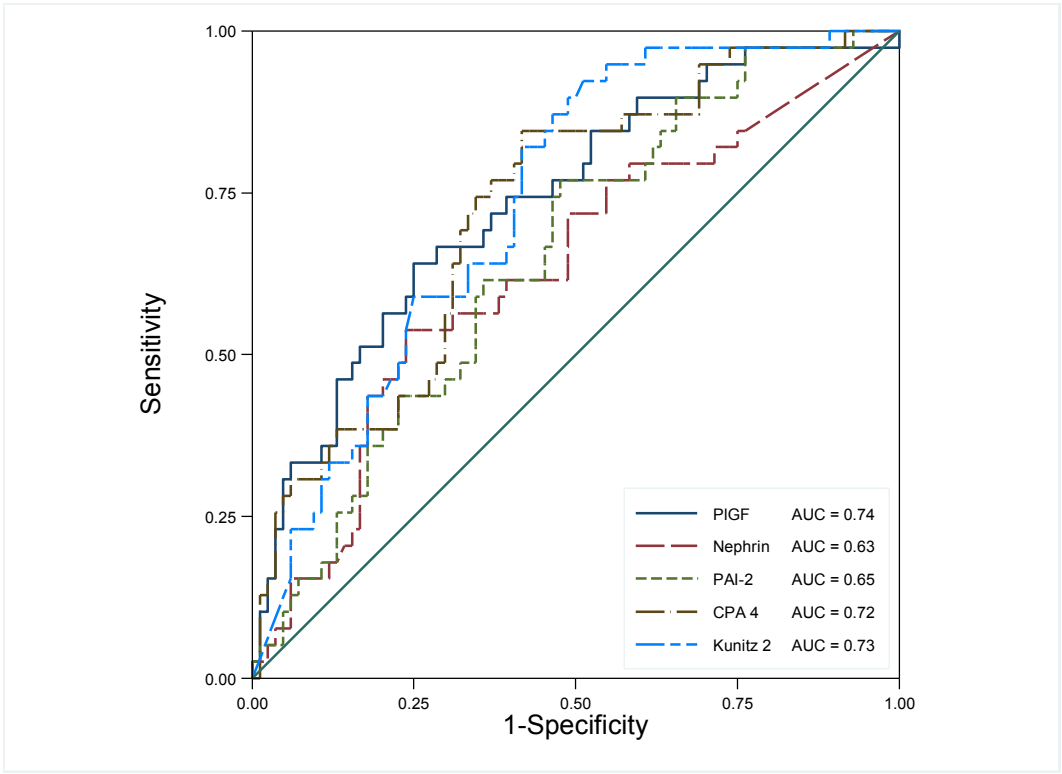
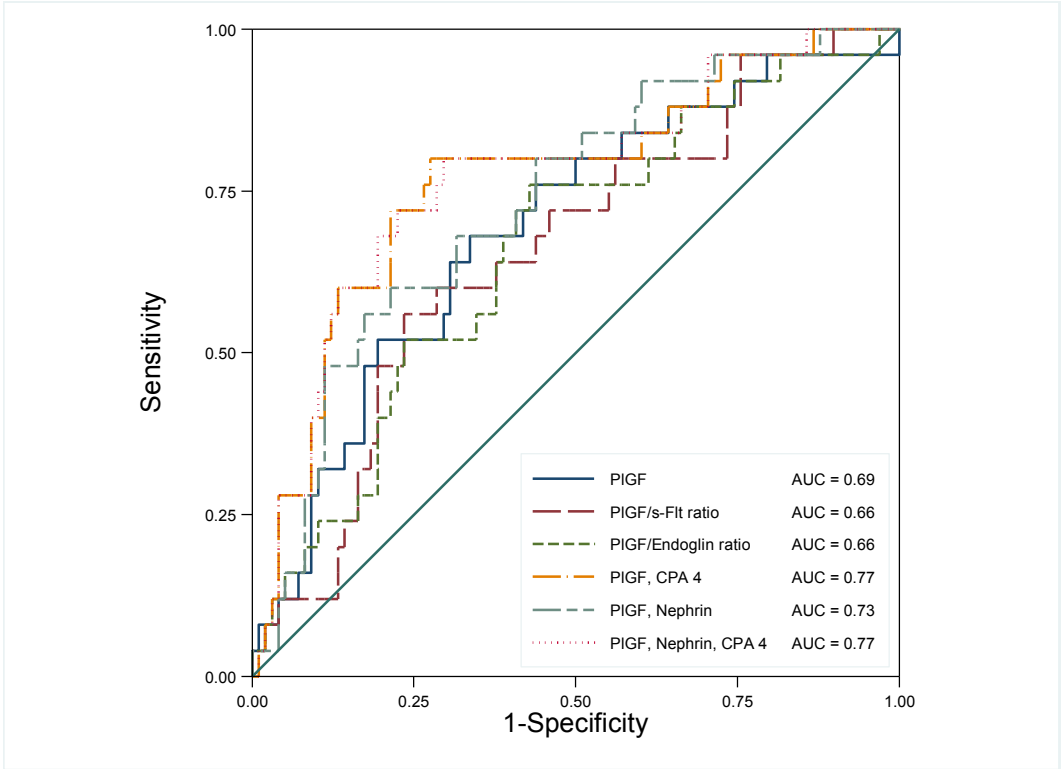
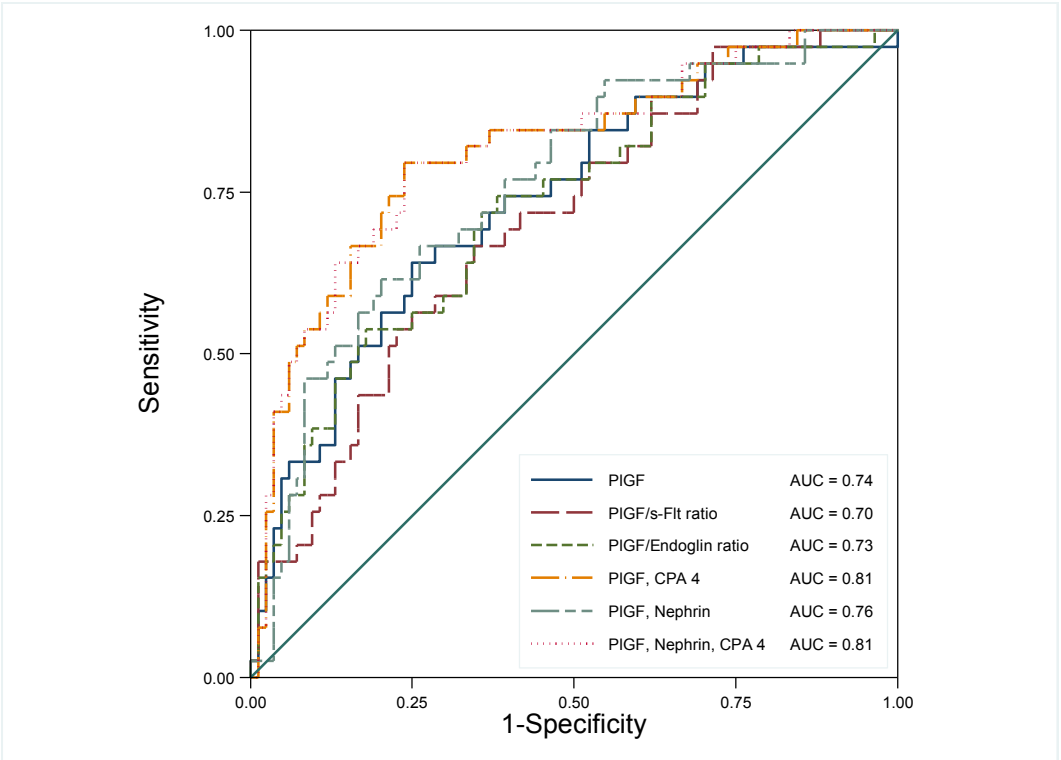


Figure 2.5: ROC areas of biomarker combinations measured between 35^{+0} and 36^{+6} weeks' gestation to determine:

SGA < 3rd centile



SGA <10th centile



Tables 2.17 and 2.18 compare test performance of currently utilised ultrasound parameters and PlGF to determine delivery of an SGA infant <3rd and <10th customised centiles respectively. Similar patterns are seen in test performance in this group as were observed in women recruited prior to 35 weeks' gestation. EFW/AC <10th centile has the best test performance of any ultrasound parameter assessed to determine delivery of an SGA infant (sensitivity 64.3%, NPV 88.1% for SGA <3rd centile) but PlGF outperforms all ultrasound parameters (sensitivity 85.7% (95% CI 57.2 to 98.2%), NPV 81.8% (95% CI 48.2 to 97.7%)) in determining subsequent delivery of an SGA infant. Addition of PlGF measurement to currently utilized ultrasound parameters increases test sensitivity to determine SGA <3rd centile from 71.4% (95% CI 41.9 to 91.6%) to 92.9% (95% CI 66.1 to 99.8%). Similar patterns are seen in Table 2.19 for prediction of adverse perinatal outcomes, where PlGF in isolation has a sensitivity of 100% in determining this endpoint.

Table 2.17: Test performance statistics for individual indicators and in combination to predict small for gestational age (SGA) <3rd customised birth weight centile in women presenting between 35+0 and 36+6 weeks' gestation (n= 53)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
AC or EFW <10th centile	64.3 (35.1 to 87.2)	94.9 (82.7 to 99.4)	81.8 (48.2 to 97.7)	88.1 (74.4 to 96.0)	12.5 (3.1 to 51.1)	0.4 (0.2 to 0.8)
Oligohydramnios	14.3 (1.8 to 42.8)	94.9 (82.7 to 99.4)	50.0 (6.8 to 93.2)	75.5 (61.1 to 86.7)	2.8 (0.4 to 17.9)	0.9 (0.7 to 1.1)
AREDF	14.3 (1.8 to 42.8)	97.4 (86.5 to 99.9)	66.7 (9.4 to 99.2)	76.0 (61.8 to 86.9)	5.6 (0.6 to 56.8)	0.9 (0.7 to 1.1)
PlGF <100 pg/ml	85.7 (57.2 to 98.2)	23.1 (11.1 to 39.3)	28.6 (15.7 to 44.6)	81.8 (48.2 to 97.7)	1.11 (0.9 to 1.5)	0.6 (0.2 to 2.5)
Combinations						
AC or EFW <10th centile or oligo or AREDF	71.4 (41.9 to 91.6)	89.7 (75.8 to 97.1)	71.4 (41.9 to 91.6)	89.7 (75.8 to 97.1)	7.0 (2.6 to 18.7)	0.32 (0.14 to 0.73)
AC or EFW <10th centile or PlGF <100 pg/ml	92.9 (66.1 to 99.8)	23.1 (11.1 to 39.3)	30.2 (17.2 to 46.1)	90.0 (55.5 to 99.7)	1.2 (1.0 to 1.5)	0.3 (0.04 to 2.2)

AC or EFW, Abdominal Circumference or Estimated Fetal Weight, Oligohydramnios defined as amniotic fluid index <5th centile for gestational age, AREDF, Absent or Reversed End Diastolic Flow in umbilical artery

Table 2.18: Test performance statistics for individual indicators and in combination to predict small for gestational age (SGA) <10th customised birth weight centile in women presenting between 35^{+0} and 36^{+6} weeks' gestation (n=53)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
AC or EFW <10th centile	52.4 (29.8 to 74.3)	100 (89.1 to 100)	100 (71.5 to 100)	76.2 (60.5 to 87.9)	0	0.5 (0.3 to 0.8)
Oligohydramnios	14.3 (3.0 to 36.3)	96.9 (83.8 to 99.9)	75.0 (19.4 to 99.4)	63.3 (48.3 to 76.6)	4.6 (0.5 to 41.1)	0.9 (0.7 to 1.1)
AREDF	9.5 (1.2 to 30.4)	96.9 (83.8 to 99.9)	66.7 (9.4 to 99.2)	62.0 (47.2 to 75.3)	3.1 (0.3 to 31.5)	0.9 (0.8 to 1.1)
PlGF <100 pg/ml	90.5 (69.6 to 98.8)	28.1 (13.7 to 46.7)	45.2 (29.8 to 61.3)	81.8 (48.2 to 97.7)	1.3 (1.0 to 1.6)	0.3 (0.1 to 1.4)
Combinations						
AC or EFW <10th centile or oligo or AREDF	57.1 (34.0 to 78.2)	93.8 (79.2 to 99.2)	85.7 (57.2 to 98.2)	76.9 (60.7 to 88.9)	9.14 (2.3 to 36.8)	0.46 (0.3 to 0.8)
AC or EFW <10th centile or PlGF <100 pg/ml	95.2 (76.2 to 99.9)	28.1 (13.7 to 46.7)	46.5 (31.2 to 62.3)	90.0 (55.5 to 99.7)	1.3 (1.1 to 1.7)	0.2 (0.02 to 1.2)

Table 2.19: Test performance statistics for individual indicators and in combination to predict adverse perinatal outcome in women presenting between 35^{+0} and 36^{+6} weeks' gestation (n=53)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
AC or EFW <10th centile	50.0 (6.8 to 93.2)	81.6 (68.0 to 91.2)	18.2 (2.3 to 51.8)	95.2 (83.8 to 99.4)
Oligohydramnios	0 (0 to 60.2)	91.8 (80.4 to 97.7)	0 (0 to 60.2)	91.8 (80.4 to 97.7)
AREDF	25.0 (0.6 to 80.6)	95.9 (86.0 to 99.5)	33.3 (0.8 to 90.6)	94.0 (83.5 to 98.7)
PlGF <100 pg/ml	100 (39.8 to 100)	22.4 (11.8 to 36.6)	9.5 (2.7 to 22.6)	100 (71.5 to 100)
Combinations				
AC or EFW <10^{th} centile or oligohydramnios or AREDF	50.0 (6.8 to 93.2)	75.5 (61.1 to 86.7)	14.3 (1.8 to 42.8)	94.9 (82.7 to 99.4)
AC or EFW <10th centile or PlGF <100 pg/ml	100 (39.8 to 100)	20.4 (10.2 to 34.3)	9.3 (2.6 to 22.1)	100 (69.2 to 100)

AC or EFW, Abdominal Circumference or Estimated Fetal Weight,

Oligohydramnios defined as amniotic fluid index <5^{th} centile for gestational age,

AREDF, Absent or Reversed End Diastolic Flow in umbilical artery

2.4 Summary

In women presenting prior to 35 weeks' gestation, PlGF outperformed all other individual biomarkers in its ability to determine delivery of an SGA infant in this cohort of women with suspected pre-eclampsia. Combinations of biomarkers added only modest rises in ROC area and are unlikely to be clinically useful.

Ultrasound parameters utilised in current clinical practice had modest sensitivity in determining delivery of an SGA infant, resulting in 33% of cases of SGA <3rd centile in this cohort remaining undetected, compared to only 7% of cases being missed if low PlGF concentration had been used as a predictive test. Addition of PlGF quantification to ultrasound parameters improved detection rates.

In addition, PlGF had high sensitivity to detect adverse perinatal outcome, which included stillbirth. The performance of PlGF to detect adverse perinatal outcome was particularly marked in women recruited between 35^{+0} and 36^{+6} weeks' gestation (100% sensitivity (39.8 to 100%)). However caution should be exercised regarding the reproducibility of these results as only 53 women recruited between 35^{+0} and 36^{+6} weeks' gestation had ultrasound data available for analysis.

Participant characteristics at booking and enrolment were similar for women presenting beyond 35 weeks' gestation to those recruited prior to 35 weeks', with new onset of hypertension and proteinuria reported more frequently in women who delivered an SGA infant, compared to those delivering AGA infants. However, in contrast to women recruited prior to 35 weeks' gestation,

onset and mode of delivery in women recruited after 35 weeks' gestation were not strongly associated with birth weight. This could be explained by women presenting at later gestations having less severe disease or that by presenting later, closer proximity to delivery limits disease progression to the same extent as in women presenting at earlier gestations. The proportion of women having a pre-labour caesarean section was much higher in women enrolled prior to 35 weeks' gestation, compared to those entering the study between 35^{+0} and 36^{+6} weeks' gestation, irrespective of birth weight. In addition to the explanations given above, the difficulties with successfully inducing labour at early gestations may also have contributed to this finding.

Trends in biomarker test performance in women recruited between 35^{+0} and 36^{+6} weeks' gestation to determine delivery of an SGA infant were similar to those observed in women recruited prior to 35 weeks' gestation with PlGF outperforming other biomarkers (ROC area 0.74 (0.64 to 0.83) for SGA $<10^{th}$ centile). However, test performance statistics were less impressive than those reported in women recruited prior to 35 weeks' gestation. Combinations of biomarkers achieved only modest improvements in test performance over PlGF alone (ROC area for combination of PlGF, Nephrin 101a, CPA-4 0.81; 95% CI 0.73 to 0.90)).

Further discussion of these findings are detailed in Chapter 4: Discussion.

Chapter 3: Biomarkers and ultrasound parameters to determine the small for gestational age infant

3.1 Introduction

Following from the positive findings of the PELICAN-PE study, where PlGF accurately predicted delivery of an SGA infant in a high-risk population with suspected pre-eclampsia, I sought to investigate whether these findings could be replicated in a more general antenatal setting. Within the UK, current clinical referral pathways rely on symphysis fundal height measurement to identify those suspected of carrying an SGA fetus. Women with reduced symphysis-fundal height measurement are then referred for further assessment, usually by ultrasound scan.

I investigated how measuring PlGF in this group of women, at time of presentation for ultrasound scan, compared to current tools and whether this could provide additional information to the clinician to improve identification of pregnancies complicated by SGA.

3.1.1 Involvement with the study

I developed the study concept and design for PELICAN FGR in conjunction with the PELICAN-PE study chief investigators. I submitted a substantial amendment to the research ethics committee (East London) via the Integrated Research Application System, to request permission to extend the PELCAN-PE study protocol to include recruitment of women with suspected SGA (as defined in section 3.2). Approval for these amendments was subsequently granted. A standard operating protocol was compiled and circulated to all study sites. The plan for statistical analysis was agreed between myself, Mr Paul Seed (medical statistician), the principal investigator for St Thomas' Hospital and the study chief investigator.

I led design of the database fields needed to collect data for women recruited with suspected SGA.

I headed a team, who undertook site initiation visits (SIVs) to train staff on use of the database and provided support to all sites as part of my role of study coordinator. In this role I worked along side an independent study monitor, who was responsible for training laboratory staff in the techniques necessary for PlGF measurement and also for downloading PlGF results, which were masked to all other staff involved in the study. I received training in the use of the PlGF Triage meter and assisted the study monitor with ensuring correct calibration of meters at enrolling sites. I also led regular study teleconferences to maintain contact and updates with all sites.

I led regular site visits to all participating sites with the exception of Vancouver, Canada, with whom I maintained regular contact.

In addition to my work coordinating other sites, I personally recruited over 100 women to this study from our antenatal clinics, ultrasound scan department and day assessment unit at St Thomas' Hospital, London. Two colleagues under my supervision and myself were responsible for inputting all data for the 177 women recruited at St Thomas' Hospital. I was responsible for data cleaning and adjudication of all final maternal diagnoses, along with senior clinicians. I undertook the statistical analysis with the medical statistician, and input from the study chief investigator and principal investigator for St Thomas' Hospital.

3.2 Methods

The PELICAN-FGR study was a prospective observational study evaluating the ability of placental growth factor (PlGF) and current ultrasound parameters to determine delivery of a small for gestational age (SGA) infant in women who presented measuring small for dates. Women were recruited from eleven consultant-led units across the United Kingdom and Canada between December 2011 and July 2013. Ethical approval was granted by East London Research Ethics Committee (ref. 10/H0701/117).

Study eligibility required women to be aged 16 years or over, with a singleton pregnancy, between 24^{+0} to 36^{+6} weeks' gestation and referred with suspected SGA by either:

1) Symphysis-fundal height (cm) measuring more than 2cm (i.e. 3cm or more) under that expected for any given gestational age (completed weeks) e.g. measuring 33cm or less at 36 weeks' gestation.

   Or

2) Symphysis-fundal height less than the 10^{th} centile on a customised chart.

The above definitions were selected for eligibility criteria as they constituted the current parameters utilised in enrolling sites for further investigation for suspected SGA. Symphysis-fundal height was measured according to recruiting site protocol for measurement. Women with multi-fetal pregnancies, confirmed SGA, major fetal anomaly, or ruptured amniotic membranes were excluded (see section 2.2.1 for definitions). Written informed consent was obtained from participants. A study specific database was designed and implemented with

training provided for all users at recruiting sites, prior to recruitment of the first participant. Baseline demographic and pregnancy specific data was entered, including ultrasound data at study enrolment. An independent data monitor was appointed to ensure accurate reporting and regular data monitoring was undertaken at all sites.

At study enrolment blood was drawn into ethylenediamine tetra-acetic acid and processed and stored as described in section 2.2. All maternal plasma samples were analysed for PlGF at the recruiting site using the Triage® PlGF Test (Alere, San Diego, CA) as described in section 2.2.2. All laboratory staff received standardised training in sample processing delivered by the study monitor. All Triage meters were programmed to produce a masked result and only provided confirmation of a satisfactory test. All laboratory staff were masked to the clinical diagnosis and all results were concealed until a final adjudicated maternal diagnosis was reached. PlGF results were classified as normal (PlGF ≥ 5^{th} centile for gestational age), low (<5^{th} centile) or very low (<12 pg/ml) i.e. below the lower limit of detection. To assess assay reproducibility, replicate samples were tested at a central laboratory. The total precision (coefficient of variation) on plasma controls at concentrations of 85 pg/ml and 1300 pg/ml were 12.8% and 13.2%, respectively.

Any subsequent hospital attendances were recorded in the study database, including repeat ultrasound assessments, details of delivery and adverse maternal and perinatal outcomes (defined in 2.2.1).

All cases were adjudicated by senior obstetricians (with review by two clinicians, and additional review by a third if discordancy in order to reach a consensus), without knowledge of PlGF concentrations, and a final maternal diagnosis was assigned according to the definitions detailed in 2.2.1 and those given below.

3.2.1 Definitions

Small for gestational age fetus:

Fetal abdominal circumference and/ or estimated fetal weight (on ultrasound assessment) less than the 10th centile for gestational age.

Small for gestational age infant:

Birth weight less than the 3rd and 10th centile by customised GROW centile.

Suspected fetal growth restriction:

Symphysis fundal height (cm) measuring more than 2cm (i.e. 3cm or more) under that expected for any given gestational age (completed weeks) e.g. measuring 33cm or less at 36 weeks' gestation

Major fetal anomaly:

Fetal malformations that affect viability and/ or the quality of life of the fetus and require intervention. (European Union Registry Of Congenital Anomalies and Twins: EUROCAT(Addor et al., 2000))

Oligohydramnios:

Amniotic fluid index less than 5 cm.

3.2.2 Sample size and power calculation

The PELICAN-FGR study was powered for the primary endpoint of delivering an SGA infant with birth weight less than the 3rd customised birth weight centile. Based on data from St Thomas' Hospital, London, 8% of women referred with suspected SGA delivered an SGA infant with birth weight less than the 3rd customised centile. The study was powered on the basis of the number of cases needed to reliably distinguish good (80%) from moderate (60%) sensitivity. 55 cases of SGA <3rd birth weight centile were needed for 90% power and 5% significance. Therefore, the planned sample size was 688. During data monitoring (whilst still masked to PlGF concentrations), the incidence of SGA <3rd birth weight centile in the study population was noted to be 13%. This resulted in recruitment of 78 cases from a total of 601 women, at which point the study was closed.

3.2.3 Statistical analysis

The primary outcome (reference standard) of delivering an SGA infant less than the 3rd customised birth weight centile, was calculated using version 6.7 of Gestation Related Optimal Weight (GROW) calculator (Gardosi and Francis), with maternal weights adjusted for normal BMI (18-30 kg/m^2). This threshold (3rd centile) was chosen as it includes fewer infants who are constitutionally small and has a stronger association with perinatal mortality (Moraitis et al.,

2014). Delivery of an SGA infant less than the 10th customised birth weight centile, and adverse perinatal outcomes were considered as secondary outcomes.

Gestational age adjusted centiles have been calculated for PlGF from a large low risk antenatal population (Knudsen et al., 2012). A PlGF concentration below the 5th centile was taken as abnormal as this has been shown to offer a combination of high sensitivity and acceptable specificity for pre-eclampsia and SGA (Chappell et al., 2013).

Sensitivity, specificity, positive and negative predictive values and positive and negative likelihood ratios (with 95% confidence intervals) were calculated for PlGF and three ultrasound parameters (estimated fetal weight <10th centile, umbilical artery Doppler pulsatility index (UAPI) > 95th centile and oligohydramnios) to determine delivery of an SGA infant <3rd and <10th customised centiles, both in isolation and in combination. To provide umbilical artery Doppler pulsatility index centiles for this analysis, gestation adjusted centiles were calculated for each observed value of UAPI based on a mean value of 0.405 -0.0134 x gestational age in weeks', and a standard deviation of 0.0794 for the $\log^{10}$ UAPI (Parra-Cordero et al., 2007).

Receiver operator characteristic (ROC) curve areas were also calculated for each individual parameter and combinations, and in a pre-defined subgroup who delivered within six weeks of PlGF sampling.

Variables related to health resource use (number of fetal ultrasounds, neonatal bed nights in Special Care Baby Unit or in Neonatal Intensive Care Unit, attendances at out-patient clinic/ Day Assessment Unit/ Antepartum Hospital Clinic after enrolment, maternal bed nights for suspected fetal compromise after enrolment, post-partum bed nights) were presented as means with 95% confidence intervals. Due to non-normal distributions, a bias corrected and accelerated bootstrap was used with 10,000 replications (Efron, 1987).

Statistical analysis was carried out in Stata statistical package (version 11.2), College Station Texas, USA. Formal significance was taken at $p<0.05$.

This study was reported in accordance with the STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines.

3.3 Results

601 women presenting with suspected SGA between 24^{+0} and 36^{+6} weeks' gestation were recruited across 11 sites between December 2011 and July 2013. We recruited all women who were approached, eligible and consented but did not document women who declined to participate. No outcome data were available on two participants and five women did not have a valid plasma PlGF result. A further two women had no ultrasound data at enrolment available. After exclusion of these nine cases, 592 women were included in this analysis. Of these women 192 delivered an SGA infant with birth weight <10th customised centile and 78 had a birth weight <3^{rd} centile (Figure 3.1).

All descriptive tables are subdivided according to subsequent birth weight. Characteristics of participants at booking are given in Table 3.1, with higher rates of smoking observed in those who delivered an SGA infant <3^{rd} and <10^{th} birth weight centiles. Baseline characteristics of participants at study enrolment are summarised in Table 3.2, with little difference in maternal factors between groups. At enrolment, only 57.9% of women who delivered an SGA infant <3^{rd} centile and 47.1% of women who delivered an SGA infant <10^{th} centile had an EFW <10^{th} centile. Details of maternal and neonatal outcomes are shown in Table 3.3. Overall, maternal and perinatal adverse outcomes were infrequent (3.2 and 2.2% respectively) but rates were numerically higher in those who delivered an SGA infant <3^{rd} and <10^{th} centile (SGA-3 6.4% and 5.1% and SGA-10 4.7% and 3.1% respectively). Induction of labour and caesarean section were more common in cases complicated by SGA in comparison to those pregnancies delivering AGA. Final maternal adjudicated diagnoses are

given in Table 3.4, with the majority of women experiencing no maternal complications during their pregnancy (n=555; 94%). Whilst the number of cases of pre-eclampsia were small (n=16) most delivered an SGA infant (n=12).

Figure 3.1: Flow diagram of participants

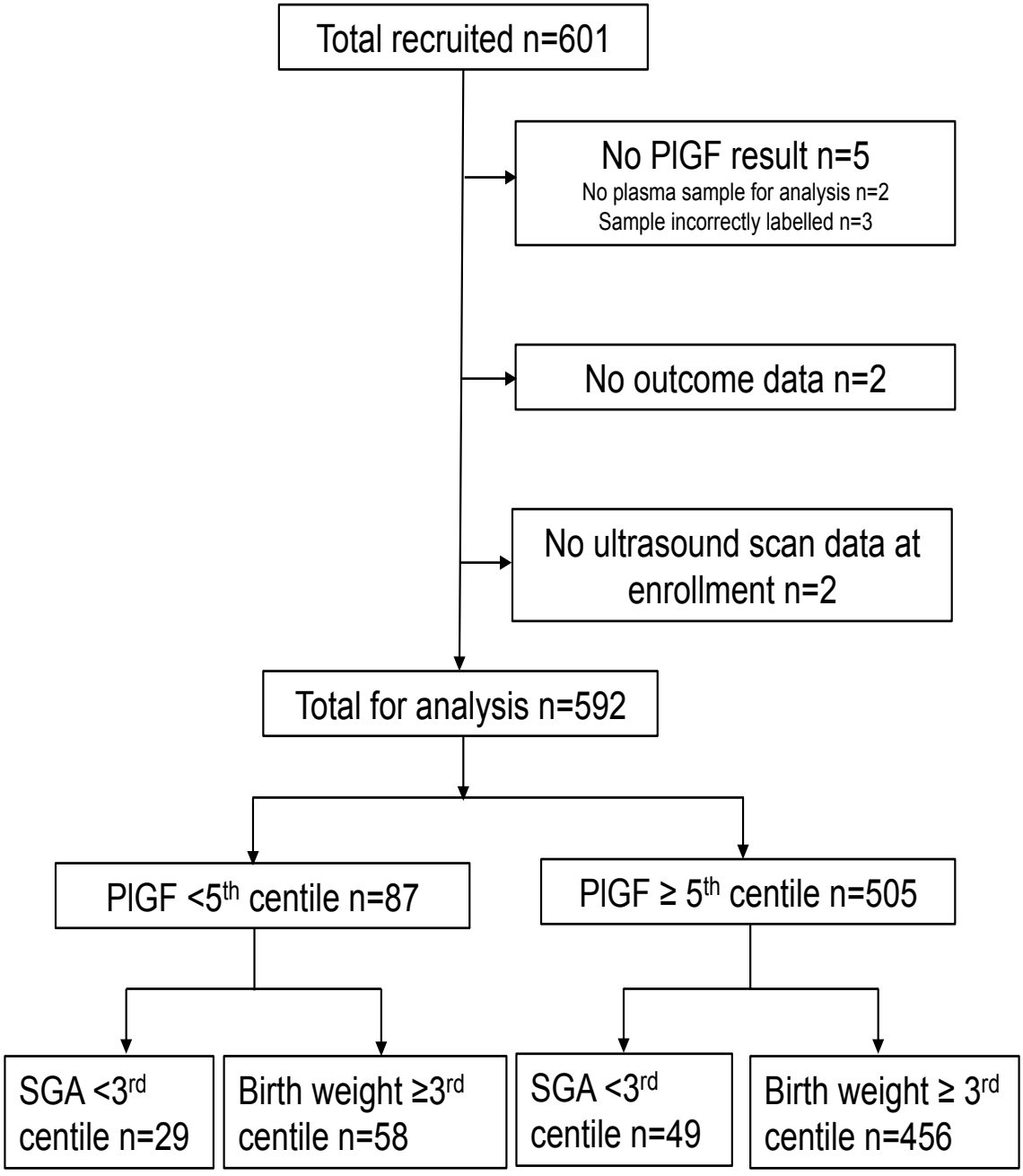


Table 3.1: Characteristics of participants at booking. Values given are median (quartiles) or n (%) as appropriate.

	Women with SGA infant <3rd centile (n=78)	Women with SGA infant <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
General maternal				
Age (years)	29.1 (24.1 to 32.9)	29.6 (24.8 to 33.5)	30.0 (25.3 to 33.7)	29.9 (25.2 to 33.6)
Body Mass Index at booking (kg/m^2) †	22.9 (20.3 to 25.2)	21.7 (20.1 to 24.1)	21.5 (20.0 to 23.4)	21.5 (20.0 to 23.6)
White ethnicity	52 (66.7)	122 (63.5)	266 (66.5)	388 (65.5)
Primiparity	65 (83.3)	163 (84.9)	344 (86.0)	507 (85.6)
Highest first trimester systolic BP (mmHg)	105 (100 to 114)	105 (100 to 114)	104 (100 to 112)	105 (100 to 112)
Highest first trimester diastolic BP (mmHg)	63 (60 to 70)	62 (60 to 70)	60 (60 to 69)	61 (60 to 70)
Smoking status				
Never smoked †	46 (59)	128 (66.7)	306 (76.5)	434 (73.3)
Quit smoking before pregnancy	9 (11.5)	22 (11.5)	31 (7.8)	53 (8.9)
Quit smoking during pregnancy	10 (12.8)	16 (8.3)	24 (6.0)	40 (6.7)
Current smoker	13 (16.7)	26 (13.5)	39 (9.8)	65 (11.0)
Drug use				
History of drug use *†	5 (6.4)	6 (3.1)	3 (0.8)	9 (1.5)
Current drug user **	1 (1.3)	2 (1.0)	0 (0.0)	2 (0.3)
Previous medical history				
Pre-eclampsia with delivery <34/40	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Chronic hypertension	0 (0)	1 (0.5)	1 (0.3)	2 (0.3)
SLE/ APS	1 (1.3)	1 (0.5)	0 (0)	1 (0.2)
Pre-existing diabetes	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Renal disease	0 (0)	0 (0)	0 (0)	0 (0)
Self-report previous SGA	9 (11.5)	22 (11.5)	27 (6.8)	49 (8.3)

BP, blood pressure

* Drugs used before pregnancy: include cannabis, cocaine, ecstasy, amphetamines (speed, crystal meth), and heroin.

** Drugs used during pregnancy: Cannabis only (rare or occasional)

SLE/ APS, Systemic lupus erythematosus/ anti-phospholipid syndrome

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 3.2: Baseline characteristics of participants at study enrolment. Values given are median (quartiles) or n (%) as appropriate.

	Women with SGA infant <3rd centile (n=78)	Women with SGA infant <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Gestational age at PlGF sampling (days)	238 (221 to 250)	235 (213 to 250)	236 (214 to 250)	236 (213 to 250)
Maternal BP				
Highest systolic BP (mmHg) †	118 (109 to129)	115 (102 to 121)	110 (101 to 118)	110 (101 to 120)
Highest diastolic BP (mmHg) †	70 (60 to 81)	70 (60 to 80)	67 (60 to 73)	68 (60 to 74)
Dipstick proteinuria				
Not done	11 (14.1)	29 (15.1)	61 (15.3)	90 (15.2)
Negative	58 (74.4)	148 (77.1)	322 (80.5)	470 (79.4)
Present (+1 or greater) †	9 (11.5)	15 (7.8)	17 (4.3)	32 (5.4)
Complications in current pregnancy				
Gestational hypertension	4 (5.1)	4 (2.1)	0 (0)	4 (0.7)
Pre-eclampsia	0 (0)	1 (0.5)	1 (0.3)	2 (0.3)
Gestational diabetes	1 (1.3)	3 (1.5)	4 (1.0)	7 (1.2)
Intrahepatic cholestasis of pregnancy	0 (0.0)	1 (0.5)	2 (0.5)	3 (0.5)

	Women with SGA infant <3rd centile (n=78)	Women with SGA infant <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Fetal				
EFW < 10th centile †	44 (57.9)	88 (47.1)	64 (16.3)	152 (25.9)
Oligohydramnios (AFI < 5 cm)	2 (3.6) (n=54)	4 (3.3) (n=118)	1 (0.4) (n=228)	5 (1.4) (n=346)
Absent/ reversed umbilical artery Doppler flow	1 (1.3) (n=76)	1 (0.6) (n=176)	1(0.3) (n= 358)	2 (0.4) (n=534)
Umbilical artery Doppler pulsatility index > 95th centile †	10 (16.1) (n=61)	12 (8.2) (n=147)	14 (4.5) (n=312)	26 (5.7) (n=458)

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 3.3: Characteristics of delivery and maternal and neonatal outcome.

Values given are median (quartiles) or n (%) as appropriate.

	Women with SGA infant <3rd centile (n=78)	**Women with SGA infant <10th centile (n=192)**	**Women with infant ≥10th centile (n=400)**	**All women (n=592)**
Median gestation at delivery (weeks) †	38.7 (37.1 to 40.1)	39.4 (38.0 to 40.4)	40.0 (39.0 to 40.9)	39.9 (38.9 to 40.7)
Maternal medications (at any point during pregnancy)				
Dexamethasone†	5 (6.4)	7 (3.6)	4 (1.0)	11 (1.8)
Betamethasone	2 (2.6)	4 (2.1)	0 (0)	4 (0.7)
Methyldopa	2 (2.6)	2 (1.0)	0 (0)	2 (0.3)
Labetalol †	6 (7.7)	9 (4.7)	2 (0.5)	11 (1.8)
Heparin	1 (1.3)	2 (1.0)	3 (0.8)	5 (0.8)
Nifedipine	1 (1.3)	2 (1.0)	1 (0.3)	3 (0.5)
Aspirin	3 (3.8)	4 (2.1)	8 (2.0)	12 (2.0)
Oral corticosteroids	0 (0)	3 (1.6)	2 (0.5)	5 (0.8)
Onset of labour				
Spontaneous †	24 (30.8)	99 (51.6)	300 (75.0)	399 (67.4)
Induced †	41 (52.6)	67 (34.9)	66 (16.5)	133 (22.5)
Pre-labour caesarean section	13 (16.7)	26 (13.5)	34 (8.5)	60 (10.1)

	Women with SGA infant <3rd centile (n=78)	Women with SGA infant <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Mode of delivery				
Spontaneous	48 (61.5)	125 (65.1)	279 (69.8)	404 (68.2)
Assisted vaginal delivery	8 (10.3)	23 (12.0)	66 (16.5)	89 (15.0)
Caesarean section	22 (28.2)	44 (22.9)	55 (13.8)	99 (16.7)
Adverse maternal outcome*	5 (6.4)	9 (4.7)	10 (2.5)	19 (3.2)
Postpartum haemorrhage	2 (2.6)	5 (2.6)	7 (1.8)	12 (2.0)
Abruption	1 (1.3)	1 (0.5)	1 (0.3)	2 (0.3)
HELLP	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Fetal				
Fetal death	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Neonatal death	0 (0)	0 (0)	0 (0)	0 (0)
Median birth weight †	2375 (2100 to 2610)	2660 (2360 to 2854)	3214 (3000 to 3470)	3050 (2740 to 3329)
Adverse perinatal outcome **	4 (5.1)	6 (3.1)	7 (1.8)	13 (2.2)

HELLP, haemolysis, elevated liver enzymes, low platelets.

* 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 $<50\times10^{9}$/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.

** 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.

† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Table 3.4: Maternal diagnosis according to birth weight and gestation at delivery

	Women with SGA infant <3rd centile (%) (n=78)		Women with SGA infant <10th centile (%) (n=192)		Women with infant ≥10th centile (%) (n=400)		All women (%) (n=592)	
	Delivery <37 weeks (n=14)	Delivery ≥ 37 weeks (n=64)	Delivery <37 weeks (n=19)	Delivery ≥ 37 weeks (n=173)	Delivery <37 weeks (n=16)	Delivery ≥ 37 weeks (n=384)	Delivery <37 weeks (n=35)	Delivery ≥ 37 weeks (n=557)
No maternal disease †	9 (64.2)	59 (92.2)	14 (73.7)	159 (91.9)	13 (81.3)	369 (96.1)	27 (77.1)	528 (94.8)
Gestational hypertension	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (2.1)	0 (0)	8 (1.4)
Chronic hypertension	0 (0)	0 (0)	0 (0)	2 (1.2)	0 (0)	0 (0)	0 (0)	2 (0.4)
Mild PE	0 (0)	0 (0)	0 (0)	0 (0)	2 (12.5)	1 (0.3)	2 (5.7)	1 (0.2)
Severe PE	2 (14.3)	2 (3.1)	2 (10.5)	6 (3.5)	0 (0)	0 (0)	2 (5.7)	6 (1.1)
Superimposed PE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Atypical PE	2 (14.3)	2 (3.1)	2 (10.5)	2 (1.2)	0 (0)	0 (0)	2 (5.7)	2 (0.4)
HELLP syndrome	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.3)	0 (0)	1 (2.9)	0 (0)
Other dx	2 (14.3)	1 (1.6)	1 (5.3)	4 (2.3)	0 (0)	6 (1.6)	3 (6.1)	10 (1.8)[1]

[1] Mild, severe, superimposed and atypical PE are defined in section 2.2.1.
† indicates p value <0.05 for comparison of women with SGA infant <3rd centile (the main outcome) to women with infant ≥ 10th centile

Given the low frequency of maternal complications and adverse perinatal outcome it is unsurprising that average length of antenatal and postnatal admissions were less than one day (Table 3.5). Length of admission (including admission to neonatal unit) was slightly longer in those delivering an SGA infant compared to pregnancies delivering AGA.

Table 3.5: Health service usage according to birth weight centile; all values are given as mean (with 95% confidence intervals).

	Women with SGA infant <3rd centile (n=78)	Women with SGA infant <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Maternal				
Antenatal bed nights after enrolment	1.1 (0.6 to 2.7)	0.6 (0.4 to 1.2)	0.2 (0.1 to 0.2)	0.3 (0.2 to 0.5)
Post-partum bed nights	2.6 (2.1 to 3.1)	2.0 (1.8 to 2.3)	1.4 (1.3 to 1.6)	1.6 (1.5 to1.8)
Neonatal				
No of ultrasound assessments	2.6 (2.3 to 3.0)	2.3 (2.1 to 2.6)	1.5 (1.4 to 1.6)	1.7 (1.6 to 1.9)
Intensive care bed nights	1.1 (0.2 to 4.7)	0.6 (0.2 to 2.0)	0.09 (0.02 to 0.3)	0.3 (0.1 to 0.7)
Special care bed nights	2.7 (1.6 to 5.3)	1.1 (0.5 to 2.3)	0.1 (0.01 to 0.6)	0.4 (0.2 to 0.8)

Test performance statistics for PlGF and ultrasound parameters to determine delivery of an SGA infant <3rd and 10th customised centiles are given in Table 3.6, with estimated fetal weight (EFW) <10th centile having the highest sensitivity and negative predictive power (sensitivity 57.9% and 47.1%; NPV 92.6 and 77.2% for SGA <3rd and SGA <10th centiles) of all parameters assessed. Addition of PlGF to ultrasound parameters currently utilised to identify SGA antenatally, altered test sensitivity from 57.7 to 69.2% (NPV 91.3 to 92.9%) in determining SGA <3rd centile and from 48.7 to 57.4% (NPV 76.6 to 78.3%) in determining SGA <10th centile. ROC curve areas for all parameters in determining SGA <3rd centile are shown in Figure 3.2. EFW <10th centile, with a ROC area of 0.79 (95% CI 0.74 to 0.84), was greater than for low PlGF (0.70; 95% CI 0.63 to 0.77). A combination of parameters increased the ROC area to 0.82 (95% CI 0.77 to 0.86).

In contrast to the findings of the PELICAN-PE study, there were no major differences in the results between women enrolled prior to 35 weeks' gestation and those enrolled between 35+0 and 36+6 weeks' gestation. The results of this study have therefore not been subdivided according to gestation at enrollment.

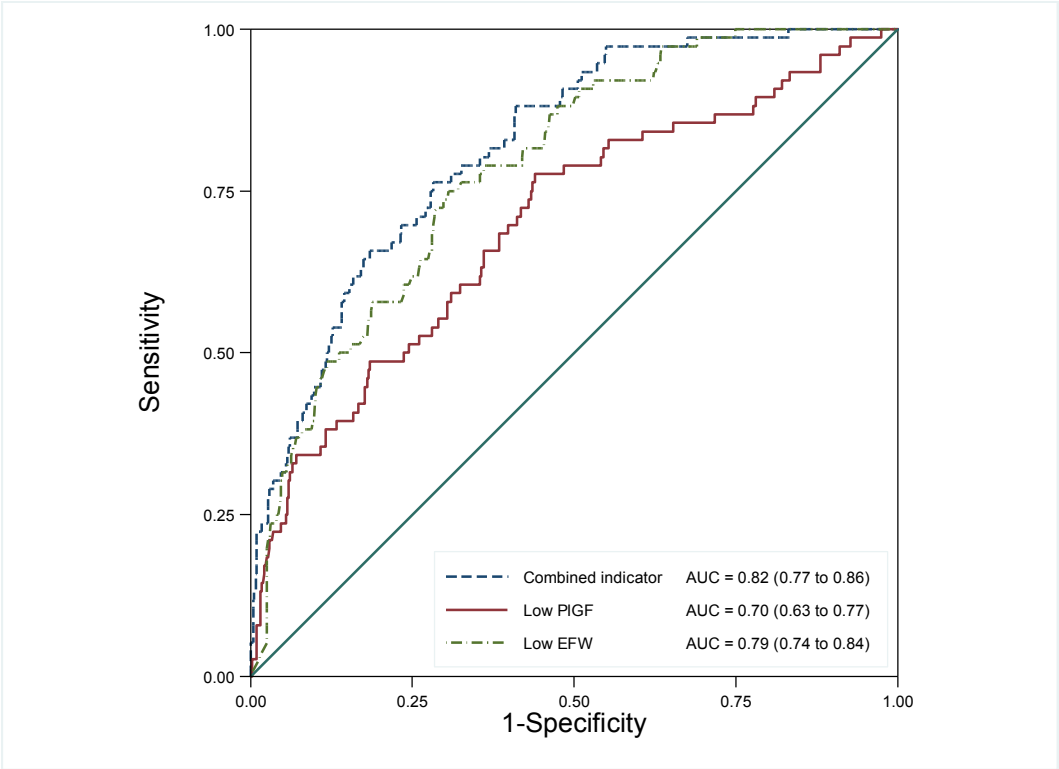
Table 3.6: Test performance statistics for PlGF and all ultrasound parameters (with 95% confidence intervals) to predict SGA <3rd and <10th centiles (n = 592)

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
SGA <3rd centile						
EFW <10th centile	57.9 (46.0 to 69.1) 44/76	78.8 (75.0 to 82.3) 402/510	28.9 (21.9 to 36.8) 44/152	92.6 (89.8 to 94.9) 402/434	2.73 (2.12 to 3.53)	0.53 (0.41 to 0.70)
Oligohydramnios (AFI < 5 cm)	3.7 (0.5 to 12.7) 2/54	99.0 (97.0 to 99.8) 289/292	40.0 (5.3 to 85.3) 2/5	84.8 (80.5 to 88.4) 289/341	3.60 (0.62 to 21.1)	0.97 (0.92 to 1.03)
Umbilical artery Doppler PI >95th centile	16.4 (8.2 to 28.1) 10/61	96.0 (93.5 to 97.7) 381/395	38.5 (20.2 to 59.4) 10/26	88.2 (84.8 to 91.1) 381/432	4.07 (1.94 to 8.55)	0.87 (0.78 to 0.98)
PlGF < 5th centile	37.2 (26.5 to 48.9) 29/78	88.7 (85.7 to 91.3) 456/514	33.3 (23.6 to 44.3) 29/87	90.3 (87.4 to 92.7) 456/505	3.29 (2.26 to 4.80)	0.71 (0.60 to 0.84)
Combinations						
Abnormal AFI or EFW	57.7 (43.2 to 71.3) 30/52	79.0 (73.9 to 83.6) 230/291	33.0 (23.5 to 43.6) 30/91	91.3 (87.1 to 94.4) 230/252	2.75 (1.99 to 3.80)	0.54 (0.39 to 0.74)
Abnormal PlGF or AFI or EFW	69.2 (54.9 to 81.3) 36/52	72.2 (66.6 to 77.2) 210/291	30.8 (22.6 to 40.0) 36/117	92.9 (88.8 to 95.9) 210/226	2.49 (1.92 to 3.22)	0.43 (0.28 to 0.64)

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
SGA <10th centile						
EFW <10th centile	47.1 (39.7 to 54.5) 88/187	84.0 (80.0 to 87.4) 335/399	57.9 (49.6 to 65.8) 88/152	77.2 (72.9 to 81.1) 335/434	2.93 (2.24 to 3.85)	0.63 (0.55 to 0.73)
Oligohydramnios (AFI < 5 cm)	3.4 (0.9 to 8.5) 4/118	99.6 (97.6 to 100.0) 227/228	80.0 (28.4 to 99.5) 4/5	66.6 (61.3 to 71.6) 227/341	7.73 (0.87 to 68.4)	0.97 (0.94 to 1.00)
Umbilical artery Doppler PI >95th centile	8.2 (4.3 to 13.8) 12/147	95.5 (92.6 to 97.5) 297/311	46.2 (26.6 to 66.6) 12/26	68.8 (64.1 to 73.1) 297/432	1.81 (0.86 to 3.82)	0.96 (0.91 to 1.01)
PlGF < 5th centile	24.5 (18.6 to 31.2) 47/192	90.0 (86.6 to 92.8) 360/400	54.0 (43.0 to 64.8) 47/87	71.3 (67.1 to 75.2) 360/505	2.45 (1.67 to 3.60)	0.84 (0.77 to 0.92)
Combinations						
Abnormal AFI or EFW	48.7 (39.3 to 58.2) 56/115	84.6 (79.3 to 89.1) 193/228	61.5 (50.8 to 71.6) 56/91	76.6 (70.9 to 81.7) 193/252	3.17 (2.22 to 4.54)	0.61 (0.50 to 0.73)
Abnormal PlGF or AFI or EFW	57.4 (47.8 to 66.6) 66/115	77.6 (71.7 to 82.9) 177/228	56.4 (46.9 to 65.6) 66/117	78.3 (72.4 to 83.5) 177/226	2.57 (1.92 to 3.42)	0.55 (0.44 to 0.69)

SGA, small for gestational age; EFW, estimated fetal weight; AFI, amniotic fluid index; PI, pulsatility index; PlGF, placental growth factor.

Figure 3.2: ROC areas for low PlGF, EFW <10th centile and combination of these parameters to predict delivery of an SGA infant <3rd birth weight centile in all women sampled (n=592)



To explore whether these predictive parameters improved if the interval between gestation at sampling to delivery was restricted, a subgroup analysis of 267 women where delivery occurred within six weeks of PlGF sampling (Table 3.7) was undertaken. ROC areas were 0.74 (95% CI 0.66 to 0.83), 0.76 (95% CI 0.69 to 0.84) and 0.81 (95% CI 0.72 to 0.88) for PlGF, low EFW, and a combination of both parameters respectively to predict delivery of an SGA infant with birth weight <3rd customised centile (Figure 3.3). These results were similar to those of the whole study population (Figure 3.2) suggesting that restricting sampling to delivery time had minimal effect in this study cohort.

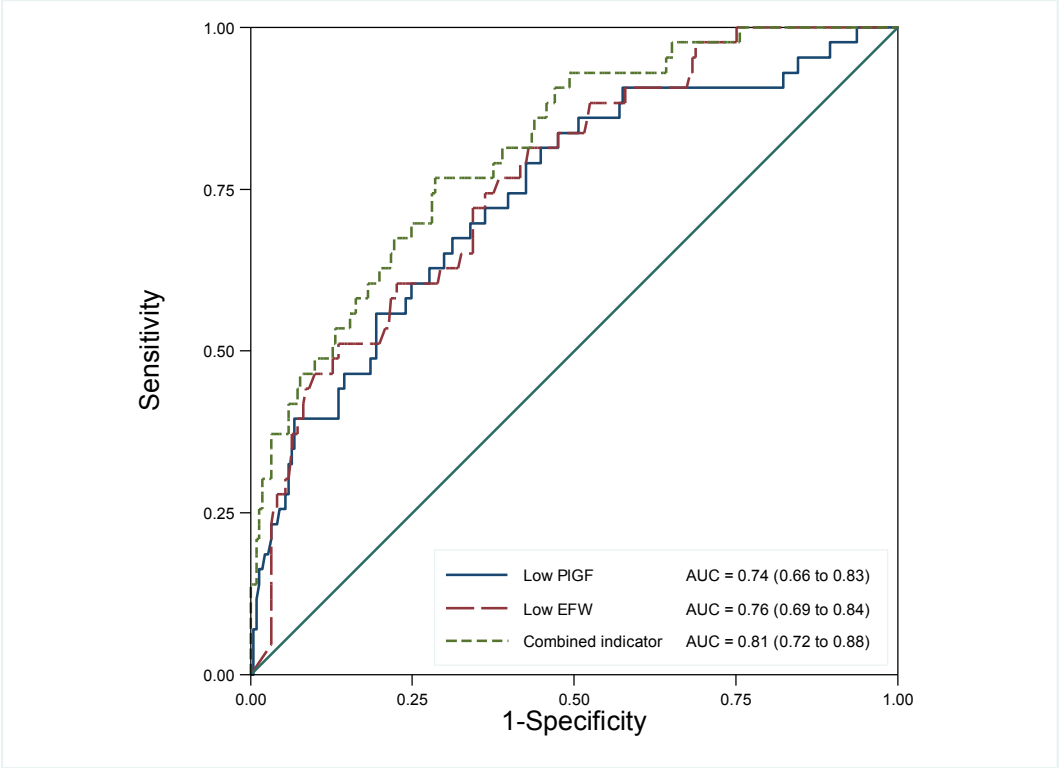
Table 3.7: Test performance statistics for PlGF and ultrasound parameters (with 95 % confidence intervals) to predict SGA <3rd and 10th centiles when PlGF sampled within six weeks of delivery (n = 267)

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
SGA<3rd centile						
EFW <10th centile	62.2 (46.5 to 76.2) 28/45	73.0 (66.6 to 78.7) 162/221	31.8 (22.3 to 42.6) 28/88	90.5 (85.2 to 94.4) 162/179	2.30 (1.68 to 3.15)	0.52 (0.35 to 0.76)
Oligohydramnios (AFI < 5 cm)	5.9 (0.7 to 19.7) 2/34	97.7 (93.4 to 99.5) 126/129	40.0 (5.3 to 85.3) 2/5	79.7 (72.6 to 85.7) 126/158	2.53 (0.44 to 14.54)	0.96 (0.88 to 1.05)
Umbilical artery Doppler PI >95th centile	22.2 (10.1 to 39.2) 8/36	96.0 (91.9 to 98.4) 167/174	53.3 (26.6 to 78.7) 8/15	85.6 (79.9 to 90.2) 167/195	5.52 (2.14 to 14.27)	0.81 (0.68 to 0.97)
PlGF < 5th centile	42.2 (27.7 to 57.8) 19/45	86.6 (81.4 to 90.8) 194/224	38.8 (25.2 to 53.8) 19/49	88.2 (83.2 to 92.1) 194/220	3.15 (1.96 to 5.08)	0.67 (0.52 to 0.86)
Combinations						
Abnormal AFI or EFW	62.5 (43.7 to 78.9) 20/32	67.2 (58.3 to 75.2) 86/128	32.3 (20.9 to 45.3) 20/62	87.8 (79.6 to 93.5) 86/98	1.90 (1.32 to 2.74)	0.56 (0.35 to 0.89)
Abnormal PlGF or AFI or EFW	70.0 (50.6 to 85.3) 21/30	58.3 (49.2 to 67.0) 74/127	28.4 (18.5 to 40.1) 21/74	89.2 (80.4 to 94.9) 74/83	1.68 (1.23 to 2.29)	0.51 (0.29 to 0.91)

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
SGA <10th centile						
EFW <10th centile	57.6 (46.9 to 67.9) 53/92	80.0 (73.3 to 85.7) 140/175	60.2 (49.2 to 70.5) 53/88	78.2 (71.4 to 84.0) 140/179	2.88 (2.04 to 4.06)	0.53 (0.41 to 0.68)
Oligohydramnios (AFI < 5 cm)	6.3 (1.7 to 15.2) 4/64	99.0 (94.5 to 100) 98/99	80.0 (28.4 to 99.5) 4/5	62.0 (54.0 to 69.6) 98/158	6.19 (0.71 to 54.12)	0.95 (0.89 to 1.01)
Umbilical artery Doppler PI >95th centile	12.2 (5.7 to 21.8) 9/74	95.6 (90.6 to 98.4) 130/136	60.0 (32.3 to 83.7) 9/15	66.7 (59.6 to 73.2) 130/195	2.76 (1.02 to 7.44)	0.92 (0.84 to 1.01)
PlGF < 5th centile	27.4 (18.7 to 37.5) 26/95	86.8 (80.8 to 91.4) 151/174	53.1 (38.3 to 67.5) 26/49	68.6 (62.1 to 74.7) 151/220	2.07 (1.25 to 3.42)	0.84 (0.73 to 0.96)
Combinations						
Abnormal AFI or EFW	62.3 (49.0 to 74.4) 38/61	75.8 (66.1 to 83.8) 75/99	61.3 (48.1 to 73.4) 38/62	76.5 (66.9 to 84.5) 75/98	2.57 (1.72 to 3.83)	0.50 (0.35 to 0.70)
Abnormal PlGF or AFI or EFW	67.8 (54.4 to 79.4) 40/59	65.3 (55.0 to 74.6) 64/98	54.1 (42.1 to 65.7) 40/74	77.1 (66.6 to 85.6) 64/83	1.95 (1.41 to 2.70)	0.49 (0.33 to 0.73)

SGA, small for gestational age; EFW, estimated fetal weight; AFI, amniotic fluid index; PlGF, placental growth factor.

Figure 3.3: ROC areas for low PlGF, EFW <10th centile and combination of these parameters to predict delivery of an SGA infant <3rd birth weight centile in women sampled within 6 weeks of delivery (n=267)



The test performance of PlGF to predict pre-eclampsia and SGA in women with suspected pre-eclampsia has previously been shown to be high when measured prior to 35 weeks' gestation and performance diminishes at gestations beyond this. The test performance of PlGF and ultrasound parameters in women sampled prior to 35 weeks' gestation were therefore calculated to evaluate whether restricting the timing of PlGF sampling improved the markers ability to determine delivery of an SGA infant. These results are summarised in Table 3.8, with EFW <10th centile having the highest sensitivity (55.6%) and NPV (93.1%) for delivery of an SGA infant <3rd birth weight centile. Whilst test sensitivity for PlGF to determine delivery of an SGA infant was lower than test performance of EFW <10th centile, it is marginally higher than when sampling is undertaken up to 37 weeks' gestation.

Table 3.8: Test performance statistics for PlGF and ultrasound parameters (with 95 % confidence intervals) to predict SGA <3rd and <10th centiles when PlGF sampled before 35 weeks' gestation (n = 388)

Biomarker/ clinical indicator SGA<3rd centile	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
EFW <10th centile	55.6 (40.0 to 70.4) 25/45	79.7 (75.0 to 83.9) 271/340	26.6 (18.0 to 36.7) 25/94	93.1 (89.6 to 95.8) 271/291	2.74 (1.96 to 3.83)	0.56 (0.40 to 0.78)
Umbilical artery Doppler PI >95th centile	16.7 (6.4 to 32.8) 6/36	96.6 (93.6 to 98.4) 252/261	40.0 (16.3 to 67.7) 6/15	89.4 (85.2 to 92.7) 252/282	4.83 (1.83 to 12.78)	0.86 (0.74 to 1.00)
PlGF < 5th centile	39.1 (25.1 to 54.6) 18/46	89.2 (85.4 to 92.3) 305/342	32.7 (20.7 to 46.7) 18/55	91.6 (88.1 to 94.3) 305/333	3.62 (2.26 to 5.80)	0.68 (0.54 to 0.86)
Combinations						
Abnormal AFI or EFW	56.3 (37.7 to 73.6) 18/32	82.8 (76.8 to 87.8) 164/198	34.6 (22.0 to 49.1) 18/52	92.1 (87.2 to 95.6) 164/178	3.28 (2.13 to 5.05)	0.53 (0.35 to 0.79)
Abnormal PlGF or AFI or EFW	71.9 (53.3 to 86.3) 23/32	77.3 (70.8 to 82.9) 153/198	33.8 (22.8 to 46.3) 23/68	94.4 (89.7 to 97.4) 153/162	3.16 (2.26 to 4.43)	0.36 (0.21 to 0.64)

Biomarker/ clinical indicator **SGA <10th centile**	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
EFW <10th centile	44.1 (34.9 to 53.5) 52/118	84.3 (79.3 to 88.4) 225/267	55.3 (44.7 to 65.6) 52/94	77.3 (72.1 to 82.0) 225/291	2.80 (1.99 to 3.95)	0.66 (0.56 to 0.79)
Umbilical artery Doppler PI >95th centile	7.9 (3.2 to 15.5) 7/89	96.2 (92.6 to 98.3) 200/208	46.7 (21.3 to 73.4) 7/15	70.9 (65.2 to 76.2) 200/282	2.04 (0.76 to 5.47)	0.96 (0.90 to 1.02)
PlGF < 5th centile	25.0 (17.5 to 33.7) 30/120	90.7 (86.5 to 93.9) 243/268	54.5 (40.6 to 68.0) 30/55	73.0 (67.9 to 77.7) 243/333	2.68 (1.65 to 4.35)	0.83 (0.74 to 0.92)
Combinations						
Abnormal AFI or EFW	43.2 (31.8 to 55.3) 32/74	7.2 (80.9 to 92.0) 136/156	61.5 (47.0 to 74.7) 32/52	76.4 (69.5 to 82.4) 136/178	3.37 (2.08 to 5.48)	0.65 (0.53 to 0.80)
Abnormal PlGF or AFI or EFW	54.1 (42.1 to 65.7) 40/74	82.1 (75.1 to 87.7) 128/156	58.8 (46.2 to 70.6) 40/68	79.0 (71.9 to 85.0) 128/162	3.01 (2.03 to 4.47)	0.56 (0.43 to 0.72)

Only one case of oligohydramnios in women sampled before 35 weeks' gestation therefore test accuracy could not be assessed

Only 13 women had an adverse perinatal outcome which included one stillbirth, four cases of respiratory distress syndrome and nine cases of admission to the neonatal intensive care unit for greater than 48 hours at term. The ability of PlGF and ultrasound parameters to determine adverse perinatal outcome was assessed, with results displayed in Table 3.9. All parameters performed poorly in determining this endpoint with EFW <10th centile having the highest sensitivity (23.1%) and negative predictive value (97.7%). When a combination of abnormal EFW, AFI and PlGF was used to predict adverse perinatal outcome, sensitivity and NPV did improve to 66.7% and 99.1% respectively.

Table 3.9: Test performance statistics for PlGF and ultrasound parameters (with 95 % confidence intervals) to predict adverse perinatal outcome

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N
EFW <10th centile	23.1 (5.0 to 53.8) 3/13	74.0 (70.2 to 77.5) 424/573	2.0 (0.4 to 5.7) 3/152	97.7 (95.8 to 98.9) 424/434
Oligohydramnios (AFI < 5 cm)	16.7 (0.4 to 64.1) 1/6	98.8 (97.0 to 99.7) 336/340	20.0 (0.5 to 71.6) 1/5	98.5 (96.6% to 99.5) 336/341
Umbilical artery Doppler PI >95th centile	0 (0 to 28.5) 0/11	94.2 (91.6 to 96.2) 421/447	0 (0 to 13.2) 0/26	97.5 (95.5 to 98.7) 421/432
PlGF < 5th centile	15.4 (1.9 to 45.4) 2/13	85.3 (82.2 to 88.1) 494/579	2.3 (0.3 to 8.1) 2/87	97.8 (96.1 to 98.9) 494/505
Combinations				
Abnormal AFI or EFW	50.0 (11.8 to 88.2) 3/6	73.9 (68.9 to 78.5) 249/337	3.3 (0.7 to 9.3) 3/91	98.8 (96.6 to 99.8) 249/252
Abnormal PlGF or AFI or EFW	66.7 (22.3 to 95.7) 4/6	66.5 (61.2 to 71.5) 224/337	3.4 (0.9 to 8.5) 4/117	99.1 (96.8 to 99.9) 224/226

Very low concentrations of PlGF (<12 pg/ml, lower level of assay detection) have previously been correlated with poor outcome (Chappell et al., 2013). 16 women in the study population had very low PlGF at enrolment and details of their pregnancies are given in Table 3.10. Of these women, 11 delivered an SGA infant and seven had hypertensive disorders (7/16; 50% versus 17/577; 3% in the rest of the cohort).

The test performance of PlGF in a subgroup of 152 women who had EFW <10th centile at study enrolment was also assessed (Table 3.11). Sensitivity was similar in this subgroup (40.9% to determine SGA <3rd birth weight centile) to that displayed in Table 3.6, for the whole study population (37.2%) but NPV was lower in the subgroup (77.0% versus 90.3%).

Table 3.10: Maternal and neonatal outcomes for women with PlGF <12 pg/ml. There were no fetal or neonatal complications in this group.

Subject ID	Gestation at sampling	Gestation at delivery	Birth weight (g)	Customised birth weight centile	Maternal complications
A	30+0	33+6	1935	10.1	HELLP Syndrome
B	31+4	33+3	1305	0	Severe pre-eclampsia
C	31+4	35+4	1825	1.2	Gestational diabetes mellitus
D	34+2	39+3	2530	2.7	Atypical pre-eclampsia
E	35+2	37+0	2225	0.6	Severe pre-eclampsia
F	35+5	36+6	2905	38.5	None
G	35+5	38+0	2330	2.6	None
H	35+6	38+0	2260	9.5	Severe pre-eclampsia
I	36+0	36+4	2525	38.4	Mild pre-eclampsia
J	36+0	37+6	1958	0.1	None
K	36+1	39+0	2765	8.8	None
L	36+3	38+3	2600	17.4	None
M	36+3	41+1	2710	1.3	None
N	36+4	37+1	2000	0.3	None
O	36+4	37+3	2398	6.0	Chronic hypertension
P	36+6	40+2	3720	63.2	Gestational hypertension

HELLP, haemolysis, elevated liver enzymes, low platelets.

Table 3.11: Test performance statistics for PlGF (with 95 % confidence intervals) to predict SGA <3rd and <10th centiles in women who had an EFW <10th centile at enrolment (n=152)

PlGF	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N
SGA <3rd centile	40.9 (26.3 to 56.8) 18/44	80.6 (71.8 to 87.5) 87/108	46.2 (30.1 to 62.8) 18/39	77.0 (68.1 to 84.4) 87/113
SGA <10th centile	29.5 (20.3 to 40.2) 26/88	79.7 (67.8 to 88.7) 51/64	66.7 (49.8 to 80.9) 26/39	45.1 (35.8 to 54.8) 51/113

In addition to assessing the ability of PlGF to determine delivery of an SGA infant, the possibility of identifying a high cut-off for PlGF measurement was explored, which would provide a threshold for use of PlGF as a “rule out” test. Table 3.12 shows the distribution of cases within the cohort according to PlGF centile and subdivides according to birth weight above or below the 3rd centile. There was no PlGF cut off that provided adequate performance to rule out delivery of an SGA infant.

Table 3.12: Distribution of cases across PlGF centiles.

(Numbers and row percentages are shown).

PlGF centile	Birth weight ≥3rd centile n (%)	Birth weight <3rd centile n (%)	Total n
<10th	93 (72.1)	36 (27.9)	129
10th-20th	65 (89.0)	8 (11.0)	73
20th-30th	48 (82.8)	10 (17.2)	58
30th-40th	44 (86.3)	7 (13.7)	51
40th-50th	32 (91.4)	3 (8.6)	35
50th-60th	39 (95.1)	2 (4.9)	41
60th-70th	42 (97.7)	1 (2.3)	43
70th-80th	49 (94.2)	3 (5.8)	52
80th-90th	55 (91.7)	5 (8.3)	60
90th-100th	47 (94.0)	3 (6.0)	50
Total	514 (86.8)	78 (13.8)	592

3.4 Summary

Within the UK, current clinical practice relies on measurement of ultrasound parameters to identify pregnancies at risk of delivering an SGA infant. In this prospective, multicentre cohort study, recruiting women with reduced symphysis-fundal height (current UK referral tool to identify women at risk of SGA), these parameters had modest test performance for predicting delivery of an SGA infant. EFW <10th centile had the highest sensitivity and negative predictive value to predict delivery of an SGA infant <3rd birth weight centile (57.9% and 92.6% respectively). Quantification of maternal plasma PlGF in this cohort performed no better than ultrasound parameters (sensitivity 37.2% and negative predictive value 90.3% for SGA <3rd centile). This is in contrast to the findings of the PELICAN-PE study, where low plasma PlGF had high sensitivity (89.6%; 95% CI 81.7 to 94.9%) and negative predictive value (91.3%; 95% CI 84.6 to 95.8%) to determine delivery of an SGA infant (birth weight <3rd customised centile).

Whilst I have focused on the ability of ultrasound parameters and PlGF to predict delivery of an SGA infant I also investigated their ability to predict adverse perinatal outcome. However, only 13 pregnancies in the whole cohort experienced an adverse perinatal outcome, making firm conclusions regarding the predictive capability of PlGF and ultrasound parameters for this endpoint impossible. The single stillbirth reported in PELICAN FGR was not SGA and had a normal PlGF concentration at enrolment.

I discuss possible explanations for differences observed between the PELICAN-PE and PELICAN-FGR studies and implications of the study findings in Chapter 4: Discussion.

Chapter 4: Discussion

4.1 Principal findings

4.1.1 PELICAN-PE study findings

In this work, I have demonstrated that the angiogenic factor, PlGF accurately identifies pregnancies delivering an SGA infant in a high-risk cohort of women presenting in the second half of pregnancy with suspected pre-eclampsia (sensitivity 93.2%, negative predictive value 89.7%, ROC area 0.83 for SGA <3rd birth weight centile in women enrolled prior to 35 weeks' gestation).

4.1.1.1 Comparison of PlGF test performance to other individual biomarkers

In women sampled before 35 weeks' gestation, PlGF outperformed all other individual biomarkers assessed, with high-test performance to predict delivery of an SGA infant. However, test performance statistics for PlGF in women enrolled beyond 35 weeks' gestation (ROC area 0.69 (95% CI 0.57 to 0.81) for SGA <3rd birth weight centile) were less impressive than those reported in women recruited prior to 35 weeks' gestation. A possible explanation for this finding is the convergence of PlGF concentrations between normal and pathological pregnancies with advancing gestation.

Of all 47 biomarkers assessed, the three markers with highest individual test performance to predict delivery of an SGA infant (PlGF, Endoglin and sFlt-1) were all angiogenic factors. Given that they are all involved in the same pathological process, this may explain why addition of Endoglin or sFlt-1 to PlGF did not improve overall test performance. The finding that the best

performing biomarkers for SGA in suspected pre-eclampsia are involved in the regulation of angiogenesis adds support to placental insufficiency being key to pathogenesis of FGR in these women.

4.1.1.2 Test performance of individual versus combinations of biomarkers

Due to the complex underlying pathophysiology of FGR, involving multiple pathological processes, I hypothesised that a panel of biomarkers reflecting differing pathways might improve test performance over individual biomarkers to predict delivery of an SGA infant.

From the analysis of women enrolled to the PELICAN-PE study prior to 35 weeks' gestation, the best performing combination of biomarkers, derived from factor analysis and multiple logistic regression included PlGF, nephrin and CPA-4 (ROC area of 0.84 for SGA <3rd birth weight centile). Nephrin is a protein essential for normal renal glomerular function and broadly reflects endothelial function, whilst CPA-4 is a metalloprotease, which cleaves angiotensin, a major component of the renin-angiotensin system, and essential for blood pressure homeostasis. Despite their diverse pathophysiology, addition of nephrin and CPA-4 to PlGF had little affect on overall test performance to determine SGA <3rd birth weight centile over that derived from PlGF measured in isolation. The high individual test performance of PlGF to determine SGA <3rd birth weight centile may explain why addition of other biomarkers (with modest individual test performance (ROC areas of 0.63 for SGA <3rd birth weight centile)) did not aid identification of the few cases undetected by PlGF alone.

In women sampled beyond 35 weeks' gestation, isolated biomarkers performed less well to determine delivery of an SGA infant, possibly due to other processes in addition to placental disease contributing to delivery of an SGA infant near term. A combination of biomarkers reflecting other pathological processes may therefore be more useful in this group. The same combination of PlGF, nephrin and CPA-4 achieved the best test performance in determining subsequent delivery of an SGA infant in women sampled beyond 35 weeks' gestation, but this combination achieved only modest improvements in test performance over PlGF alone (ROC area for combination of PlGF, Nephrin, CPA-4 0.77 (95% CI 0.66 to 0.88) for delivery of an SGA infant <3rd birth weight centile).

Using single, rather than multiple biomarkers, has significant advantages when translating this work to a clinical setting. It is likely to be more cost effective and will reduce the overall error associated with using multiple assays. It will also facilitate future comparisons between datasets as reproducibility is likely to be easier.

4.1.1.3 Comparison of PlGF test performance with ultrasound parameters to determine delivery of an SGA infant and predict adverse perinatal outcome in women presenting with suspected pre-eclampsia

After identifying PlGF as the most promising biomarker for prediction of delivering an SGA infant in these high-risk women, I compared its test performance to currently utilised ultrasound parameters. I have shown that PlGF outperformed all ultrasound parameters in predicting subsequent delivery of an SGA infant in women presenting with suspected pre-eclampsia. PlGF also

had high sensitivity and negative predictive value to detect adverse perinatal outcome, which included stillbirth. A systematic review and meta-analysis of risk factors for stillbirth in high-income countries reported SGA as having the highest population attributable risk (23%) for this devastating outcome (Flenady et al., 2011a). There were six cases of stillbirth in this cohort, with five cases delivering an infant with birth weight <3rd centile. Only three of these cases were detected antenatally by ultrasound scan but all had a low PlGF at study enrolment. This supports the hypothesis that PlGF measurement would aid identification of such cases early enough to allow appropriate surveillance and timely delivery, with the aim of avoiding this major adverse outcome.

The high sensitivity of PlGF in this population is only slightly increased by addition of ultrasound parameters. However, ultrasound may give additional information that may aid decisions regarding timing of delivery, such as absent or reversed umbilical artery end-diastolic flow as PlGF is not yet validated as a predictor of stillbirth. Therefore, in women presenting with high risk features for pre-eclampsia, I propose combining PlGF and ultrasound data to generate a test that has very high negative predictive value. Addition of PlGF to current ultrasound parameters has the potential to increase detection of SGA in high-risk women, allowing appropriate follow up and targeted intervention with the aim of reducing adverse perinatal outcomes.

4.1.2 PELICAN-FGR study findings

While PlGF has been shown to work well in high-risk women, it would be very advantageous for a biomarker to predict SGA in a more general antenatal population. I therefore investigated use of PlGF in women with reduced symphysis-fundal height measurement, a referral trigger for suspected SGA in the UK. I compared the test performance of PlGF to predict subsequent delivery of an SGA infant to the performance of ultrasound parameters used in current clinical practice.

4.1.2.1 Comparison of PlGF test performance with ultrasound parameters to determine delivery of an SGA infant and predict adverse perinatal outcome

In this work I demonstrated that ultrasound parameters used in current clinical practice to identify pregnancies at risk of delivering an SGA infant had modest test performance. EFW <10th centile had the best test sensitivity of all ultrasound parameters assessed but only identified 58% of cases antenatally who delivered an infant with a birth weight <3rd centile (sensitivity 57.9% and NPV 92.6%). This finding is similar to results of a systematic review and subsequent retrospective cohort study which both assessed the predictive capability of EFW <10th centile to determine delivery of an SGA infant in high risk populations (including women with suspected SGA), and published sensitivities of 33-89%, 68% and specificities of 54-91%, 89% respectively (Chang et al., 1992, De Jong et al., 2000). Sensitivity to detect SGA in unselected populations have been reported as lower than those seen in the PELICAN-FGR study (21-47%) (David et al., 1996, Ben-Haroush et al., 2007,

Souka et al., 2012). In contrast to the findings in the high-risk PELICAN-PE cohort, PlGF quantification performed no better than ultrasound parameters in predicting delivery of an SGA infant in this population (sensitivity 37.2% and NPV 90.3% for SGA <3rd centile).

Whilst EFW <10th centile had the highest sensitivity and negative predictive values of all ultrasound parameters evaluated, oligohydramnios and umbilical artery Doppler PI >95th centile had high specificity for SGA (99% and 96% respectively for SGA <3rd birth weight centile). This corroborates the findings of a recent meta-analysis and systematic review evaluating the association of amniotic fluid index and adverse perinatal outcome, where there was a strong association between oligohydramnios and delivery of an SGA infant and mortality (Morris et al., 2014). However the predictive accuracy for perinatal outcome was poor. The very low sensitivities for oligohydramnios and umbilical artery Doppler >95th centile to predict delivery of an SGA infant <3rd birth weight centile (3.7% and 16.4% respectively) in the PELICAN-FGR cohort, limit their use in clinical practice as isolated predictors of SGA but their high specificity for identifying pregnancies delivering an SGA infant, makes them useful indicators for timing of delivery, as discussed in section 4.1.1.3.

In addition to evaluating the ability of ultrasound parameters and PlGF to predict delivery of an SGA infant, I also investigated their ability to predict adverse perinatal outcome. Previously, three Cochrane systematic reviews evaluating symphysis-fundal height measurement (Neilson, 2000), routine ultrasound measurement (Bricker et al., 2008) and umbilical artery doppler assessment in normal pregnancies (Alfirevic et al., 2010) concluded that none of these

techniques reduced adverse perinatal outcome. However preliminary data from a recent large prospective cohort study reported increased sensitivity of screening (79%) vs. selective (32%) sonography in the third trimester in an unselected nulliparous population for prediction of severe SGA (Sovio et al., 2014).

The PELICAN-FGR study was powered for the primary endpoint of delivering an SGA infant, assuming a rate of SGA <3rd birth weight centile of 8% in women referred with suspected SGA (based on data from St Thomas' Hospital, London). The incidence of SGA <3rd centile in the study cohort was higher than this at 13% but the low incidence of adverse perinatal outcome (a secondary outcome) in the study cohort (with only 13 cases) made conclusions regarding the ability of any of the parameters to determine adverse perinatal outcome impossible. This is in contrast to the PELICAN-PE study where 19% of the population had an adverse perinatal outcome, including nine cases of stillbirth/neonatal death. The single stillbirth reported in PELICAN-FGR was not SGA and had a normal PlGF concentration at enrolment. It is therefore unlikely that placental disease was the underlying pathological process in this case.

4.2 Comparison of PELICAN-PE and PELICAN-FGR studies

The PELICAN-PE study reported high-test performance statistics for the ability of low concentrations of maternal plasma PlGF to predict delivery of an SGA infant in women presenting with suspected pre-eclampsia (Chappell et al.,

2013). A previous study had reported similar test performance statistics for low plasma PlGF concentrations in women delivering an SGA infant with associated placental pathology (Benton et al., 2012). In both these studies placental dysfunction is the likely major underlying pathological process. PlGF is an angiogenic factor primarily released by trophoblast cells and concentrations reflect placental function. Therefore if placental dysfunction is the predominant pathological process contributing to delivery of an SGA infant, concentrations would be expected to closely correlate with presence and severity of SGA, as was observed in these studies.

In the PELICAN-FGR study, I sought to investigate whether the findings of these two studies could be applied to a lower risk population presenting with reduced symphysis-fundal height measurement. Whilst acknowledging that other pathological processes maybe involved in subsequent delivery of an SGA infant in this population, I hypothesised that placental dysfunction was still likely to be the major pathological process resulting in SGA. Therefore, low plasma PlGF could still be a useful screening tool to identify pregnancies at risk of delivering an SGA infant in this cohort. However, the findings of the PELICAN-FGR study did not support this hypothesis, with PlGF performing no better than ultrasound parameters utilised in current clinical practice to identify pregnancies at risk of delivering an SGA infant. Here I will discuss possible explanations for the contrasting findings of the PELICAN-PE and PELICAN-FGR studies.

The primary aim of the PELICAN-PE study was to assess the diagnostic accuracy of PlGF to determine need for delivery for confirmed pre-eclampsia in women presenting with suspected pre-eclampsia. As a secondary outcome, the

ability of PlGF to predict delivery of an SGA infant with birth weight <3rd customised birth weight centile was assessed. The women enrolled in this study were high-risk, with 61% of those recruited prior to 35 weeks' gestation developing pre-eclampsia (Chappell et al., 2013). This is in contrast to the low frequency of coexisting maternal complications of pregnancy in the PELICAN-FGR study (555/592, 93% had no coexisting maternal complications of pregnancy), where only 4% were diagnosed with a hypertensive disorder. I have searched the literature for similar cohorts to that described in these studies and data for comparison is sparse. Anumba and colleagues reported an incidence of pre-eclampsia of 26% in women presenting to an obstetric day assessment unit with gestational hypertension (diastolic >90mmHg in community) (Anumba et al., 2010). This study excluded women presenting with coexisting FGR, diabetes mellitus and severe hypertension, which may give some explanation for the lower incidence than that reported in the PELICAN-PE study. The PELICAN co-investigator group anticipated that not all women enrolled with suspected pre-eclampsia would develop the disease, in line with the findings of Anumba et al.

It has been suggested that different pathological processes may occur in the placentas of women with hypertensive disorders, especially if early onset compared to those who remain normotensive but deliver an SGA infant (Redline, 2008). Redline has described five chronic patterns of placental injury in placentas from pregnancies complicated by growth restriction, including; maternal vascular obstruction, fetal vascular obstruction, villitis of unknown aetiology, perivillous fibrin deposition and chronic abruption. He suggests that

maternal vascular disorders are more common in those pregnancies complicated by hypertensive disorders, whilst villitis of unknown aetiology is more common in those who remain normotensive but deliver an SGA infant. It is possible that concentrations of individual markers may vary in differing pathological processes therefore offering some explanation why the ability of PlGF to distinguish SGA in those with associated hypertensive disorders and in those who remain normotensive but deliver an SGA infant is not comparable.

Whilst SGA was defined as birth weight less <3rd customised centile in both studies, in PELICAN-FGR this encompassed a more heterogeneous population whose underlying pathology was not necessarily restricted to placental disease. It is possible that the contribution of placental dysfunction in this study population was less than I anticipated, offering an explanation as to why PlGF, a marker of placental function, may not be as accurate a predictor of delivering an SGA infant in a population whose pathology is not limited to placental disease.

Gestational age at sampling differed by three weeks between the two studies (average gestational age at sampling in those women delivering an SGA infant with birth weight <3rd centile was 31 weeks' gestation in the PELICAN-PE study compared to 34 weeks' gestation in PELICAN-FGR). In women delivering an infant with birth weight <3rd customised centile, gestational age at delivery was also markedly different in the two studies (33.8 weeks in the PELICAN-PE study (Table 2.6) and 38.7 weeks in the PELICAN-FGR study (Table 3.3). PlGF concentration peaks at approximately 32 weeks' gestation and then declines towards term (Knudsen et al., 2012). The clinical application of PlGF appears

less efficacious if sampling occurs later in the third trimester and if women deliver closer to term, likely secondary to the convergence of values between normal and pathological pregnancies with advancing gestation (Knudsen et al., 2012).

4.3 Strengths and limitations of the studies

The PELICAN-PE and PELICAN-FGR studies are the largest reported prospective multi-centre studies evaluating the ability of PlGF, measured in the third trimester, to predict delivery of an SGA infant in women presenting with suspected pre-eclampsia or reduced symphysis-fundal height respectively. The PELICAN-PE study evaluated a large panel of biomarkers chosen for their biological relevance to placental disease and pre-eclampsia and no previous studies have compared such a diverse panel of biomarkers to predict subsequent delivery of an SGA infant.

Both studies were large multicentre, multinational prospective cohort studies enrolling women from diverse geographical and ethnic backgrounds, aiding generalisabilty of the findings. The entry criteria for both studies were chosen due to their clinical relevance, being common referral triggers for obstetric assessment within the United Kingdom. However, I acknowledge that in other healthcare settings symphysis-fundal height measurement is not part of routine antenatal care. In such settings, third trimester ultrasound assessment is offered in all pregnancies and therefore the findings of the PELICAN-FGR study may be less applicable.

The studies took a pragmatic approach, in order to evaluate the real life applications of these tests, and recruited women who were referred for obstetric assessment with either a broad range of signs or symptoms of suspected pre-eclampsia or suspected SGA on symphysis-fundal height measurement and included women with underlying medical conditions. This approach was chosen in an attempt to more closely reflect the test performance in a clinical setting, rather than evaluating the test against normal healthy pregnant women, in a case-control design. However, use of reduced symphysis-fundal height as the inclusion criterion for the PELICAN-FGR study identified a heterogeneous group, with few cases complicated by placental disease (i.e. pre-eclampsia) and may explain why the performance of PlGF, an angiogenic factor, to predict delivery of an SGA infant, was lower that I anticipated. Using PlGF in a more targeted subgroup may be more appropriate.

PlGF measurement was undertaken at the enrolling site as would occur if the test was to be adopted clinically and results were validated by paired sample testing at a central laboratory. All additional biomarkers in the PELICAN-PE study were measured at the same central laboratory. All final maternal diagnoses were made by a panel of senior clinicians, without prior knowledge of the PlGF result. All clinical and laboratory staff were masked to biomarker results until study completion.

With the exception of PlGF, test results were not validated by a paired sample and there was no comparative testing at a second laboratory. In addition, both studies only measured PlGF at study enrolment. It could be informative to

evaluate serial measurements to assess whether longitudinal changes in PlGF concentrations correlate with evolving placental dysfunction. In the PELICAN-PE study serial plasma sampling would also have had the added benefit of allowing testing of all additional biomarkers at advancing gestations, as some biomarkers may only become clinically significant closer to outcome. However, with regards to PlGF measurement in the PELICAN-PE study, despite varying timescales between plasma sampling and delivery, PlGF remained a strong indicator of subsequent delivery of an SGA infant.

To allow comparison of ultrasound parameters to biomarkers measured at study enrolment, inclusion was restricted to women recruited to the PELICAN-PE study who had a recorded ultrasound scan at or within 14 days of study enrolment. Of the 397 women recruited prior to 37 weeks' gestation less than half (n=179) had ultrasound scan data available within this timeframe and data relating to umbilical artery Doppler were limited to whether there was absent/ reversed end diastolic flow. In women enrolled prior to 35 weeks' there was a disparity in the incidence of pre-eclampsia and delivery of an SGA infant with birth weight <3rd centile, with higher incidence of both in women who had ultrasound scanning (incidence of pre-eclampsia at delivery and SGA <3rd centile 74% and 46% respectively in women with ultrasound within 14 days compared to 36% and 10% in women with no ultrasound data). This has the potential to bias results.

Absent/ reversed end diastolic flow in the umbilical artery Doppler was only present in 16 cases included in this analysis (13 prior to 35 weeks' gestation and 3 cases enrolled between 35^{+0} and 36^{+6} weeks' gestation). Absent/

reversed end diastolic flow in the umbilical artery Doppler is a relatively uncommon finding even in high-risk populations and occurs late in the pathological process. It is used as an end point to aid decisions regarding timing of delivery. It may have been more informative to record umbilical artery Doppler pulsatility index (and then transformed them to allow for gestational age changes), as alterations in pulsatility index are usually observed prior to the development of absent/reversed end diastolic flow and raised pulsatility index can therefore be used as an earlier indicator of adverse outcome. These data were collected in the PELICAN-FGR cohort but there were only 26 cases with umbilical artery Doppler PI >95% centile in the whole cohort (n=592). Data of ultrasound scans conducted after enrolment were recorded but there were insufficient number of cases in both studies with additional ultrasound scan data to justify further analysis. As with serial PlGF measurements, serial ultrasound assessment would provide data to allow assessment as to whether longitudinal changes in ultrasound parameters can improve identification of pregnancies at risk of delivering an SGA infant.

4.4 Significance of findings

To date, no published studies have compared the performance of currently utlilised ultrasound parameters and such a diverse panel of biomarkers in the third trimester to predict delivery of an SGA infant. In this work I have demonstrated that third trimester PlGF quantification in a high-risk cohort of women accurately predicts delivery of an SGA infant. I have shown that PlGF

outperforms all other individual biomarkers evaluated. Combining the results of two or more biomarkers, regardless of their pathogenesis, add little to overall test performance. Given that impaired placental function contributes to a substantial proportion of cases of SGA (Redline, 2008), an angiogenic placental factor such as PlGF has biological plausibility for prediction.

4.4.1 Comparison of PELICAN-PE study findings to other studies evaluating the ability of PlGF to predict delivery of an SGA infant

Previous reports of the ability of PlGF to determine pregnancies at risk of delivering an SGA infant are conflicting. Most early studies concentrated on sampling in the first and second trimesters. Initial small case-control studies in the first half of pregnancy found no significant relationship between PlGF concentration and subsequent delivery of an SGA infant (Vandenberghe et al., 2011, Steinberg et al., 2010, Bersinger and Odegard, 2005) but larger case-control studies (Karagiannis et al., 2011, Asvold et al., 2011, Romero et al., 2008, Thadhani et al., 2004) and several prospective cohort studies measuring PlGF in the first (Poon et al., 2013, Poon et al., 2008b) and second trimesters (Espinoza et al., 2007) have reported an association between low plasma PlGF concentrations and delivery of an SGA infant. The few small (n=21 or less) mainly case-control studies that have evaluated PlGF quantification in the third trimester (including at time of delivery) to predict delivery of an SGA infant agree with the findings of the PELICAN-PE study where low PlGF concentrations are associated with subsequent delivery of an SGA infant (Benton et al., 2012, Wallner et al., 2007, Shibata et al., 2005, Taylor, 2003).

Comparison between studies investigating the ability of PlGF to identify pregnancies at risk of delivering an SGA infant is further confounded by use of a variety of PlGF assays. The majority of studies discussed above used a PlGF assay marketed by R&D systems (USA), which is not automated. At present there are only two commercially available PlGF immunoassays, which could be implemented directly into clinical practice; the Triage placental growth factor assay (Alere, USA) and the Elecsys soluble Fms-like tyrosine kinase-1/placental growth factor ratio (Roche Diagnostics, Germany). A small case control study, compared the performance of these two assays as diagnostic tests for pre-eclampsia, and reported superior performance for the former assay (Benton et al., 2011). The Alere Triage assay was used in the PELICAN-PE and PELICAN-FGR studies but was only used in one of the studies discussed above (Benton et al., 2012).

4.4.2 PlGF as a predictor of adverse perinatal outcome in the PELICAN-PE study

In addition to demonstrating the ability of third trimester PlGF sampling to predict delivery of an SGA infant in a high-risk cohort, low concentrations of this marker also identified cases complicated by adverse perinatal outcome. This finding is supported by two studies (Smith et al., 2007, Sibiude et al., 2012), but the first was a nested case-control study, measuring PlGF in the first trimester and the second reported a combined maternal and perinatal adverse outcome. Both studies included delivery of an SGA infant within their definitions of adverse outcome. In contrast, in the PELICAN-PE study, delivery of an SGA infant was a separate secondary endpoint. This allowed identification of an

association between low PlGF measurement and adverse perinatal outcome, independent of that demonstrated between low PlGF and delivery of an SGA infant. This finding has not previously been published in any study evaluating PlGF to predict delivery of an SGA infant.

4.4.3 Comparing the performance of PlGF and other individual biomarkers and biomarker combinations/ ratios in the PELICAN-PE study to other studies investigating biomarker prediction of delivering an SGA infant

Whilst many publications have linked altered concentrations of biomarkers with delivery of an SGA infant, there is a paucity of published data regarding the test performance of biomarkers, especially measured in the third trimester, to predict this endpoint. Most evidence has evaluated the ability of angiogenic biomarkers to determine delivery of an SGA infant but few studies have published test performance statistics to allow any comparison.

With the exception of PlGF, s-Flt is one of the only markers with published test performance statistics for determining delivery of an SGA infant. Stepan et al. measured s-Flt in the second trimester of pregnancy in women with abnormal uterine artery Doppler flow and reported sensitivity of 64% with specificity of 54% to predict delivery of an SGA infant with birth weight <5th centile (Stepan et al., 2007). Using a different definition for SGA, Bersinger et al. published a sensitivity of 56% (95% CI 27 to 81%) and specificity of 88% (95% CI 74 to 95%) for s-Flt to predict delivery of an SGA infant (Bersinger and Odegard, 2005). This study was limited by small sample size, (only nine cases of SGA)

which is likely to explain the wide confidence intervals quoted. In the PELICAN-PE cohort the ROC area for s-Flt to predict delivery of an SGA infant with birth weight <10th centile was 0.69 (95% CI 0.63 to 0.76), lower than that of PlGF (0.79; 95% CI 0.73 to 0.84). The sample size of our study population was much greater than any previously published data regarding the performance of s-Flt as a predictor of delivering an SGA infant and therefore our data are likely to offer more accurate information regarding the ability of s-Flt to determine this outcome.

Of the other angiogenic biomarkers discussed in detail in section 1.4.1, Asvold et al. have published test performance statistics for endoglin to predict delivery of an SGA infant with birth weight <2.5th centile in women without pre-eclampsia (Asvold et al., 2011). They report a sensitivity of 61% (95% CI 52 to 69%) and specificity of 67% (95% CI 60 to 73%) for endoglin to predict delivery of an SGA infant. In the PELICAN-PE cohort the ROC area for endoglin to predict delivery of an SGA infant with birth weight <3rd centile was 0.74 (95% CI 0.68 to 0.80), less than that for PlGF for the same endpoint (0.83; 95% CI 0.78 to 0.88). Asvold et al. sampled s-Flt in the second trimester, which may give some explanation for the differing results published compared to the PELICAN-PE data.

A recent systematic review and meta-analysis investigating novel biomarkers for predicting intrauterine growth restriction concluded that PlGF was the most promising of all 37 biomarkers evaluated (Conde-Agudelo et al., 2013). This review included 53 studies mostly evaluating biomarker measurement in the

first and second trimesters to predict delivery of an SGA infant and no studies evaluated PlGF in a similar cohort to this study. Of the 13 studies in this review reporting test performance for PlGF to predict delivery of an SGA infant, only one used a commercially available automated immunoassay (Benton et al., 2012) and only five recruited women over 20 weeks' gestation. Within this review, a subgroup analysis including the five studies enroling women over 20 weeks' gestation reported a pooled sensitivity for PlGF (at various thresholds and using two different assays) for prediction of 'IUGR' (by differing definitions) of 49% (95% CI 44 to 53%). Given the considerable difference between studies included in this analysis and the fact that only one followed a cohort design, comparisons between studies was very difficult. In the single cohort study included in the subgroup analysis of women enrolled beyond 20 weeks' gestation, delivery of an SGA infant was a secondary endpoint (primary outcome was pre-eclampsia). No cohort studies evaluating PlGF quantification in the third trimester were included in this review.

The gross heterogeneity of the studies included in this systematic review with differing inclusion criteria, study design and use of various PlGF assays, gives some explanation as to why the findings were markedly different to that of the PELICAN-PE study and emphasise the importance of this study, being the only large prospective cohort study evaluating a wide range of third trimester biomarkers in predicting delivery of an SGA infant and adverse perinatal outcome.

Subsequent to publication of this systematic review, a large prospective cohort study on an unselected population evaluated maternal plasma angiogenic

markers (PlGF, sFlt-1 and soluble endoglin) at a fixed time point (30-34 weeks' gestation) to determine delivery of an SGA infant. This group reported an increased adjusted odds ratio for PlGF/sFlt-1 ratio of 5.5 (95% CI 2.3 to 13.1%) to determine delivery of an SGA infant <3rd birth weight centile. Unfortunately they did not publish any standard test performance statistics to allow comparison to the PELICAN-PE or PELICAN-FGR datasets (Chaiworapongsa et al., 2013). Interestingly, the data from the PELICAN-PE study showed that use of a PlGF/sFlt-1 ratio added nothing to PlGF measurement alone and in fact had a lower ROC area (0.80 (95% CI 0.75-0.85) Table 2.8) than PlGF measurement in isolation (0.83 (95% CI 0.78-0.88)) in predicting delivery of an SGA infant with birth weight <3rd centile.

4.4.4 Significance of PELICAN-FGR study findings

Whilst the results of the PELICAN-FGR study do not support extension of the findings of the PELICAN-PE study in a more general antenatal population, this study is the largest prospective cohort study to evaluate the diagnostic accuracy of currently used ultrasound parameters and maternal plasma PlGF concentration in women presenting with reduced symphysis-fundal height measurement. Publication of the results of the PELICAN-FGR study will ensure avoidance of inappropriate extrapolation of the findings of the PELICAN-PE study into an unselected population. I have demonstrated that currently utilised ultrasound parameters had only modest predictive ability for delivery of an SGA infant, in line with previous publications (David et al., 1996, De Jong et al., 2000, Frøen et al., 2004, Chang et al., 1992). I have also shown that whilst oligohydramnios and umbilical artery Doppler >95% centile had low sensitivity for predicting delivery of an SGA infant, limiting their clinical utility as isolated

tools, they were both highly specific for this condition. This corroborates the findings of a recent meta-analysis and systematic review evaluating the association of amniotic fluid index and adverse perinatal outcome, where there was a strong association between oligohydramnios and delivery of an SGA infant and mortality (Morris et al., 2014).

4.5 Clinical application of findings, unanswered questions and future research

Whilst the results of my work do not support the widespread addition of PlGF to current ultrasound parameters to identify pregnancies at risk of delivering an SGA infant in an unselected population, its use as a screening tool in high- risk women presenting with signs and symptoms of pre-eclampsia is likely to be beneficial. However, in the work presented here I did not investigate whether adoption of PlGF into clinical practice would alter clinical outcome.

4.5.1 Assessment of clinical utility of PlGF guided management in suspected pre-eclampsia

I hypothesise that improved identification of those at greatest risk of delivering an SGA infant and associated adverse outcome will facilitate appropriate targeting of resources and timely intervention with subsequent improvements in maternal and neonatal outcome. A randomised controlled trial comparing current clinical practice to PlGF guided management should be performed to demonstrate effectiveness prior to widespread adoption of PlGF as a screening

tool for SGA in a high-risk population presenting with suspected pre-eclampsia. Such a study should include a comprehensive economic analysis to enable comparison to current care. We have recently received funding from the National Institute for Health Research, Research for Patient Benefit programme to perform a stepped wedge randomised trial comparing PlGF guided clinical management against current practice in women presenting with suspected pre-eclampsia. All women will have plasma PlGF measured at study entry. The two study arms will follow either;

1) current clinical practice according to NICE guidance with PlGF result being concealed from the clinician

or

2) PlGF guided management where the clinician would be informed of the PlGF result and if this is <12 pg/ml the patient should be treated according to NICE guidance on the management of pre-eclampsia, including close surveillance, irrespective of their clinical presentation. If PlGF result is >100 pg/ml then women will be offered outpatient consultant-led follow up.

The primary outcome is the proportion of women in each arm with composite maternal adverse outcome. A full economic analysis is planned and the study is powered for neonatal outcome with delivery of an SGA infant with birth weight <3rd customised centile as a secondary endpoint.

4.5.2 Use of PlGF in maternal renal disease

In section 1.1.3 I discussed the many risk factors contributing to the development of FGR and the particular importance of maternal factors, which mainly predispose to placental disease. Within the PELICAN-PE study I have demonstrated that PlGF accurately predicts delivery of an SGA infant in a high-risk cohort of women with suspected pre-eclampsia. I hypothesise that PlGF may have similar ability to predict delivery of an SGA infant and adverse perinatal outcome in other high-risk cohorts at risk of placental disease, such as women with diabetes mellitus, renal and vascular disease. Diagnosis of pre-eclampsia using conventional criteria is particularly challenging in women with pre-existing hypertension and proteinuria and if PlGF quantification in this group of women can identify those at most risk of developing pre-eclampsia and delivering an SGA infant, targeted intervention has a real opportunity to improve outcome.

Whilst some women included in the PELICAN-PE study initial analysis (n=274) had pre-existing medical conditions including diabetes mellitus (n=6) and renal disease (n=19), numbers were insufficient to draw any substantive conclusions. Further prospective cohort studies investigating the utility of PlGF as a screening tool in these populations are warranted. Bramham et al. have recently presented data assessing the diagnostic accuracy of PlGF in 129 women with chronic kidney disease or hypertension and suspected pre-eclampsia to determine pre-eclampsia or superimposed pre-eclampsia. The ROC area for low PlGF for the diagnosis of pre-eclampsia was high (0.89 (SE 0.07) for women with pre-existing hypertension and 0.98 (SE 0.02) for women with chronic kidney disease (with or without chronic hypertension) (Bramham et

al., 2014). The number of cases within this cohort, delivering an SGA infant was too small for further analysis. In future work, this group is evaluating the predictive value of PlGF for adverse pregnancy outcomes in women with chronic kidney disease and/or chronic hypertension.

4.5.3 Use of PlGF in women with a previous history of pre-eclampsia or delivery of an SGA infant

In addition to maternal factors highlighted above, women with a previous history of pre-eclampsia or delivery of an SGA infant are at increased risk of recurrence (Tejani, 1982). The absolute risk of delivering an SGA infant in subsequent pregnancies is likely to be lower than that observed in the high-risk cohort of the PELICAN-PE study but higher than that seen in the PELICAN-FGR study. Therefore, use of PlGF as a predictor of delivering an SGA infant in women who had previous pre-eclampsia or delivery of an SGA infant would require assessment as it may be a useful tool. Deciding the most appropriate gestation at which to measure PlGF is challenging, as sampling at earlier gestations has the benefit of identifying high-risk pregnancies for intensive monitoring, facilitating timely intervention. However, test performance diminishes with greater interval between sampling and delivery.

It was not possible to assess the ability of PlGF to predict delivery of an SGA infant in women with a previous history of pre-eclampsia or delivery of an SGA infant witihin the PELICAN-FGR study as only one woman enrolled reported a previous history of pre-eclampsia and whilst 49 women reported delivering a previous SGA infant, in those where actual birth weights were available, few had a birth weight <10th customized birth weight centile for gestational age.

4.5.4 Use of PlGF in maternal obesity

Maternal obesity is a growing problem, presenting multiple challenges for the clinician. Obesity in pregnancy is associated with many complications including pre-eclampsia. In obese pregnant women, identifying pregnancies at risk of FGR presents a particular challenge due to the difficulties with applying current techniques for screening and diagnosing SGA. Symphysis-fundal height is not an appropriate screening tool in obese women and whilst most local guidelines on the management of obesity in pregnancy recommend routine third trimester ultrasound scanning, obtaining optimal views for accurate measurement of ultrasound parameters is often very difficult. Unlike other biomarkers such as leptin, maternal weight does not have a significant effect on PlGF concentrations and therefore PlGF may offer a useful adjunct to ultrasound scan to identify pregnancies at risk of delivering an SGA infant. I propose further assessment of the ability of PlGF in an obese pregnant population to predict delivery of an SGA infant.

4.4.5 Use of PlGF with Doppler assessment

The UK National Screening Committee and Royal College of Obstetricians and Gynaecologists guidelines do not recommend routine uterine artery Doppler screening in mid-trimester for prediction of pre-eclampsia or FGR (UK National Screening Committee, 2011, Robson et al., 2013). However, as part of a research programme, some UK centres offer second trimester uterine artery Doppler assessment to all primiparous women. Whilst screening low-risk pregnancies using isolated uterine artery Doppler analysis has not been shown

to improve maternal or fetal outcome (Stampalija et al., 2010), its use in a combined screening programme with PlGF may be beneficial. Exploring the ability of combined second trimester uterine artery Doppler measurement and early third trimester PlGF quantification to predict delivery of an SGA infant in an unselected population would be an interesting area of future research. An economical analysis would be necessary as part of such an evaluation as introducing widespread measurement of uterine artery Doppler indices would have significant cost implications, although this could be incorporated into second trimester anomaly scanning. As the majority of centres enrolling women in the PELICAN-FGR study did not routinely undertake second trimester uterine artery Doppler screening, these data were not available for analysis.

Within the UK, uterine artery Doppler assessment is only offered (outside a research setting) in pregnancies with pre-existing risk factors for delivery of an SGA infant. However, many women who subsequently deliver an SGA infant have no pre-existing risk factors. Accurately identifying those at risk of delivering an SGA infant remains challenging. Gardosi et al. suggest that wide spread implementation of a comprehensive growth assessment protocol (GAP), involving use of customised symphysis-fundal height measurement and growth charts, aids antenatal identification of pregnancies delivering an SGA infant (Gardosi et al., 2014). Umbilical artery Doppler measurement in high-risk pregnancies, including those with suspected SGA, has been shown to reduce perinatal death (Alfirevic and Neilson, 1995). Gardosi et al. suggests that by better identification of at risk pregnancies, allowing targeted monitoring with ultrasound and umbilical Doppler measurement; stillbirth rates are reduced (Gardosi et al., 2014). Prior to widespread implementation of these findings, I suggest they are verified in a large-scale randomised control trial.

Current national guidelines suggest that if Doppler measurement is abnormal then serial ultrasound and umbilical artery Doppler measurement should be conducted from 26-28 weeks' gestation (Robson et al., 2013). Given the modest test performance of ultrasound parameters to predict delivery of an SGA infant, using PlGF quantification in this high-risk cohort might improve detection and further investigation is recommended.

4.6 Conclusion

The results of the PELICAN-PE study suggest that PlGF measurement could be a useful adjunct to current ultrasound parameters in predicting subsequent delivery of an SGA infant in a high-risk cohort of women. However the findings of PELICAN-FGR do not support its use in a more generalised setting, where women presented with reduced symphysis-fundal height. Whilst current ultrasound parameters provide only modest prediction of delivering an SGA infant (EFW <10th centile, ROC area 0.79 for SGA <3rd centile), addition of PlGF to these parameters provided minimal improvement in test performance (PlGF and EFW<10th centile ROC area 0.82 for SGA <3rd centile), insufficient to recommend inclusion into clinical practice. The contrasting findings of PELICAN-PE and PELICAN-FGR emphasises the importance of not generalising the findings in one study population to another and highlights the need for caution regarding use of new biomarkers in clinical practice without validation in the appropriate setting.

Publications and presentations

Publications

Griffin MJ, Seed PT, Webster L, Myers J, Mackillop L, Simpson N, Anumba D, Khalil A, Denbow M, Sau A, Hinshaw K, Von Dadelszen P, Girling J, Redman CW, Chappell LC, Shennan AH. Diagnostic accuracy of placental growth factor and ultrasound parameters to predict the small for gestational age infant in women presenting with reduced symphysis-fundal height measurement (Accepted for publication *Ultrasound in Obstetrics & Gynecology*, 31st March 2015, in Appendix)

Griffin M, Seed PT, Duckworth S, North R, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Chappell LC, Shennan AH. Ultrasound Parameters And Biomarkers For Determination Of The Small For Gestational Age Infant. (Resubmitted to *Hypertension*, March 2015)

Duckworth S, **Griffin M**, Seed PT, North R, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Chappell LC, Shennan AH. Prognostic Biomarkers In Women With Suspected Preeclampsia In A Prospective Multicentre Study **(**In revision for *Hypertension*, April 2015)

Chappell LC, Duckworth S, Seed PT, **Griffin M**, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Shennan AH. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: A prospective multicenter study. *Circulation*. 2013;128:2121-2131 (in Appendix)

International oral presentations

2014 **M Griffin**, P Seed, L Webster, H Tarft, L Chappell, A Shennan on behalf of the PELICAN FGR study consortium. Placental Growth Factor (PlGF) and ultrasound parameters for predicting the small for gestational age infant (SGA) in suspected SGA: PELICAN FGR study. Oral presentation, *Perinatal Medicine*, Harrogate.

2013 **M Griffin**, S Duckworth, P Seed, L Chappell, C Redman, A Shennan on behalf of the PELICAN study consortium Biomarkers determining pre-eclampsia and the small for gestational age infant: The PELICAN study. Poster and Oral presentation, *Biomarkers for a successful pregnancy, Pregnancy Summit,* The O2, London.

International poster presentations

2014 **M Griffin**, P Seed, L Webster, H Tarft, L Chappell, A Shennan on behalf of the PELICAN FGR study consortium. Placental Growth factor (PlGF) and ultrasound parameters for predicting the small for gestational age infant (SGA) in suspected small for gestational age: PELICAN FGR study. Poster presentation, *European Congress Perinatal Medicine*, Florence, Italy.

National oral presentations

2013 **Griffin, M**, Duckworth S, Webster L, Seed PT, Chappell LC, Redman CW, Shennan AH, on behalf of the PELICAN study consortium. Comparison of PlGF and other biomarkers against current ultrasound parameters for determining delivery of small for gestational age (SGA) infants in women with suspected pre-eclampsia: the PELICAN study. Oral poster presentation, *Blair Bell Annual Academic Meeting*, RCOG, London. Published in *BJOG*. 2014; 121(7); e6

2012 Duckworth S, Chappell LC, **Griffin M**, Seed PT, Redman CW, Shennan AH Plasma Placental Growth Factor (PlGF) in the diagnosis of women with pre-eclampsia requiring delivery within 14 days: the PELICAN study. Oral presentation by Dr Chappell *Blair Bell Annual Academic Meeting*, RCOG, London. Published in *BJOG*. 2013; 120(9); e1

Regional presentations

2015 Placental growth factor (PlGF) and ultrasound parameters for predicting the small for gestational age (SGA) infant and adverse perinatal outcome in suspected pre-eclampsia: a secondary analysis of the PELICAN study, Oral presentation, South West Obstetric Trainees Annual General Meeting, Bristol.

References

ACOG PRACTICE BULLETIN 2002. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol,* 99**,** 159-167.

ADDOR, M. C., PESCIA, G. & SCHORDERET, D. F. 2000. Registration of congenital anomalies in Switzerland by EUROCAT. *Schweiz Med Wochenschr,* 130**,** 1319-25.

ALFIREVIC, Z. & NEILSON, J. P. 1995. Doppler ultrasonography in high-risk pregnancies: systematic review with meta-analysis. *Am J Obstet Gynecol,* 172**,** 1379-87.

ALFIREVIC, Z., STAMPALIJA, T. & GYTE, G. M. 2010. Fetal and umbilical Doppler ultrasound in normal pregnancy. *Cochrane Database Syst Rev***,** CD001450.

ALLEN, V. M., JOSEPH, K., MURPHY, K. E., MAGEE, L. A. & OHLSSON, A. 2004. The effect of hypertensive disorders in pregnancy on small for gestational age and stillbirth: a population based study. *BMC Pregnancy Childbirth,* 4**,** 17.

ANANTH, C. V., BERKOWITZ, G. S., SAVITZ, D. A. & LAPINSKI, R. H. 1999. Placental abruption and adverse perinatal outcomes. *JAMA,* 282**,** 1646-51.

ANUMBA, D. O., LINCOLN, K. & ROBSON, S. C. 2010. Predictive value of clinical and laboratory indices at first assessment in women referred with suspected gestational hypertension. *Hypertens Pregnancy,* 29**,** 163-79.

ARIAS, F., ROMERO, R., JOIST, H. & KRAUS, F. T. 1998. Thrombophilia: a mechanism of disease in women with adverse pregnancy outcome and thrombotic lesions in the placenta. *J Matern Fetal Med,* 7**,** 277-86.

ASVOLD, O. B., VATTEN, L. J., ROMUNDSTAD, P. R., JENUM, P. A., KARUMANCHI, S. A. & ESKILD, A. 2011. Angiogenic Factors in Maternal Circulation and the Risk of Severe Fetal Growth Restriction. *American Journal of Epidemiology,* 173**,** 630-639.

AUTIERO, M., LUTTUN, A., TJWA, M. & CARMELIET, P. 2003a. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. *J Thromb Haemost,* 1**,** 1356-70.

AUTIERO, M., WALTENBERGER, J., COMMUNI, D., KRANZ, A., MOONS, L., LAMBRECHTS, D., KROLL, J., PLAISANCE, S., DE MOL, M., BONO, F., KLICHE, S., FELLBRICH, G., BALLMER-HOFER, K., MAGLIONE, D., MAYR-BEYRLE, U., DEWERCHIN, M., DOMBROWSKI, S., STANIMIROVIC, D., VAN HUMMELEN, P., DEHIO, C., HICKLIN, D. J., PERSICO, G., HERBERT, J. M., COMMUNI, D., SHIBUYA, M., COLLEN, D., CONWAY, E. M. & CARMELIET, P. 2003b. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med,* 9**,** 936-43.

BAIS, J. M., ESKES, M., PEL, M., BONSEL, G. J. & BLEKER, O. P. 2004. Effectiveness of detection of intrauterine growth retardation by abdominal palpation as screening test in a low risk population: an observational study. *Eur J Obstet Gynecol Reprod Biol,* 116**,** 164-9.

BAKER, P. N., WHEELER, S. J., SANDERS, T. A., THOMAS, J. E., HUTCHINSON, C. J., CLARKE, K., BERRY, J. L., JONES, R. L., SEED, P. T. & POSTON, L. 2009. A prospective study of micronutrient status in adolescent pregnancy. *Am J Clin Nutr,* 89**,** 1114-24.

BAKKETEIG, L. S., JACOBSEN, G., HOFFMAN, H. J., LINDMARK, G., BERGSJO, P., MOLNE, K. & RODSTEN, J. 1993. Pre-pregnancy risk factors of small-for-gestational age births among parous women in Scandinavia. *Acta Obstet Gynecol Scand,* 72**,** 273-9.

BARFIELD, W. D., BARRADAS, D. T., MANNING, S. E., KOTELCHUCK, M. & SHAPIRO-MENDOZA, C. K. 2010. Sickle cell disease and pregnancy outcomes: women of African descent. *Am J Prev Med,* 38**,** S542-9.

BARKER, D. J. P. 2006. Adult consequences of fetal growth restriction. *Clin obstet gynecol,* 49**,** 270-283.

BATES, D. O. 2011. An unexpected tail of VEGF and PlGF in pre-eclampsia. *Biochem Soc Trans,* 39**,** 1576-82.

BATES, D. O. & HARPER, S. J. 2002. Regulation of vascular permeability by vascular endothelial growth factors. *Vascul Pharmacol,* 39**,** 225-37.

BATES, D. O., HILLMAN, N. J., WILLIAMS, B., NEAL, C. R. & POCOCK, T. M. 2002. Regulation of microvascular permeability by vascular endothelial growth factors. *J Anat,* 200**,** 581-97.

BEN-HAROUSH, A., YOGEV, Y., HOD, M. & BAR, J. 2007. Predictive value of a single early fetal weight estimate in normal pregnancies. *Eur J Obstet Gynecol and Reprod Biol,* 130**,** 187-192.

BENTON, S. J., HU, Y., XIE, F., KUPFER, K., LEE, S.-W., MAGEE, L. A. & VON DADELSZEN, P. 2012. Can placental growth factor in maternal circulation identify fetuses with placental intrauterine growth restriction? *Am J Obstet Gynecol,* 206**,** 163.e1-e7.

BENTON, S. J., HU, Y., XIE, F., KUPFER, K., LEE, S. W., MAGEE, L. A. & VON DADELSZEN, P. 2011. Angiogenic factors as diagnostic tests for preeclampsia: a performance comparison between two commercial immunoassays. *Am J Obstet Gynecol,* 205**,** 469.e1-8.

BERNSTEIN, I. M., HORBAR, J. D., BADGER, G. J., OHLSSON, A. & GOLAN, A. 2013. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. *Am J Obstet Gynecol,* 182**,** 198-206.

BERSINGER, N. A. & ODEGARD, R. A. 2004. Second‐and third‐trimester serum levels of placental proteins in preeclampsia and small‐for‐gestational age pregnancies. *Acta Obstetricia et Gynecologica Scandinavica,* 83**,** 37-45.

BERSINGER, N. A. & ODEGARD, R. A. 2005. Serum Levels of Macrophage Colony Stimulating, Vascular Endothelial, and Placenta Growth Factor in Relation to Later Clinical Onset of Pre-Eclampsia and a Small-for-Gestational Age Birth. *Am J Repro Imm,* 54**,** 77-83.

BONAMY, A. K., PARIKH, N. I., CNATTINGIUS, S., LUDVIGSSON, J. F. & INGELSSON, E. 2011. Birth characteristics and subsequent risks of maternal cardiovascular disease: effects of gestational age and fetal growth. *Circulation,* 124**,** 2839-46.

BRAMHAM, K., SEED, P., NELSON-PIERCY, C., LIGHTSTONE, L., ASHFORD, L., BUTLER, J., POSTON, L. & CHAPPELL, L. C. 2014. Diagnostic accuracy of Placental Growth Factor in women with chronic kidney disease or hypertension and suspected preeclampsia: a

prospective cohort study. *World Congress for the Study of Hypertension in Pregnancy,* Oral presentation: 042.
BRICKER, L., NEILSON, J. P. & DOWSWELL, T. 2008. Routine ultrasound in late pregnancy (after 24 weeks' gestation). *Cochrane Database Syst Rev*, CD001451.
BROWN, M. A., LINDHEIMER, M. D., DE SWIET, M., VAN ASSCHE, A. & MOUTQUIN, J. M. 2001. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertension,* 20, IX-XIV.
BUKOWSKI, R., BURGETT, A. D., GEI, A., SAADE, G. R. & HANKINS, G. D. 2003. Impairment of fetal growth potential and neonatal encephalopathy. *Am J Obstet Gynecol,* 188, 1011-5.
CHAIWORAPONGSA, T., ROMERO, R., KORZENIEWSKI, S. J., KUSANOVIC, J. P., SOTO, E., LAM, J., DONG, Z., THAN, N. G., YEO, L., HERNANDEZ-ANDRADE, E., CONDE-AGUDELO, A. & HASSAN, S. S. 2013. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. *Am J Obstet Gynecol,* 208, 287.e1-e15.
CHANG, T. C., ROBSON, S. C., BOYS, R. J. & SPENCER, J. A. 1992. Prediction of the small for gestational age infant: which ultrasonic measurement is best? *Obstet Gynecol,* 80, 1030-1038.
CHAPPELL, L. C., DUCKWORTH, S., SEED, P. T., GRIFFIN, M., MYERS, J., MACKILLOP, L., SIMPSON, N., WAUGH, J., ANUMBA, D., KENNY, L. C., REDMAN, C. W. G. & SHENNAN, A. H. 2013. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation,* 128, 2121-2131.
CHAPPELL, L. C., SEED, P. T., BRILEY, A., KELLY, F. J., HUNT, B. J., CHARNOCK-JONES, D. S., MALLET, A. I. & POSTON, L. 2002. A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol,* 187, 127-36.
CHAUHAN, S. P., MAGANN, E. F., DOHERTY, D. A., ENNEN, C. S., NIEDERHAUSER, A. & MORRISON, J. C. 2008. Prediction of small for gestational age newborns using ultrasound estimated and actual amniotic fluid volume: Published data revisited. *Aust N Z J Obstet Gynaecol,* 48, 160-164.
CHIESA, C., OSBORN, J. F., HAASS, C., NATALE, F., SPINELLI, M., SCAPILLATI, E., SPINELLI, A. & PACIFICO, L. 2008. Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? *Clin Chem,* 54, 550-8.
CLARK, D. E., SMITH, S. K., HE, Y., DAY, K. A., LICENCE, D. R., CORPS, A. N., LAMMOGLIA, R. & CHARNOCK-JONES, D. S. 1998a. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod,* 59, 1540-8.
CLARK, D. E., SMITH, S. K., LICENCE, D., EVANS, A. L. & CHARNOCK-JONES, D. S. 1998b. Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. *J Endocrinol,* 159, 459-67.

CLAUSSON, B., GARDOSI, J., FRANCIS, A. & CNATTINGIUS, S. 2001. Perinatal outcome in SGA births defined by customised versus population-based birthweight standards. *Br J Obstet Gynaecol,* 108**,** 830-834.

CNOSSEN, J. S., MORRIS, R. K., TER RIET, G., MOL, B. W. J., VAN DER POST, J. A. M., COOMARASAMY, A., ZWINDERMAN, A. H., ROBSON, S. C., BINDELS, P. J. E., KLEIJNEN, J. & KHAN, K. S. 2008. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *Canadian Medical Association Journal,* 178**,** 701-711.

CONDE-AGUDELO, A., PAPAGEORGHIOU, A. T., KENNEDY, S. H. & VILLAR, J. 2013. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. *Br J Obstet Gynaecol,* 120**,** 681-94.

COSTELLO, A. B. & OSBORNE, J. W. 2005. Best Practices in Exploratory Factor Analysis: Four Recommendations for Getting the Most From Your Analysis. *Practical Assessment Research & Evaluation,* 10**,** 1-9.

COWANS, N. J. & SPENCER, K. 2007. First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. *Prenat Diagn,* 27**,** 264-71.

COZZI, V., GARLANDA, C., NEBULONI, M., MAINA, V., MARTINELLI, A., CALABRESE, S. & CETIN, I. 2012. PTX3 as a potential endothelial dysfunction biomarker for severity of preeclampsia and IUGR. *Placenta,* 33**,** 1039-1044.

CURHAN, G. C., CHERTOW, G. M., WILLETT, W. C., SPIEGELMAN, D., COLDITZ, G. A., MANSON, J. E., SPEIZER, F. E. & STAMPFER, M. J. 1996. Birth weight and adult hypertension and obesity in women. *Circulation,* 94**,** 1310-5.

DAVID, C., TAGLIAVINI, G., PILU, G., RUDENHOLZ, A. & BOVICELLI, L. 1996. Receiver-operator characteristic curves for the ultrasonographic prediction of small-for-gestational-age fetuses in low-risk pregnancies. *Am J Obstet Gynecol,* 174**,** 1037-1042.

DE JONG, C. L., FRANCIS, A., VAN GEIJN, H. P. & GARDOSI, J. 2000. Customized fetal weight limits for antenatal detection of fetal growth restriction. *Ultrasound Obstet Gynecol,* 15**,** 36-40.

DESSI, A. & FANOS, V. 2013. Myoinositol: a new marker of intrauterine growth restriction? *J Obstet Gynaecol,* 33**,** 776-80.

DIAB, A., EL-BEHERY, M. M., EBRAHIEM, M. A. & SHEHATA, A. E. 2008. Angiogenic factors for the prediction of pre-eclampsia in women with abnormal midtrimester uterine artery Doppler velocimetry. *International Journal of Gynecology and Obstetrics,* 102**,** 146-151.

EFRON, B. 1987. Better Bootstrap Confidence-Intervals. *Journal of the American Statistical Association,* 82**,** 171-185.

EREZ, O., ROMERO, R., ESPINOZA, J., FU, W., TODEM, D., KUSANOVIC, J. P., GOTSCH, F., EDWIN, S., NIEN, J. K., CHAIWORAPONGSA, T., MITTAL, P., MAZAKI-TOVI, S., THAN, N. G., GOMEZ, R. & HASSAN, S. S. 2008. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. *Journal of Maternal-Fetal and Neonatal Medicine,* 21**,** 279-287.

ERIKSSON, J., FORSEN, T., TUOMILEHTO, J., OSMOND, C. & BARKER, D. 2000. Fetal and childhood growth and hypertension in adult life. *Hypertension,* 36**,** 790-4.

ESPINOZA, J., ROMERO, R., NIEN, J. K., GOMEZ, R., KUSANOVIC, J. P., GONÇALVES, L. F., MEDINA, L., EDWIN, S., HASSAN, S., CARSTENS, M. & GONZALEZ, R. 2007. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. *Am J Obstet Gynecol,* 196**,** 326.e1-e13.

FACCO, F., YOU, W. & GROBMAN, W. 2009. Genetic thrombophilias and intrauterine growth restriction: a meta-analysis. *Obstet Gynecol,* 113**,** 1206-16.

FIGUERAS, F., FIGUERAS, J., MELER, E., EIXARCH, E., COLL, O., GRATACOS, E., GARDOSI, J. & CARBONELL, X. 2007. Customised birthweight standards accurately predict perinatal morbidity. *Arch Dis Child Fetal Neonatal Ed,* 92**,** F277-80.

FINK, J. C., SCHWARTZ, S. M., BENEDETTI, T. J. & STEHMAN-BREEN, C. O. 1998. Increased risk of adverse maternal and infant outcomes among women with renal disease. *Paediatr Perinat Epidemiol,* 12**,** 277-87.

FISCHER, C., MAZZONE, M., JONCKX, B. & CARMELIET, P. 2008. FLT1 and its ligands VEGFB and PIGF: drug targets for anti-angiogenic therapy? *Nat Rev Cancer,* 8**,** 942-56.

FLENADY, V., KOOPMANS, L., MIDDLETON, P., FRØEN, J. F., SMITH, G. C. S., GIBBONS, K., COORY, M., GORDON, A., ELLWOOD, D., MCINTYRE, H. D., FRETTS, R. & EZZATI, M. 2011a. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet,* 377**,** 1331-1340.

FLENADY, V., MIDDLETON, P., SMITH, G. C., DUKE, W., ERWICH, J. J., KHONG, T. Y., NEILSON, J., EZZATI, M., KOOPMANS, L., ELLWOOD, D., FRETTS, R., FROEN, J. F. & LANCET'S STILLBIRTHS SERIES STEERING COMMITTEE 2011b. Stillbirths: the way forward in high-income countries. *Lancet,* 377**,** 1703-17.

FORSEN, T., ERIKSSON, J., TUOMILEHTO, J., REUNANEN, A., OSMOND, C. & BARKER, D. 2000. The fetal and childhood growth of persons who develop type 2 diabetes. *Ann Intern Med,* 133**,** 176-82.

FRANKEL, S., ELWOOD, P., SWEETNAM, P., YARNELL, J. & SMITH, G. D. 1996. Birthweight, body-mass index in middle age, and incident coronary heart disease. *Lancet,* 348**,** 1478-80.

FRASER, A. 2006. Alcohol Consumption and the Outcomes of Pregnancy (RCOG statement 5). *RCOG - Green top guideline.*

FREEMAN, K., OAKLEY, L., POLLAK, A., BUFFOLANO, W., PETERSEN, E., SEMPRINI, A. E., SALT, A., GILBERT, R. & EUROPEAN MULTICENTRE STUDY ON CONGENITAL TOXOPLASMOSIS 2005. Association between congenital toxoplasmosis and preterm birth, low birthweight and small for gestational age birth. *Br J Obstet Gynaecol,* 112**,** 31-7.

FRØEN, J. F., GARDOSI, J. O., THURMANN, A., FRANCIS, A. & STRAY-PEDERSEN, B. 2004. Restricted fetal growth in sudden intrauterine unexplained death. *Acta Obstetricia et Gynecologica Scandinavica,* 83**,** 801-807.

FULROTH, R., PHILLIPS, B. & DURAND, D. J. 1989. Perinatal outcome of infants exposed to cocaine and/or heroin in utero. *Am J Dis Child,* 143**,** 905-10.
GALLO, V., EGGER, M., MCCORMACK, V., FARMER, P. B., IOANNIDIS, J. P. A., KIRSCH-VOLDERS, M., MATULLO, G., PHILLIPS, D. H., SCHOKET, B., STROMBERG, U., VERMEULEN, R., WILD, C., PORTA, M. & VINEIS, P. 2011. STrengthening the Reporting of OBservational studies in Epidemiology – Molecular Epidemiology (STROBE-ME): An Extension of the STROBE Statement. *PLoS Medicine,* 8**,** e1001117.
GARDOSI, J., CHANG, A., KALYAN, B., SAHOTA, D. & SYMONDS, E. M. 1992. Customised antenatal growth charts. *Lancet,* 339**,** 283-287.
GARDOSI, J. & FRANCIS, A. Software program for the calculation of customised birth weight centiles. Version 6.7, 2000-2009.
GARDOSI, J. & FRANCIS, A. 1999. Controlled trial of fundal height measurement plotted on customised antenatal growth charts. *Br J Obstet Gynaecol,* 106**,** 309-317.
GARDOSI, J. & FRANCIS, A. 2009. Adverse pregnancy outcome and association with small for gestational age birthweight by customized and population-based percentiles. *Am J Obstet Gynecol,* 201**,** 28 e1-8.
GARDOSI, J., GIDDINGS, S., BULLER, S., SOUTHAM, M. & WILLIAMS, M. 2014. Preventing stillbirths through improved antenatal recognition of pregnancies at risk due to fetal growth restriction. *Public Health,* 128**,** 698-702.
GARDOSI, J., GIDDINGS, S., CLIFFORD, S., WOOD, L. & FRANCIS, A. 2013a. Association between reduced stillbirth rates in England and regional uptake of accreditation training in customised fetal growth assessment. *BMJ Open,* 3**,** e003942.
GARDOSI, J., MADURASINGHE, V., WILLIAMS, M., MALIK, A. & FRANCIS, A. 2013b. Maternal and fetal risk factors for stillbirth: population based study. *BMJ,* 346**,** f108.
GOLDENBERG, R. L., MCCLURE, E. M., BHUTTA, Z. A., BELIZAN, J. M., REDDY, U. M., RUBENS, C. E., MABEYA, H., FLENADY, V., DARMSTADT, G. L. & LANCET'S STILLBIRTHS SERIES STEERING, C. 2011. Stillbirths: the vision for 2020. *Lancet,* 377**,** 1798-805.
HADLOCK, F. P., HARRIST, R. B., SHARMAN, R. S., DETER, R. L. & PARK, S. K. 1985. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. *Am J Obstet Gynecol,* 151**,** 333-7.
HALL, M. H., CHNG, P. K. & MACGILLIVRAY, I. 1980. Is routine antenatal care worth while? *Lancet,* 2**,** 78-80.
HAN, Z., MULLA, S., BEYENE, J., LIAO, G., MCDONALD, S. D. & KNOWLEDGE SYNTHESIS, G. 2011. Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. *Int J Epidemiol,* 40**,** 65-101.
HARPER, L. M., ODIBO, A. O., MACONES, G. A., CRANE, J. P. & CAHILL, A. G. 2010. Effect of placenta previa on fetal growth. *Am J Obstet Gynecol,* 203**,** 330 e1-5.
HASHIMOTO, K., KASDAGLIS, T., JAIN, S., ATKINS, K., HARMAN, C. R. & BASCHAT, A. A. 2013. Isolated low-normal amniotic fluid volume in the early third trimester: association with adverse perinatal outcomes. *J Perinat Med,* 41**,** 349-53.

HAUSER, S. & WEICH, H. A. 1993. A heparin-binding form of placenta growth factor (PlGF-2) is expressed in human umbilical vein endothelial cells and in placenta. *Growth Factors,* 9**,** 259-68.
HEINONEN, S., RYYNANEN, M., KIRKINEN, P. & SAARIKOSKI, S. 1996. Perinatal diagnostic evaluation of velamentous umbilical cord insertion: clinical, Doppler, and ultrasonic findings. *Obstet Gynecol,* 87**,** 112-7.
HENDRIX, N. & BERGHELLA, V. 2008. Non-placental causes of intrauterine growth restriction. *Semin Perinatol,* 32**,** 161-5.
HOWARTH, C., GAZIS, A. & JAMES, D. 2007. Associations of Type 1 diabetes mellitus, maternal vascular disease and complications of pregnancy. *Diabet Med,* 24**,** 1229-34.
HUI, D., OKUN, N., MURPHY, K., KINGDOM, J., ULERYK, E. & SHAH, P. S. 2012. Combinations of maternal serum markers to predict preeclampsia, small for gestational age, and stillbirth: a systematic review. *J Obstet Gynaecol Can,* 34**,** 142-53.
HUPPERTZ, B. 2008. Placental origins of preeclampsia: challenging the current hypothesis. *Hypertension,* 51**,** 970-5.
HUPPERTZ, B. 2011. Placental pathology in pregnancy complications. *Thromb Res,* 127 Suppl 3**,** S96-9.
HUTCHEON, J. A., ZHANG, X., CNATTINGIUS, S., KRAMER, M. S. & PLATT, R. W. 2008. Customised birthweight percentiles: does adjusting for maternal characteristics matter? *Br J Obstet Gynaecol,* 115**,** 1397-404.
HUXLEY, R. R., SHIELL, A. W. & LAW, C. M. 2000. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens,* 18**,** 815-31.
IYER, S., LEONIDAS, D. D., SWAMINATHAN, G. J., MAGLIONE, D., BATTISTI, M., TUCCI, M., PERSICO, M. G. & ACHARYA, K. R. 2001. The crystal structure of human placenta growth factor-1 (PlGF-1), an angiogenic protein, at 2.0 A resolution. *J Biol Chem,* 276**,** 12153-61.
JACKSON, R. A., GIBSON, K. A., WU, Y. W. & CROUGHAN, M. S. 2004. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol,* 103**,** 551-63.
JACOBSSON, B., AHLIN, K., FRANCIS, A., HAGBERG, G., HAGBERG, H. & GARDOSI, J. 2008. Cerebral palsy and restricted growth status at birth: population-based case-control study. *Br J Obstet Gynaecol,* 115**,** 1250-1255.
KARAGIANNIS, G., AKOLEKAR, R., SARQUIS, R., WRIGHT, D. & NICOLAIDES, K. H. 2011. Prediction of Small-for-Gestation Neonates from Biophysical and Biochemical Markers at 11–13 Weeks. *Fetal diagnosis and therapy,* 29**,** 148-154.
KATZ, J., LEE, A. C., KOZUKI, N., LAWN, J. E., COUSENS, S., BLENCOWE, H., EZZATI, M., BHUTTA, Z. A., MARCHANT, T., WILLEY, B. A., ADAIR, L., BARROS, F., BAQUI, A. H., CHRISTIAN, P., FAWZI, W., GONZALEZ, R., HUMPHREY, J., HUYBREGTS, L., KOLSTEREN, P., MONGKOLCHATI, A., MULLANY, L. C., NDYOMUGYENYI, R., NIEN, J. K., OSRIN, D., ROBERFROID, D., SANIA, A., SCHMIEGELOW, C., SILVEIRA, M. F., TIELSCH, J., VAIDYA, A., VELAPHI, S. C., VICTORA, C. G., WATSON-JONES, D. & BLACK, R. E. 2013. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet,* 382**,** 417-25.

KAUFMANN, P., BLACK, S. & HUPPERTZ, B. 2003. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod,* 69**,** 1-7.
KEAN, L. H. & LIU, D. 1996. Antenatal care as a screening tool for the detection of small for gestational age babies in the low risk population. *Journal of Obstetrics & Gynecology,* 16**,** 77-82.
KHADER, Y. S. & TA'ANI, Q. 2005. Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. *J Periodontol,* 76**,** 161-5.
KHOURY, M. J., ERICKSON, J. D., CORDERO, J. F. & MCCARTHY, B. J. 1988. Congenital malformations and intrauterine growth retardation: a population study. *Pediatrics,* 82**,** 83-90.
KIM, S. M., PARK, J. S., NORWITZ, E. R., JUNG, H. J., KIM, B. J., PARK, C. W. & JUN, J. K. 2013. Circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) correlate with the presence and severity of preeclampsia. *Reprod Sci,* 20**,** 1083-9.
KIM, Y. J., PARK, H., LEE, H. Y., AHN, Y. M., HA, E. H., SUH, S. H. & PANG, M. G. 2007. Paraoxonase gene polymorphism, serum lipid, and oxidized low-density lipoprotein in preeclampsia. *Eur J Obstet Gynecol Reprod Biol,* 133**,** 47-52.
KLOVERPRIS, S., GAIDAMAUSKAS, E., RASMUSSEN, L. C., OVERGAARD, M. T., KRONBORG, C., KNUDSEN, U. B., CHRISTIANSEN, M., KUMAR, A. & OXVIG, C. 2013. A robust immunoassay for pregnancy-associated plasma protein-A2 based on analysis of circulating antigen: establishment of normal ranges in pregnancy. *Mol Hum Reprod,* 19**,** 756-63.
KNUDSEN, U. B., KRONBORG, C. S., VON DADELSZEN, P., KUPFER, K., LEE, S.-W., VITTINGHUS, E., ALLEN, J. G. & REDMAN, C. W. 2012. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. *Pregnancy Hypertension,* 2**,** 8-15.
KOOPMANS, C. M., BIJLENGA, D., GROEN, H., VIJGEN, S. M., AARNOUDSE, J. G., BEKEDAM, D. J., VAN DEN BERG, P. P., DE BOER, K., BURGGRAAFF, J. M., BLOEMENKAMP, K. W., DROGTROP, A. P., FRANX, A., DE GROOT, C. J., HUISJES, A. J., KWEE, A., VAN LOON, A. J., LUB, A., PAPATSONIS, D. N., VAN DER POST, J. A., ROUMEN, F. J., SCHEEPERS, H. C., WILLEKES, C., MOL, B. W., VAN PAMPUS, M. G. & FOR THE HYPITAT STUDY GROUP 2009. Induction of labour versus expectant monitoring for gestational hypertension or mild pre-eclampsia after 36 weeks' gestation (HYPITAT): a multicentre, open-label randomised controlled trial. *Lancet,* 374**,** 979-988.
KRAMPL, E. 2002. Pregnancy at high altitude. *Ultrasound Obstet Gynecol,* 19**,** 535-9.
KUNTZ, T. B., CHRISTENSEN, R. D., STEGNER, J., DUFF, P. & KOENIG, J. M. 2001. Fas and Fas ligand expression in maternal blood and in umbilical cord blood in preeclampsia. *Pediatr Res,* 50**,** 743-9.
LACKMAN, F., CAPEWELL, V., RICHARDSON, B., DASILVA, O. & GAGNON, R. 2001. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. *Am J Obstet Gynecol,* 184**,** 946-953.

LALOR, J. G., FAWOLE, B., ALFIREVIC, Z. & DEVANE, D. 2008. Biophysical profile for fetal assessment in high risk pregnancies. *Cochrane Database Syst Rev*, CD000038.

LANG, J. M., LIEBERMAN, E. & COHEN, A. 1996. A comparison of risk factors for preterm labor and term small-for-gestational-age birth. *Epidemiology,* 7, 369-76.

LASKOWSKA, M., LASKOWSKA, K., LESZCZYNSKA-GORZELAK, B. & OLESZCZUK, J. 2006. Evaluation of the maternal and umbilical vein serum sFas/sFasL system in pregnancies complicated by preeclampsia with intrauterine growth retardation. *Eur J Obstet Gynecol Reprod Biol,* 126, 155-9.

LATAL-HAJNAL, B., VON SIEBENTHAL, K., KOVARI, H., BUCHER, H. U. & LARGO, R. H. 2003. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. *The Journal of Pediatrics,* 143, 163-170.

LAWN, J. E., BLENCOWE, H., PATTINSON, R., COUSENS, S., KUMAR, R., IBIEBELE, I., GARDOSI, J., DAY, L. T., STANTON, C. & LANCET'S STILLBIRTHS SERIES STEERING, C. 2011. Stillbirths: Where? When? Why? How to make the data count? *Lancet,* 377, 1448-63.

LAWN, J. E., COUSENS, S. & ZUPAN, J. 2005. 4 million neonatal deaths: when? Where? Why? *Lancet,* 365, 891-900.

LEE, K. S., FERGUSON, R. M., CORPUZ, M. & GARTNER, L. M. 1988. Maternal age and incidence of low birth weight at term: a population study. *Am J Obstet Gynecol,* 158, 84-9.

LEON, D. A., LITHELL, H. O., VAGERO, D., KOUPILOVA, I., MOHSEN, R., BERGLUND, L., LITHELL, U. B. & MCKEIGUE, P. M. 1998. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *BMJ,* 317, 241-5.

LINDQVIST, P. G. & MOLIN, J. 2005. Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound Obstet Gynecol,* 25, 258-64.

LIPSCOMB, W. N., REEKE, G. N., JR., HARTSUCK, J. A., QUIOCHO, F. A. & BETHGE, P. H. 1970. The structure of carboxypeptidase A. 8. Atomic interpretation at 0.2 nm resolution, a new study of the complex of glycyl-L-tyrosine with CPA, and mechanistic deductions. *Philos Trans R Soc Lond B Biol Sci,* 257, 177-214.

LITHELL, H. O., MCKEIGUE, P. M., BERGLUND, L., MOHSEN, R., LITHELL, U. B. & LEON, D. A. 1996. Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. *BMJ,* 312, 406-10.

MAGANN, E. F., HAAS, D. M., HILL, J. B., CHAUHAN, S. P., WATSON, E. M. & LEARMAN, L. A. 2011. Oligohydramnios, small for gestational age and pregnancy outcomes: an analysis using precise measures. *Gynecol Obstet Invest,* 72, 239-44.

MAGLIONE, D., GUERRIERO, V., VIGLIETTO, G., DELLI-BOVI, P. & PERSICO, M. G. 1991. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci U S A,* 88, 9267-71.

MAGLIONE, D., GUERRIERO, V., VIGLIETTO, G., FERRARO, M. G., APRELIKOVA, O., ALITALO, K., DEL VECCHIO, S., LEI, K. J., CHOU, J. Y. & PERSICO, M. G. 1993. Two alternative mRNAs coding for the

angiogenic factor, placenta growth factor (PIGF), are transcribed from a single gene of chromosome 14. *Oncogene,* 8**,** 925-31.
MALIN, G. L., MORRIS, R. K., RILEY, R., TEUNE, M. J. & KHAN, K. S. 2014. When is birthweight at term abnormally low? A systematic review and meta-analysis of the association and predictive ability of current birthweight standards for neonatal outcomes. *Br J Obstet Gynaecol,* 121**,** 515-26.
MANNING, F. A. 2002. Fetal biophysical profile: a critical appraisal. *Clin Obstet Gynecol,* 45**,** 975-85.
MARCELLINI, M., DE LUCA, N., RICCIONI, T., CIUCCI, A., ORECCHIA, A., LACAL, P. M., RUFFINI, F., PESCE, M., CIANFARANI, F., ZAMBRUNO, G., ORLANDI, A. & FAILLA, C. M. 2006. Increased melanoma growth and metastasis spreading in mice overexpressing placenta growth factor. *Am J Pathol,* 169**,** 643-54.
MAULIK, D. 2006. Fetal growth restriction: the etiology. *Clin Obstet Gynecol,* 49**,** 228-35.
MAULIK, D., FRANCES EVANS, J. & RAGOLIA, L. 2006. Fetal growth restriction: pathogenic mechanisms. *Clin Obstet Gynecol,* 49**,** 219-227.
MAYER, C. & JOSEPH, K. S. 2013. Fetal growth: a review of terms, concepts and issues relevant to obstetrics. *Ultrasound Obstet Gynecol,* 41**,** 136-145.
MCCORMICK, M. C., WORKMAN-DANIELS, K. & BROOKS-GUNN, J. 1996. The behavioral and emotional well-being of school-age children with different birth weights. *Pediatrics,* 97**,** 18-25.
MCCOWAN, L., ROBERTS, C. T., DEKKER, G. A., TAYLOR, R. S., CHAN, E., KENNY, L. C., BAKER, P. N., MOSS-MORRIS, R., CHAPPELL, L. C., NORTH, R. A. & ON BEHALF OF THE SCOPE CONSORTIUM 2010. Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study. *Br J Obstet Gynaecol,* 117**,** 1599-1607.
MCCOWAN, L. M., DEKKER, G. A., CHAN, E., STEWART, A., CHAPPELL, L. C., HUNTER, M., MOSS-MORRIS, R., NORTH, R. A. & SCOPE CONSORTIUM 2009. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. *BMJ,* 338**,** b1081.
MCINTIRE, D. D., BLOOM, S. L., CASEY, B. M. & LEVENO, K. J. 1999. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med,* 340**,** 1234-1238.
MILLS, J. L., GRAUBARD, B. I., HARLEY, E. E., RHOADS, G. G. & BERENDES, H. W. 1984. Maternal alcohol consumption and birth weight. How much drinking during pregnancy is safe? *JAMA,* 252**,** 1875-9.
MISTRY, H. D., KURLAK, L. O., YOUNG, S. D., BRILEY, A. L., PIPKIN, F. B., BAKER, P. N. & POSTON, L. 2014. Maternal selenium, copper and zinc concentrations in pregnancy associated with small-for-gestational-age infants. *Matern Child Nutr,* 10**,** 327-34.
MISTRY, H. D. & WILLIAMS, P. J. 2011. The importance of antioxidant micronutrients in pregnancy. *Oxid Med Cell Longev,* 2011**,** 841749.
MORAITIS, A. A., WOOD, A. M., FLEMING, M. & SMITH, G. C. 2014. Birth weight percentile and the risk of term perinatal death. *Obstet Gynecol,* 124**,** 274-83.

MORRIS, R. K., CNOSSEN, J. S., LANGEJANS, M., ROBSON, S. C., KLEIJNEN, J., TER RIET, G., MOL, B. W., VAN DER POST, J. A. & KHAN, K. S. 2008. Serum Screening with Down's Syndrome Markers to Predict Pre-eclampsia and Small for gestational age: Systematic Review and Meta-analysis. *BMC Pregnancy and Childbirth,* 8**,** 33.

MORRIS, R. K., MELLER, C. H., TAMBLYN, J., MALIN, G. M., RILEY, R. D., KILBY, M. D., ROBSON, S. C. & KHAN, K. S. 2014. Association and prediction of amniotic fluid measurements for adverse pregnancy outcome: systematic review and meta-analysis. *Br J Obstet Gynaecol,* 121**,** 686-99.

MURPHY, V. E., SMITH, R., GILES, W. B. & CLIFTON, V. L. 2006. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev,* 27**,** 141-69.

MYERS, J. E., MERCHANT, S. J., MACLEOD, M., MIRES, G. J., BAKER, P. N. & DAVIDGE, S. T. 2005. MMP-2 levels are elevated in the plasma of women who subsequently develop preeclampsia. *Hypertens Pregnancy,* 24**,** 103-15.

NAEYE, R. L., BLANC, W., LEBLANC, W. & KHATAMEE, M. A. 1973. Fetal complications of maternal heroin addiction: abnormal growth, infections, and episodes of stress. *J Pediatr,* 83**,** 1055-61.

NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE 2008. *Antenatal Care: Routine care for the healthy pregnant woman.* London: NICE.

NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE 2010. *Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy.* London: NICE.

NEILSON, J. P. 2000. Symphysis-fundal height measurement in pregnancy. *Cochrane Database Syst Rev***,** CD000944.

NEVO, O., SOLEYMANLOU, N., WU, Y., XU, J., KINGDOM, J., MANY, A., ZAMUDIO, S. & CANIGGIA, I. 2006. Increased expression of sFlt-1 in in vivo and in vitro models of human placental hypoxia is mediated by HIF-1. *Am J Physiol Regul Integr Comp Physiol,* 291**,** R1085-93.

NEWSOME, C. A., SHIELL, A. W., FALL, C. H., PHILLIPS, D. I., SHIER, R. & LAW, C. M. 2003. Is birth weight related to later glucose and insulin metabolism?--A systematic review. *Diabet Med,* 20**,** 339-48.

NIELSEN, J. S. & MCNAGNY, K. M. 2009. The role of podocalyxin in health and disease. *J Am Soc Nephrol,* 20**,** 1669-76.

NIKNAFS, P. & SIBBALD, J. 2013. Accuracy of single ultrasound parameters in detection of fetal growth restriction. *Am J Perinatol,* 18**,** 325-334.

ODEGARD, R. A., VATTEN, L. J., NILSEN, S. T., SALVESEN, K. A. & AUSTGULEN, R. 2000. Preeclampsia and fetal growth. *Obstet Gynecol,* 96**,** 950-5.

ODIBO, A. O., CAHILL, A. G., ODIBO, L., ROEHL, K. & MACONES, G. A. 2011. Prediction of intrauterine fetal death in small-for gestational-age fetuses: impact of including ultrasound biometry in customized models. *Ultrasound Obstet Gynecol,* 39**,** 288-292.

ODUM, L., ANDERSEN, A. S. & HVIID, T. V. 2014. Urinary neutrophil gelatinase-associated lipocalin (NGAL) excretion increases in normal pregnancy but not in preeclampsia. *Clin Chem Lab Med,* 52**,** 221-5.

PAPASTEFANOU, I., SOUKA, A. P., PILALIS, A., ELEFTHERIADES, M., MICHALITSI, V. & KASSANOS, D. 2012. First trimester prediction of small- and large-for-gestation neonates by an integrated model

incorporating ultrasound parameters, biochemical indices and maternal characteristics. *Acta Obstet Gynecol Scand,* 91**,** 104-11.
PARK, J. E., CHEN, H. H., WINER, J., HOUCK, K. A. & FERRARA, N. 1994. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem,* 269**,** 25646-54.
PARK, J. K., KANG, M. Y., KIM, Y. H., JO, H. C., SHIN, J. K., CHOI, W. J., LEE, S. A., LEE, J. H., CHOI, W. S. & PAIK, W. Y. 2008. PKC delta in preeclamptic placentas promotes Bax dissociation from 14-3-3 zeta through 14-3-3 zeta phosphorylation. *Placenta,* 29**,** 584-92.
PARRA-CORDERO, M., LEES, C., MISSFELDER-LOBOS, H., SEED, P. & HARRIS, C. 2007. Fetal arterial and venous Doppler pulsatility index and time averaged velocity ranges. *Prenat Diagn,* 27**,** 1251-7.
PATRA, J., BAKKER, R., IRVING, H., JADDOE, V. W., MALINI, S. & REHM, J. 2011. Dose-response relationship between alcohol consumption before and during pregnancy and the risks of low birthweight, preterm birth and small for gestational age (SGA)-a systematic review and meta-analyses. *Br J Obstet Gynaecol,* 118**,** 1411-21.
PATTON, D. E., LEE, W., COTTON, D. B., MILLER, J., CARPENTER, R. J., JR., HUHTA, J. & HANKINS, G. 1990. Cyanotic maternal heart disease in pregnancy. *Obstet Gynecol Surv,* 45**,** 594-600.
PECKS, U., CASPERS, R., SCHIESSL, B., BAUERSCHLAG, D., PIROTH, D., MAASS, N. & RATH, W. 2012. The evaluation of the oxidative state of low-density lipoproteins in intrauterine growth restriction and preeclampsia. *Hypertens Pregnancy,* 31**,** 156-65.
PEREIRA, L., PETITT, M., FONG, A., TSUGE, M., TABATA, T., FANG-HOOVER, J., MAIDJI, E., ZYDEK, M., ZHOU, Y., INOUE, N., LOGHAVI, S., PEPKOWITZ, S., KAUVAR, L. M. & OGUNYEMI, D. 2014. Intrauterine growth restriction caused by underlying congenital cytomegalovirus infection. *J Infect Dis,* 209**,** 1573-84.
PERSSON, B., STANGENBERG, M., LUNELL, N. O., BRODIN, U., HOLMBERG, N. G. & VACLAVINKOVA, V. 1986. Prediction of size of infants at birth by measurement of symphysis fundus height. *Br J Obstet Gynaecol,* 93**,** 206-211.
PHAM, T., STEELE, J., STAYBOLDT, C., CHAN, L. & BENIRSCHKE, K. 2006. Placental mesenchymal dysplasia is associated with high rates of intrauterine growth restriction and fetal demise: A report of 11 new cases and a review of the literature. *Am J Clin Pathol,* 126**,** 67-78.
PHILLIPS, D. I. 1996. Insulin resistance as a programmed response to fetal undernutrition. *Diabetologia,* 39**,** 1119-22.
PIHL, K., LARSEN, T., KREBS, L. & CHRISTIANSEN, M. 2008. First trimester maternal serum PAPP-A, β-hCG and ADAM12 in prediction of small-for-gestational-age fetuses. *Prenat Diagn,* 28**,** 1131-1135.
POON, L. C., NEKRASOVA, E., ANASTASSOPOULOS, P., LIVANOS, P. & NICOLAIDES, K. H. 2009. First-trimester maternal serum matrix metalloproteinase-9 (MMP-9) and adverse pregnancy outcome. *Prenat Diagn,* 29**,** 553-9.
POON, L. C. Y., CHELEMEN, T., GRANVILLANO, O., PANDEVA, I. & NICOLAIDES, K. 2008a. First-trimester maternal serum a disintegrin and metalloprotease 12 (ADAM12) and adverse pregnancy outcome. *Obstet Gynecol,* 112**,** 1082-1090.

POON, L. C. Y., SYNGELAKI, A., AKOLEKAR, R., LAI, J. & NICOLAIDES, K. H. 2013. Combined Screening for Preeclampsia and Small for Gestational Age at 11-13 Weeks. *Fetal diagnosis and therapy,* 33**,** 16-27.
POON, L. C. Y., ZARAGOZA, E., AKOLEKAR, R., ANAGNOSTOPOULOS, E. & NICOLAIDES, K. H. 2008b. Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11+0 to 13+6 weeks of gestation. *Prenat Diagn,* 28**,** 1110-1115.
POSTON, L., IGOSHEVA, N., MISTRY, H. D., SEED, P. T., SHENNAN, A. H., RANA, S., KARUMANCHI, S. A. & CHAPPELL, L. C. 2011. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. *Am J Clin Nutr,* 94**,** 1980S-1985S.
PRYOR, J., SILVA, P. A. & BROOKE, M. 1995. Growth, development and behaviour in adolescents born small-for-gestational-age. *J Paediatr Child Health,* 31**,** 403-7.
RAHIMI, Z., RAHIMI, Z., SHAHSAVANDI, M. O., BIDOKI, K. & REZAEI, M. 2013. MMP-9 (-1562 C:T) polymorphism as a biomarker of susceptibility to severe pre-eclampsia. *Biomarkers in Medicine,* 7**,** 93-98.
RAIJMAKERS, M. T. M., DECHEND, R. & POSTON, L. 2004. Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension,* 44**,** 374-380.
RAJAKUMAR, A., JEYABALAN, A., MARKOVIC, N., NESS, R., GILMOUR, C. & CONRAD, K. P. 2007. Placental HIF-1 alpha, HIF-2 alpha, membrane and soluble VEGF receptor-1 proteins are not increased in normotensive pregnancies complicated by late-onset intrauterine growth restriction. *Am J Physiol Regul Integr Comp Physiol,* 293**,** R766-74.
REDLINE, R. W. 2005. Severe fetal placental vascular lesions in term infants with neurologic impairment. *Am J Obstet Gynecol,* 192**,** 452-7.
REDLINE, R. W. 2008. Placental Pathology: A Systematic Approach with Clinical Correlations. *Placenta,* 29**,** 86-91.
REDLINE, R. W. & O'RIORDAN, M. A. 2000. Placental lesions associated with cerebral palsy and neurologic impairment following term birth. *Arch Pathol Lab Med,* 124**,** 1785-91.
REDMAN, C. W. G. & SARGENT, I. L. 2009. Placental Stress and Pre-eclampsia: A Revised View. *Placenta,* 30**,** 38-42.
RESNIK, R. 2002. Intrauterine growth restriction. *Obstet Gynecol,* 99**,** 490-6.
RICH-EDWARDS, J. W., COLDITZ, G. A., STAMPFER, M. J., WILLETT, W. C., GILLMAN, M. W., HENNEKENS, C. H., SPEIZER, F. E. & MANSON, J. E. 1999. Birthweight and the risk for type 2 diabetes mellitus in adult women. *Ann Intern Med,* 130**,** 278-84.
RINGHOLM, L., PEDERSEN-BJERGAARD, U., THORSTEINSSON, B., BOOMSMA, F., DAMM, P. & MATHIESEN, E. R. 2011. Atrial Natriuretic Peptide (ANP) in early pregnancy is associated with development of preeclampsia in type 1 diabetes. *Diabetes Res Clin Pract,* 93**,** e106-9.
ROBSON, S. C., MARTIN, W. L. & MORRIS, R. K. 2013. The Investigation and Management of the Small–for–Gestational–Age Fetus. *RCOG - Green top guidelines***,** 1-34.
ROEX, A., NIKPOOR, P., VAN EERD, E., HODYL, N. & DEKKER, G. 2012. Serial plotting on customised fundal height charts results in doubling of the antenatal detection of small for gestational age fetuses in nulliparous women. *Aust N Z J Obstet Gynaecol,* 52**,** 78-82.
ROLFO, A., MANY, A., RACANO, A., TAL, R., TAGLIAFERRO, A., IETTA, F., WANG, J., POST, M. & CANIGGIA, I. 2010. Abnormalities in oxygen

sensing define early and late onset preeclampsia as distinct pathologies. *PLoS One,* 5**,** e13288.
ROMERO, R., NIEN, J. K., ESPINOZA, J., TODEM, D., FU, W., CHUNG, H., KUSANOVIC, J. P., GOTSCH, F., EREZ, O., MAZAKI-TOVI, S., GOMEZ, R., EDWIN, S., CHAIWORAPONGSA, T., LEVINE, R. J. & KARUMANCHI, S. A. 2008. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *Journal of Maternal-Fetal and Neonatal Medicine,* 21**,** 9-23.
RONNENBERG, A. G., WANG, X., XING, H., CHEN, C., CHEN, D., GUANG, W., GUANG, A., WANG, L., RYAN, L. & XU, X. 2003. Low preconception body mass index is associated with birth outcome in a prospective cohort of Chinese women. *J Nutr,* 133**,** 3449-55.
ROSENBERG, K., GRANT, J. M. & HEPBURN, M. 1982. Antenatal detection of growth retardation: actual practice in a large maternity hospital. *Br J Obstet Gynaecol,* 89**,** 12-5.
ROVERE-QUERINI, P., ANTONACCI, S., DELL'ANTONIO, G., ANGELI, A., ALMIRANTE, G., CIN, E. D., VALSECCHI, L., LANZANI, C., SABBADINI, M. G., DOGLIONI, C., MANFREDI, A. A. & CASTIGLIONI, M. T. 2006. Plasma and tissue expression of the long pentraxin 3 during normal pregnancy and preeclampsia. *Obstet Gynecol,* 108**,** 148-55.
ROY, H., BHARDWAJ, S., BABU, M., JAUHIAINEN, S., HERZIG, K. H., BELLU, A. R., HAISMA, H. J., CARMELIET, P., ALITALO, K. & YLA-HERTTUALA, S. 2005. Adenovirus-mediated gene transfer of placental growth factor to perivascular tissue induces angiogenesis via upregulation of the expression of endogenous vascular endothelial growth factor-A. *Hum Gene Ther,* 16**,** 1422-8.
SABOGAL, J. 2007. *Fetal growth restriction,* London, Informa Healthcare.
SALEH, S., ANTONIOU, A., HARRINGTON, K. & AQUILINA, J. 2010. Second trimester maternal serum cystatin C levels in preeclamptic and normotensive pregnancies: A small case-control study. *Hypertens Pregnancy,* 29**,** 112-9.
SANKARALINGAM, S., XU, H., JIANG, Y., SAWAMURA, T. & DAVIDGE, S. T. 2009. Evidence for increased methylglyoxal in the vasculature of women with preeclampsia: role in upregulation of LOX-1 and arginase. *Hypertension,* 54**,** 897-904.
SCHIEVE, L. A., MEIKLE, S. F., FERRE, C., PETERSON, H. B., JENG, G. & WILCOX, L. S. 2002. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med,* 346**,** 731-7.
SCHOENFELD, J., LESSAN, K., JOHNSON, N. A., CHARNOCK-JONES, D. S., EVANS, A., VOURVOUHAKI, E., SCOTT, L., STEPHENS, R., FREEMAN, T. C., SAIDI, S. A., TOM, B., WESTON, G. C., ROGERS, P., SMITH, S. K. & PRINT, C. G. 2004. Bioinformatic analysis of primary endothelial cell gene array data illustrated by the analysis of transcriptome changes in endothelial cells exposed to VEGF-A and PlGF. *Angiogenesis,* 7**,** 143-56.
SCHOTHORST, P. F. & VAN ENGELAND, H. 1996. Long-term behavioral sequelae of prematurity. *J Am Acad Child Adolesc Psychiatry,* 35**,** 175-83.

SECHER, N. J., HANSEN, P. K., LENSTRUP, C., ERIKSEN, P. S., THOMSEN, B. L. & KEIDING, N. 1987. On the evaluation of routine ultrasound screening in the third trimester for detection of light for gestational age (LGA) infants. *Acta Obstetricia et Gynecologica Scandinavica,* 66**,** 463-471.

SHAARAWY, M. & ABDEL-MAGID, A. M. 2000. Plasma endothelin-1 and mean arterial pressure in the prediction of pre-eclampsia. *International Journal of Gynaecology and Obstetrics,* 68**,** 105-111.

SHAARAWY, M., AL-SOKKARY, F., SHEBA, M., WAHBA, O., KANDIL, H. O. & ABDEL-MOHSEN, I. 2005. Angiogenin and vascular endothelial growth factor in pregnancies complicated by preeclampsia. *International Journal of Gynecology and Obstetrics,* 88**,** 112-117.

SHIBATA, E., RAJAKUMAR, A., POWERS, R. W., LARKIN, R. W., GILMOUR, C., BODNAR, L. M., CROMBLEHOLME, W. R., NESS, R. B., ROBERTS, J. M. & HUBEL, C. A. 2005. Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. *Journal of Clinical Endocrinology & Metabolism,* 90**,** 4895-4903.

SIBIUDE, J., GUIBOURDENCHE, J., DIONNE, M.-D., LE RAY, C., ANSELEM, O., SERREAU, R., GOFFINET, F. & TSATSARIS, V. 2012. Placental Growth Factor for the Prediction of Adverse Outcomes in Patients with Suspected Preeclampsia or Intrauterine Growth Restriction. *PLoS ONE,* 7**,** e50208.

SMITH, G. C., STENHOUSE, E. J., CROSSLEY, J. A., AITKEN, D. A., CAMERON, A. D. & CONNOR, J. M. 2002. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *Journal of Clinical Endocrinology & Metabolism,* 87**,** 1762-1767.

SMITH, G. C. S., CROSSLEY, J. A., AITKEN, D. A., JENKINS, N., LYALL, F., CAMERON, A. D., CONNOR, J. M. & DOBBIE, R. 2007. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. *Obstet Gynecol,* 109**,** 1316-1324.

SMITH, G. D., HARDING, S. & ROSATO, M. 2000. Relation between infants' birth weight and mothers' mortality: prospective observational study. *BMJ,* 320**,** 839-40.

SNIJDERS, R. J., SHERROD, C., GOSDEN, C. M. & NICOLAIDES, K. H. 1993. Fetal growth retardation: associated malformations and chromosomal abnormalities. *Am J Obstet Gynecol,* 168**,** 547-55.

SOUKA, A. P., PAPASTEFANOU, I., PILALIS, A., MICHALITSI, V. & KASSANOS, D. 2012. Performance of third-trimester ultrasound for prediction of small-for-gestational-age neonates and evaluation of contingency screening policies. *Ultrasound Obstet Gynecol,* 39**,** 535-42.

SOVIO, U., SMITH, G. & DACEY, A. 2014. Level 1 evidence for the diagnostic effectiveness of routine sonography as a screening test for small for gestational age (SGA) infants. *Am J Obstet Gynecol,* 210(S)**,** S408.

SPENCER, K., COWANS, N. J. & STAMATOPOULOU, A. 2008. ADAM12s in maternal serum as a potential marker of pre-eclampsia. *Prenatal Diagnosis,* 28**,** 212-216.

STAMPALIJA, T., GYTE, G. M. & ALFIREVIC, Z. 2010. Utero-placental Doppler ultrasound for improving pregnancy outcome. *Cochrane Database Syst Rev*, CD008363.
STEINBERG, G., LEE, C., RAUH-HAIN, J. A., ECKER, J., KHANKIN, E. V., HSU, C. D., COHEN, B., RANA, S., KARUMANCHI, S. A., THADHANI, R., HACKER, M. R. & LIM, K. H. 2010. Early-pregnancy soluble Fas levels in idiopathic small-for-gestational-age pregnancies. *Am J Obstet Gynecol,* 202, 299.e1-e7.
STEPAN, H., UNVERSUCHT, A., WESSEL, N. & FABER, R. 2007. Predictive Value of Maternal Angiogenic Factors in Second Trimester Pregnancies With Abnormal Uterine Perfusion. *Hypertension,* 49, 818-824.
STRAUSS, R. S. & DIETZ, W. H. 1998. Growth and development of term children born with low birth weight: effects of genetic and environmental factors. *The Journal of Pediatrics,* 133, 67-72.
SURVEILLANCE OF CEREBRAL PALSY IN EUROPE 2000. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). *Dev Med Child Neurol,* 42, 816-24.
SZABO, G., MOLVAREC, A., STENCZER, B., RIGO, J., JR. & NAGY, B. 2011. Natriuretic peptide precursor B gene (TTTC)(n) microsatellite polymorphism in pre-eclampsia. *Clin Chim Acta,* 412, 1371-5.
TAYLOR, R. 2003. Longitudinal serum concentrations of placental growth factor: Evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol,* 188, 177-182.
TEJANI, N. A. 1982. Recurrence of intrauterine growth retardation. *Obstet Gynecol,* 59, 329-31.
THADHANI, R., MUTTER, W. P., WOLF, M., LEVINE, R. J., TAYLOR, R. N., SUKHATME, V. P., ECKER, J. & KARUMANCHI, S. A. 2004. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *Journal of Clinical Endocrinology & Metabolism,* 89, 770-775.
THILAGANATHAN, B., RALPH, E., PAPAGEORGHIOU, A. T., MELCHIORRE, K. & SHELDON, J. 2009. Raised maternal serum cystatin C: an early pregnancy marker for preeclampsia. *Reprod Sci,* 16, 788-93.
THILAGANATHAN, B., WORMALD, B., ZANARDINI, C., SHELDON, J., RALPH, E. & PAPAGEORGHIOU, A. T. 2010. Early-pregnancy multiple serum markers and second-trimester uterine artery Doppler in predicting preeclampsia. *Obstet Gynecol,* 115, 1233-8.
TJOA, M. L., VAN VUGT, J. M., MULDERS, M. A., SCHUTGENS, R. B., OUDEJANS, C. B. & VAN WIJK, I. J. 2001. Plasma placenta growth factor levels in midtrimester pregnancies. *Obstet Gynecol,* 98, 600-607.
UK NATIONAL SCREENING COMMITTEE 2011. The UK NSC recommendation on Pre-eclampsia screening in pregnancy. *Public Health England*, 1-4.
UNICEF 2004. State of the World's Children 2005. New York: UNICEF.
UNTERSCHEIDER, J., DALY, S., GEARY, M. P., KENNELLY, M. M., MCAULIFFE, F. M., O'DONOGHUE, K., HUNTER, A., MORRISON, J. J., BURKE, G., DICKER, P., TULLY, E. C. & MALONE, F. D. 2013. Optimizing the definition of intrauterine growth restriction: the multicenter prospective PORTO Study. *Am J Obstet Gynecol,* 208, 290 e1-6.
UZUN, H., BENIAN, A., MADAZLI, R., TOPCUOGLU, M. A., AYDIN, S. & ALBAYRAK, M. 2005. Circulating oxidized low-density lipoprotein and

paraoxonase activity in preeclampsia. *Gynecol Obstet Invest,* 60**,** 195-200.
VANDENBERGHE, G., MENSINK, I., TWISK, J. W. R., BLANKENSTEIN, M. A., HEIJBOER, A. C. & VAN VUGT, J. M. G. 2011. First trimester screening for intra-uterine growth restriction and early-onset pre-eclampsia. *Prenat Diagn,* 31**,** 955-961.
VEDMEDOVSKA, N., REZEBERGA, D., TEIBE, U., MELDERIS, I. & DONDERS, G. G. 2011. Placental pathology in fetal growth restriction. *Eur J Obstet Gynecol Reprod Biol,* 155**,** 36-40.
VENKATESHA, S., TOPORSIAN, M., LAM, C., HANAI, J., MAMMOTO, T., KIM, Y. M., BDOLAH, Y., LIM, K. H., YUAN, H. T., LIBERMANN, T. A., STILLMAN, I. E., ROBERTS, D., D'AMORE, P. A., EPSTEIN, F. H., SELLKE, F. W., ROMERO, R., SUKHATME, V. P., LETARTE, M. & KARUMANCHI, S. A. 2006. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med,* 12**,** 642-9.
VILLAR, J., PAPAGEORGHIOU, A. T., PANG, R., OHUMA, E. O., ISMAIL, L. C., BARROS, F. C., LAMBERT, A., CARVALHO, M., JAFFER, Y. A., BERTINO, E., GRAVETT, M. G., ALTMAN, D. G., PURWAR, M., FREDERICK, I. O., NOBLE, J. A., VICTORA, C. G., BHUTTA, Z. A., KENNEDY, S. H. & FOR THE INTERNATIONAL FETAL NEWBORN GROWTH CONSORTIUM FOR THE 21ST CENTURY 2014. The likeness of fetal growth and newborn size across non-isolated populations in the INTERGROWTH-21 Project: the Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. *Lancet Diabetes Endocrinol*.
WALKER, D.-M. & MARLOW, N. 2007. Neurocognitive outcome following fetal growth restriction. *Archives of Disease in Childhood - Fetal and Neonatal Edition,* 93**,** F322-F325.
WALLNER, W., SENGENBERGER, R., STRICK, R., STRISSEL, P. L., MEURER, B., BECKMANN, M. W. & SCHLEMBACH, D. 2007. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clinical Science,* 112**,** 51.
WANG, L., FENG, Y., ZHANG, Y., ZHOU, H., JIANG, S., NIU, T., WEI, L. J., XU, X., XU, X. & WANG, X. 2006. Prolylcarboxypeptidase gene, chronic hypertension, and risk of preeclampsia. *Am J Obstet Gynecol,* 195**,** 162-71.
WHO. 2006a. Assessment of differences in linear growth among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl,* 450**,** 56-65.
WHO. 2006b. Enrolment and baseline characteristics in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl,* 450**,** 7-15.
WHO. 2006c. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl,* 450**,** 76-85.
WILCOX, M. A., SMITH, S. J., JOHNSON, I. R., MAYNARD, P. V. & CHILVERS, C. E. 1995. The effect of social deprivation on birthweight, excluding physiological and pathological effects. *Br J Obstet Gynaecol,* 102**,** 918-24.
WU, Y. W., MARCH, W. M., CROEN, L. A., GRETHER, J. K., ESCOBAR, G. J. & NEWMAN, T. B. 2004. Perinatal stroke in children with motor impairment: a population-based study. *Pediatrics,* 114**,** 612-9.

XIONG, X., DEMIANCZUK, N. N., SAUNDERS, L. D., WANG, F. L. & FRASER, W. D. 2002. Impact of preeclampsia and gestational hypertension on birth weight by gestational age. *Am J Epidemiol,* 155**,** 203-9.
YAMASHIRO, C., OHNISHI, Y., KANENISHI, K., HAYASHI, K., YANAGIHARA, T. & HATA, T. 2000. Maternal and fetal circulating angiogenin levels in pregnancies with small-for-gestational-Age and appropriate-for-gestational-Age infants. *Gynecol Obstet Invest,* 49**,** 156-9.
YANG, W., AHN, H., HINRICHS, M., TORRY, R. J. & TORRY, D. S. 2003. Evidence of a novel isoform of placenta growth factor (PlGF-4) expressed in human trophoblast and endothelial cells. *J Reprod Immunol,* 60**,** 53-60.
YASMEEN, S., WILKINS, E. E., FIELD, N. T., SHEIKH, R. A. & GILBERT, W. M. 2001. Pregnancy outcomes in women with systemic lupus erythematosus. *J Matern Fetal Med,* 10**,** 91-6.
YASUDA, M., TAKAKUWA, K., TOKUNAGA, A. & TANAKA, K. 1995. Prospective studies of the association between anticardiolipin antibody and outcome of pregnancy. *Obstet Gynecol,* 86**,** 555-9.
YINON, Y., NEVO, O., XU, J., MANY, A., ROLFO, A., TODROS, T., POST, M. & CANIGGIA, I. 2008. Severe intrauterine growth restriction pregnancies have increased placental endoglin levels: hypoxic regulation via transforming growth factor-beta 3. *Am J Pathol,* 172**,** 77-85.
ZALEL, Y., WEISZ, B., GAMZU, R., SCHIFF, E., SHALMON, B. & ACHIRON, R. 2002. Chorioangiomas of the placenta: sonographic and Doppler flow characteristics. *J Ultrasound Med,* 21**,** 909-13.
ZENG, X., SUN, Y., YANG, H., LI, D., LI, Y., LIAO, Q. & WANG, Y. 2009. Plasma level of soluble c-Met is tightly associated with the clinical risk of preeclampsia. *Am J Obstet Gynecol,* 201**,** 618.e1-618.e7.
ZHENG, M., LV, L. L., NI, J., NI, H. F., LI, Q., MA, K. L. & LIU, B. C. 2011. Urinary podocyte-associated mRNA profile in various stages of diabetic nephropathy. *PLoS One,* 6**,** e20431.
ZHU, B. P., ROLFS, R. T., NANGLE, B. E. & HORAN, J. M. 1999. Effect of the interval between pregnancies on perinatal outcomes. *N Engl J Med,* 340**,** 589-94.

Appendix

Hypertension

Diagnostic Accuracy of Placental Growth Factor in Women With Suspected Preeclampsia

A Prospective Multicenter Study

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Background—Hypertensive disorders of pregnancy are a major contributor to death and disability for pregnant women and their infants. The diagnosis of preeclampsia by using blood pressure and proteinuria is of limited use because they are tertiary, downstream features of the disease. Placental growth factor (PlGF) is an angiogenic factor, a secondary marker of associated placental dysfunction in preeclampsia, with known low plasma concentrations in the disease.

Methods and Results—In a prospective multicenter study, we studied the diagnostic accuracy of low plasma PlGF concentration (<5th centile for gestation, Alere Triage assay) in women presenting with suspected preeclampsia between 20 and 35 weeks' gestation (and up to 41 weeks' gestation as a secondary analysis). The outcome was delivery for confirmed preeclampsia within 14 days. Of 625 women, 346 (55%) developed confirmed preeclampsia. In 287 women enrolled before 35 weeks' gestation, PlGF <5th centile had high sensitivity (0.96; 95% confidence interval, 0.89–0.99) and negative predictive value (0.98; 0.93–0.995) for preeclampsia within 14 days; specificity was lower (0.55; 0.48–0.61). Area under the receiver operating characteristic curve for low PlGF (0.87, standard error 0.03) for predicting preeclampsia 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 preeclampsia (P<0.001 for all comparisons).

Conclusions—In women presenting before 35 weeks' gestation with suspected preeclampsia, low PlGF has high sensitivity and negative predictive value for preeclampsia within 14 days, is better than other currently used tests, and presents an innovative adjunct to management of such women. **(*Circulation*. 2013;128:2121-2131.)**

Key Words: angiogenesis inducers ■ diagnosis ■ hypertension ■ pregnancy

Preeclampsia complicates 2% to 8% of all pregnancies and is characterized by placental and maternal vascular dysfunction and associated adverse outcomes.[1] 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.[2] This clinical uncertainty leads to overuse of ancillary testing and intervention, with associated expense of antenatal monitoring and inpatient admissions, placing considerable burden on pregnant women and their families. In the United States, preeclampsia is the most common reason for iatrogenic preterm delivery.[3] Evaluation of biomarkers and imaging techniques has shown that none have adequate sensitivity, specificity, and convenience for diagnosis or prediction of preeclampsia or complications,[2,4] the majority identifying advanced disease with established end-organ damage.

Clinical Perspective on p 2131

Recent advances in understanding preeclampsia and fetal growth restriction have elucidated important biological roles for placentally derived angiogenic factors.[5] In normal pregnancy, placental growth factor (PlGF), synthesized by syncytiotrophoblast,[6] increases with gestation in maternal circulation, with concentrations peaking at 26 to 30 weeks[7] and declining toward term. PlGF is abnormally low in women with preeclampsia in comparison with gestational age-matched controls[8] and is reduced further in severe preeclampsia.[9] Development of a test for preeclampsia with the use of a pathophysiologically relevant biomarker, such as PlGF, may

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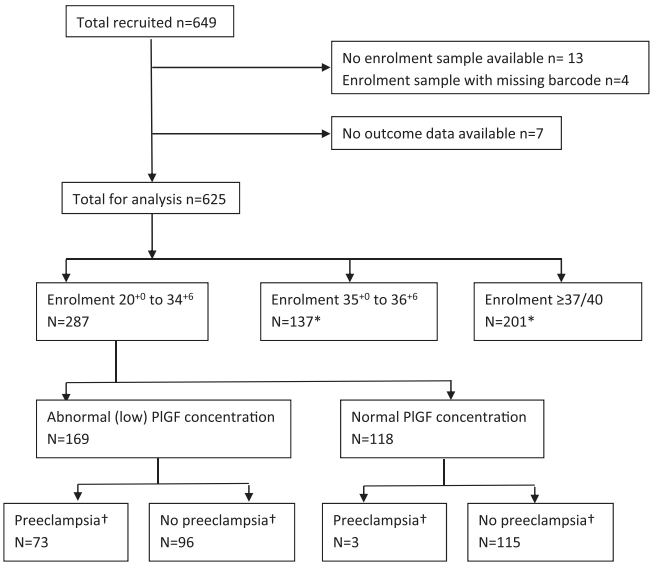


Figure 1. Flow diagram of participants. *Details given in Figure 2. †Preeclampsia requiring delivery within 14 days. PlGF indicates placental growth factor.

have advantages over blood pressure and urinary protein, which are the consequences of established disease. Because earlier gestation of preeclampsia onset is associated with greater maternal and perinatal risks,[1] and the difference in PlGF concentrations between normal and preeclamptic pregnancies is most marked before 35 weeks, PlGF has potential to aid the diagnosis of hypertensive disorders of pregnancy at gestations critical to clinical outcome. The most clinically relevant test for health professionals would identify women with preeclampsia associated with deteriorating disease requiring iatrogenic delivery. Because women with suspected hypertensive disease are routinely monitored every 2 weeks, a

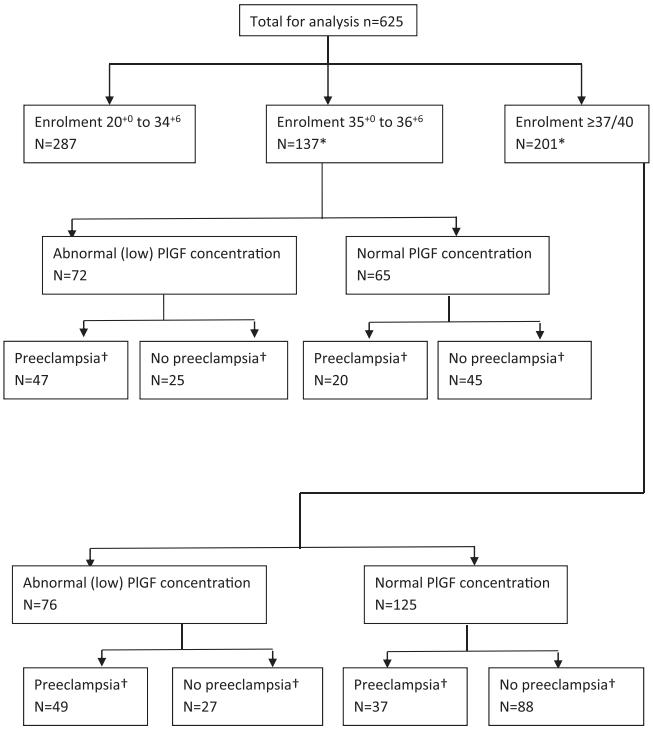


Figure 2. Flow diagram of participants in study enrolled after 35 weeks' gestation. †Preeclampsia requiring delivery within 14 days.

Table 1. Characteristics at Booking and Enrollment

	Gestation at Enrollment (weeks, days)		
	<35^{+0}	35^{+0} to 36^{+6}	≥37^{+0}
	n=287	n=137	n=201
Age, y, median (IQR)	31.9 (27.0–35.9)	32.4 (27.5–35.4)	32.1 (27.5–36.0)
Body mass index, kg/m^2, median (IQR)	28.6 (24.2–33.6)	28.6 (24.4–32.7)	26.9 (23.1–31.2)
Nulliparous	123 (43)	60 (44)	89 (44)
Singleton pregnancy	275 (96)	123 (90)	198 (99)
White ethnicity	187 (65)	88 (64)	151 (75)
Black ethnicity	70 (24)	27 (20)	25 (12)
Asian ethnicity	19 (7)	13 (9)	12 (6)
Other ethnicity	11 (4)	9 (7)	13 (7)
Highest 1st trimester systolic BP, mm Hg, median (IQR)	120 (110–130)	118 (110–127)	120 (108–123)
Highest 1st trimester diastolic BP, mm Hg, median (IQR)	74 (66–81)	70 (65–80)	72 (65–80)
Current smoking	24 (8)	10 (7)	19 (9)
Quit smoking	52 (19)	22 (17)	30 (15)
Never smoked	204 (73)	101 (76)	151 (76)
Previous medical history			
Previous preeclampsia	55 (20)	17 (12)	30 (15)
Previous preeclampsia requiring delivery <34/40	30 (11)	6 (4.4)	9 (4.5)
Chronic hypertension	45 (17)	10 (7.9)	8 (4.5)
Systemic lupus erythematosus/antiphospholipid syndrome	12 (4.5)	0	1 (0.6)
Pregestational diabetes mellitus	6 (2.2)	4 (3.2)	0
Renal disease	19 (7.1)	4 (3.2)	6 (3.4)
At enrollment in assessment unit			
Gestational age, wk, median (IQR)	31.0 (27.9–33.4)	36.0 (35.4–36.4)	38.4 (37.6–39.6)
Signs/ symptoms of suspected preeclampsia (non exclusive)			
New onset of hypertension	155 (54)	92 (67)	133 (66)
Worsening of underlying hypertension	56 (20)	21 (15)	39 (19)
New onset of dipstick proteinuria	161 (56)	85 (62)	108 (54)
Persistent epigastric/ right upper quadrant pain	18 (6)	8 (6)	13 (6)
Headaches	84 (29)	44 (32)	77 (38)
Suspected fetal growth restriction	25 (9)	4 (3)	2 (1)
Highest systolic BP, median (IQR)	144 (131–159)	144 (132–153)	145 (135–155)
Highest diastolic BP, median (IQR)	92 (82–100)	94 (86–100)	95 (87–100)
Dipstick proteinuria			
Not done	38 (13)	19 (14)	15 (8)
Negative	103 (36)	34 (25)	81 (40)
Present (1+ or greater)	146 (51)	84 (61)	105 (52)
Alanine transaminase, U/L, median (IQR)	14 (11–20) (n=248)	15 (11–21) (n=123)	14 (11–19) (n=177)
Creatinine, μmol/ L, median (IQR)	51 (44–62) (n=267)	55 (47–66) (n=128)	55 (49–64) (n=194)
Uric acid, μmol/ L, median (IQR)	257 (189–330) (n=188)	315 (237–360) (n=96)	310 (253–380) (n=149)
Platelet count, 10^9/L, median (IQR)	233 (196–271) (n=269)	213 (175–263) (n=132)	215 (177–270) (n=194)

Values are given as number (percentage) unless stated otherwise. BP indicates blood pressure; and IQR, interquartile range.

test should be applicable for a subsequent 14-day window to impact management strategies.

The primary aim of this study was to evaluate the diagnostic accuracy of plasma PlGF concentrations in women presenting with suspected preeclampsia between 20 and 35 weeks' gestation (up to 40^{+6} weeks as a secondary analysis) in determining the need for delivery for preeclampsia within 14 days of testing (preeclampsia-D14).

Methods

This prospective observational study was undertaken between January 2011 and February 2012 in 7 consultant-led maternity units in the

Table 2. Characteristics at Delivery for Women in Each Gestational Age Group

	Gestation at Enrollment (weeks, days)		
	<35^{+0}	35^{+0} to 36^{+6}	≥37^{+0}
Total number of women	n=287	n=137	n=201
Maternal characteristics			
Final diagnosis (exclusive), preeclampsia	176 (61)	81 (59)	89 (44)
Final diagnosis (exclusive)			
Mild preeclampsia	25 (9)	24 (18)	40 (20)
Severe preeclampsia	76 (26)	31 (23)	23 (11)
Superimposed preeclampsia	40 (11)	10 (6)	7 (3)
Atypical preeclampsia	32 (14)	15 (12)	19 (9)
Eclampsia	1 (0)	1 (1)	0 (0)
HELLP syndrome	2 (1)	0 (0)	0 (0)
Gestational hypertension	27 (9)	14 (10)	42 (21)
Chronic hypertension only	28 (10)	9 (7)	18 (9)
Isolated proteinuria only	10 (3)	6 (4)	10 (5)
Isolated SGA (<10th customized birthweight centile)	8 (3)	3 (2)	5 (2)
Transient hypertension	14 (5)	17 (12)	24 (12)
Normal	22 (8)	5 (4)	12 (6)
Other	2 (1)	2 (1)	1 (0)
Antihypertensive use			
1 drug	51 (18)	31 (23)	42 (21)
2 drugs	53 (18)	9 (7)	16 (8)
≥3 drugs	19 (7)	8 (6)	7 (4)
Magnesium sulfate use	6 (2)	4 (3)	0
Onset of labor			
Spontaneous labor	42 (15)	25 (19)	59 (29)
Induced labor	108 (38)	75 (55)	111 (55)
Prelabor cesarean delivery	134 (47)	36 (26)	31 (16)
Adverse maternal outcome*	122 (43)	44 (32)	53 (26)
Neonatal characteristics	n=299	n=151	n=204
Gestation at delivery, wk, median (IQR)	36.7 (33.6–38.6)	37.3 (36.6–38.4)	39.4 (38.6–40.3)
Preterm delivery <37/40	158 (53)	55 (36)	0
Mode of delivery			
Spontaneous vaginal delivery	72 (27)	54 (41)	98 (50)
Assisted vaginal delivery	31 (11)	13 (9.9)	29 (15)
Cesarean delivery	169 (62)	64 (49)	70 (35)
Fetal death	7	0	1
Neonatal death	2	0	0
Birth weight, g, median (IQR)	2420 (1620–3125)	2820 (2340–3340)	3278 (2980–3560)
SGA (<10th customized birth weight centile)	142 (47)	57 (38)	52 (25)
SGA (<3rd customized birth weight centile)	108 (36)	39 (26)	25 (12)
SGA (<1st customized birth weight centile)	78 (26)	19 (13)	15 (7.3)
Adverse perinatal outcome†	69 (23)	13 (8.6)	13 (6.4)

Values are given as number (percentage) unless stated otherwise. HELLP indicates hemolysis, elevated liver enzymes, and low platelet count; IQR, interquartile range; and SGA, small for gestational age infant.

*Defined in Table I in the online-only Data Supplement.

†Defined in Table II in the online-only Data Supplement.

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 ≥16 years of age. Symptoms or signs included headache, visual disturbances, epigastric or right upper quadrant pain, hypertension, dipstick proteinuria, 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 enrollment was not eligible. A woman could only be enrolled

Table 3. PlGF Concentrations in Women by Final Diagnosis and by Adverse Events, Stratified by Gestational Age Group

	Gestation at enrollment (weeks, days)		
	<35^{+0}	35^{+0} to 36^{+6}	≥37^{+0}
Total number of women	n=287	n=137	n=201
By diagnosis			
Mild preeclampsia	51 (20–228) n=25	29 (15–65) n=24	20 (12–30) n =40
Severe preeclampsia	10 (10–25) n =79	16 (10–28) n =32	15 (10–21) n =23
Superimposed preeclampsia	43 (10–432) n =40	54 (28–100) n =10	16 (10–120) n =7
Atypical preeclampsia	29 (10–106) n =32	14 (12–52) n =15	34 (14–73) n =19
Gestational hypertension	153 (59–407) n =27	29 (23–97) n =14	27 (20–64) n =42
All other diagnoses	291 (143–542) n =84	104 (36–273) n =42	52 (28–116) n =7
By adverse events			
No event	107 (20–365) n=168	40 (15–146) n=95	31 (15–81) n=150
Systolic blood pressure ≥160 mm Hg only	32 (10–140) n=80	25 (14–51) n=28	21 (16–31) n=31
All other adverse events	19 (10–132) n=39	36 (15–100) n=14	29 (10–92) n=20

The values stated are PlGF concentrations (pg/mL), median (IQR). IQR indicates interquartile range; and PlGF, placental growth factor.

once. Written informed consent was obtained, and baseline demographic and pregnancy-specific information was entered onto the study database (finalized before the first participant being enrolled). Fifteen milliliters of blood (additional to routine blood samples) were drawn into ethylenediamine tetra-acetic acid and transported to the laboratory within 1 hour, and plasma stored until analysis (−80°C). Pregnancy outcome details for mother and infant were obtained from case note and electronic database review. Participants were recruited until sufficient numbers in each prespecified gestational age range had been reached. The study was approved by East London Research Ethics Committee (ref. 10/H0701/117).

Definitions and outcomes were prespecified in the study protocol. The primary analysis was of diagnostic accuracy of low plasma PlGF (<5th centile for gestational age) to predict the need to deliver for preeclampsia within 14 days of testing in women with suspected preeclampsia before 35 weeks' gestation. The prespecified secondary analyses included women presenting later (35–36^{+6}; ≥37 weeks), or by 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; superimposed preeclampsia was defined as new-onset proteinuria in women with hypertension before 20 weeks, a sudden increase in proteinuria if already present in early gestation, a sudden increase in hypertension, or the development of hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome.[10] Atypical preeclampsia was defined by the International and Australasian Societies for the Study of Hypertension in Pregnancy[11] as gestational hypertension without proteinuria but with other multiorgan involvement or fetal growth restriction (<10th customized birthweight centile). The latter[12] was calculated by using the Gestation Related Optimal Weight method by freely available software.[13]

The final adjudicated diagnosis of pregnancy outcome was the reference standard for evaluating PlGF test accuracy. This was determined by 2 independent senior physicians requiring documentation of end points required to fulfill the diagnostic criteria; disagreement was resolved by a third adjudicator. All adjudicators were masked to PlGF values when assigning a final diagnosis; PlGF measurements were not revealed until all subject adjudication was complete. Ten percent of database records were verified against source data by an independent assessor.

Plasma samples were tested, using the Triage PlGF Test (Alere, San Diego, CA), at each study center according to the manufacturer's instructions. All meters were programmed for 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 labeled recombinant murine monoclonal antibodies and detects PlGF specifically and quantitatively, in the range of 12 to 3000 pg/mL, in ≈15 minutes. The Total Precision (coefficient of variation) on plasma controls at concentrations of 85 and 1300 pg/mL is 12.8% and 13.2%, respectively, based on the manufacturer's package insert generated before the study.

As prespecified, women were classified according to the gestation of the test, <35, 35 to 36^{+6}, and ≥37 weeks; the test result, normal (≥5th centile for gestation), low (<5th centile), and very low (<12 pg/mL); and the principal outcome, preeclampsia-D14. A positive test was PlGF concentration <5th centile for gestational age for normal controls (calculated from 247 women with normal pregnancies contributing 1366 samples between 20 and 40 weeks' gestation).[14] Test performance was evaluated as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and receiver operating characteristics (ROC) areas. Kaplan-Meier survival curves of gestational age at delivery were produced, treating data as left-censored before the gestation at which the test was conducted or at 24 weeks (to avoid very low numbers at gestation of the first delivery). The curves represent the probability of delivery conditional on no delivery before the gestation of the earliest test. Median and interquartile ranges for the time from PlGF test to delivery were calculated. Logistic regression was used to consider whether the utility of PlGF was limited to women delivering small for gestational age infants. Comparison of PlGF with other standard tests for preeclampsia (systolic and diastolic blood pressure, proteinuria, uric acid, alanine transaminase) was performed for the primary outcome by using unadjusted PlGF concentrations. The 4 tests (excluding proteinuria, which amounts to confirmation of diagnosis) were combined into a single predictor by using logistic regression, and ROC areas were compared for the prediction of the primary end point. For implementation to clinical practice in women <37 weeks' gestation, an exploratory analysis was conducted with the aim of identifying a single threshold (independent of gestation) with test performance statistics similar to 5th centile, while retaining the properties of a clinically relevant test (high sensitivity, high negative predictive value). We evaluated test biochemical reproducibility by analyzing all samples a second time in 1 central laboratory. The required sample sizes were calculated for accurate estimation of the sensitivity (within 10%) and specificity (within 6%) of PlGF in determining the primary end point. We assumed a sensitivity of 0.90, specificity 0.90, and 95% confidence intervals (2-tailed), requiring 62 preeclampsia cases and 150 nonpreeclamptic women. Because adjudication of the final diagnosis (after delivery) lagged behind enrollment, 287 women were recruited before 35 weeks' gestation before enrollment was stopped. Statistical analysis was performed in the statistical package Stata (version 11.2), College Station, TX. The study is reported in accordance with STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines.

Results

Between January 2011 and February 2012, 649 women were recruited (Figures 1 and 2). We recruited all those who were approached, eligible, and consented but did not document women who declined to take part. 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 in Table 1, maternal and infant outcomes are in Table 2, details of adverse maternal and perinatal outcomes

Table 4. Test Performance Statistics for Low PlGF in Prediction of Adverse Outcomes

	Gestation at Enrollment (weeks, days)		
	<35^{+0}	35^{+0} to 36^{+6}	≥37^{+0}
	n=287	n=137	n=201
PlGF <5th centile for gestation	Preeclampsia requiring delivery within 14 days		
Sensitivity n/N	0.96 (0.89–0.99) 73/76	0.70 (0.58–0.81) 47/67	0.57 (0.46–0.68) 49/86
Specificity n/N	0.55 (0.48–0.61) 115/211	0.64 (0.52–0.75) 45/70	0.77 (0.68–0.84) 88/115
Positive predictive value n/N	0.43 (0.36–0.51) 73/169	0.65 (0.53–0.76) 47/72	0.65 (0.53–0.75) 49/76
Negative predictive value n/N	0.98 (0.93–0.995) 115/118	0.69 (0.57–0.80) 45/65	0.70 (0.62–0.78) 88/125
Positive likelihood ratio	2.1 (1.8–2.5)	2.0 (1.4–2.8)	2.4 (1.7–3.5)
Negative likelihood ratio	0.07 (0.02–0.22)	0.46 (0.31–0.71)	0.56 (0.43–0.73)
PlGF <12 pg/mL	Preeclampsia requiring delivery within 14 days		
Sensitivity n/N	0.63 (0.51–0.74) 48/76	0.22 (0.13–0.34) 15/67	0.26 (0.17–0·36) 22/86
Specificity n/N	0.90 (0.85–0.94) 190/211	0.91 (0.82–0.97) 64/70	0.89 (0.81–0.94) 102/115
Positive predictive value n/N	0.70 (0.57–0.80) 48/69	0.71 (0.48–0.89) 15/21	0.63 (0.45–0.79) 22/35
Negative predictive value n/N	0.87 (0.82–0.91) 190/218	0.55 (0.46–0.64) 64/116	0.61 (0.54–0.69) 102/166
Positive likelihood ratio	6.4 (4.1–9.9)	2.6 (1.1–6.3)	2.3 (1.2–42)
Negative likelihood ratio	0.41 (0.30–0.55)	0.85 (0.73–0.98)	0.84 (0.73–0.97)
	Preeclampsia requiring delivery		
PlGF <100 pg/mL	Within 14 days	Before 37 wk	
	n=287	n=137	
Sensitivity n/N	0.96 (0.89–0.99) 73/76	0.95 (0.83–0.99) 37/39	
Specificity n/N	0.56 (0.49–0.63) 118/211	0.32 (0.22–0.42) 31/98	
Positive predictive value n/N	0.44 (0.36–0.52) 73/166	0.36 (0.26–0.44) 37/104	
Negative predictive value n/N	0.98 (0.93–0.995) 118/121	0.94 (0.80–0.99) 31/33	
Positive likelihood ratio	2.2 (1.9–2.6)	1.4 (1.2–1.6)	
Negative likelihood ratio	0.07 (0.02–0.22)	0.16 (0.04–0.64)	
PlGF <5th centile for gestation	Small for gestational age singleton infants <1st centile		
	n=275	n=123	
Sensitivity n/N	0.93 (0.84–0.98) 63/68	0.91 (0.59–0.99) 10/11	
Specificity n/N	0.53 (0.46–0.60) 110/207	0.51 (0.41–0.61) 57/112	
Positive predictive value n/N	0.39 (0.32–0.47) 63/160	0.15 (0.08–0.27) 10/65	
Negative predictive value n/N	0.96 (0.90–0.99) 110/115	0.98 (0.91–1.00) 57/58	
Positive likelihood ratio	2.0 (1.7–2.3)	1.9 (1.4–2.4)	
Negative likelihood ratio	0.14 (0.06–0.3)	0.2 (0.03–1.2)	

PlGF indicates placental growth factor.

are in Tables I and II in the online-only Data Supplement, respectively, and unadjusted PlGF concentrations by final diagnosis are shown in Table 3.

The diagnostic accuracy of PlGF for predicting preeclampsia-D14 (primary outcome) is shown in Table 4, with the use of prespecified thresholds of <5th centile and <12 pg/mL.

Table 5. False Negatives, Cases With Very Low PlGF and Term Delivery, and Antepartum Deaths in Women Presenting <35 Weeks' Gestation

Subject	Gestation (Sampling)	Gestation (Delivery)	[PlGF], pg/mL	Birth Weight	BW Centile	Final Adjudicated Diagnosis and Other Details
False negative (PlGF normal and delivered within 14 days of sampling with final diagnosis of preeclampsia)						
A	28^{+2}	29^{+5}	1224	1330	29	Superimposed preeclampsia; SPPROM, spontaneous labor, cesarean delivery
B	29^{+6}	30^{+0}	160	1095	1	Atypical preeclampsia; reduced fetal movements and prelabor cesarean delivery
C	33^{+2}	34^{+4}	218	2020	5	Severe preeclampsia; previous history of early-onset preeclampsia
PlGF very low and not delivered preterm <37/40						
D	33^{+6}	37^{+5}	<12	2900	34	Severe preeclampsia
E	34^{+1}	38^{+1}	<12	2350	3	Severe preeclampsia
F	34^{+2}	37^{+0}	<12	2310	5	Severe preeclampsia
G	34^{+2}	37^{+2}	<12	1805	0	Severe preeclampsia
Antepartum deaths						
H	23^{+0}	23^{+1}	<12	374	0	Severe preeclampsia
I	25^{+3}	26^{+6}	<12	690	0.5	Severe preeclampsia with placental abruption
J	27^{+5}	29^{+4}	<12	570	0	Superimposed preeclampsia
K	28^{+0}	30^{+2}	59	480	0	Twin pregnancy; severe preeclampsia, and discordant FGR
L	28^{+0}	35^{+4}	17	2210	1.7	Chronic hypertension with increase in blood pressure; FGR not suspected antenatally
M	30^{+4}	35^{+5}	<12	2220	12	Chronic hypertension with placental abruption
N	33^{+2}	38^{+6}	39	1900	0	Gestational hypertension; FGR not suspected antenatally

BW indicates birth; FGR, fetal growth restriction; and SPPROM, spontaneous preterm prelabor rupture of membranes.

All values, including outliers, were included. Low PlGF <5th centile 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 <100 pg/mL predicted preeclampsia-D14 or before 37 weeks' gestation (whichever was sooner) with sensitivity and negative predictive values similar to diagnostic accuracy estimates obtained by using a <5th centile cutoff (Table III in the online-only Data Supplement). Raw values of PlGF had higher ROC areas for determination of preeclampsia-D14 than PlGF categorized by centiles in women before 35 weeks' gestation (Figure I in the online-only Data Supplement).

PlGF <5th centile also had good test accuracy for predicting subsequent delivery of a small for gestational age infant <1st centile (not restricted to diagnosis within 14 days of testing). The sensitivity of PlGF for determining preeclampsia-D14 was similar if the infant was subsequently born appropriate-for-gestational age (before 35 weeks 0.94 [0.71–0.99]; 35^{+0} to 36^{+6} weeks 0.88 [0.73–0.97]). There was no interaction between PlGF and small for gestational age as a predictor of preeclampsia-D14 on formal testing with the use of logistic regression.

For women presenting before 35 weeks' gestation, there were 3 cases with false-negative results (≥5th centile), all with an additional indication for early delivery; 4 cases with PlGF <12 pg/mL were delivered after 37 weeks with severe preeclampsia, 3 of whom delivered infants ≤5th customized birthweight centile, suggesting placental disease (Table 5). PlGF was <5th centile in all cases and <12 pg/mL in 4 of the 7 cases of antepartum fetal death (occurring after enrollment; Table 5). PlGF <5th centile predicted intrauterine fetal death with sensitivity 1.00 (95% confidence interval, 0.71–1.00); specificity 0.48 (0.44–0.52); positive predictive value 0.03 (0.02–0.05); negative predictive value 1.00 (0.99–1.00). In 5 cases, testing at enrollment predated Doppler ultrasound abnormalities by 7 to 39 days and predated the stillbirth by 10 to 53 days.

The area under the ROC curve for low PlGF in predicting preeclampsia-D14 was greater than all other commonly used tests, either singly or in combination ($P<0.001$ for all comparisons; Figure 3). The addition of blood pressure or other blood tests currently used did not increase the ROC area further in comparison with PlGF alone. PlGF was a consistently good predictor of preeclampsia-D14 in women with and without ≥ 1+ dipstick proteinuria. The times (in days) to delivery for the 3 PlGF groups are presented for women with all diagnoses and for preeclampsia cases (Figure 4).

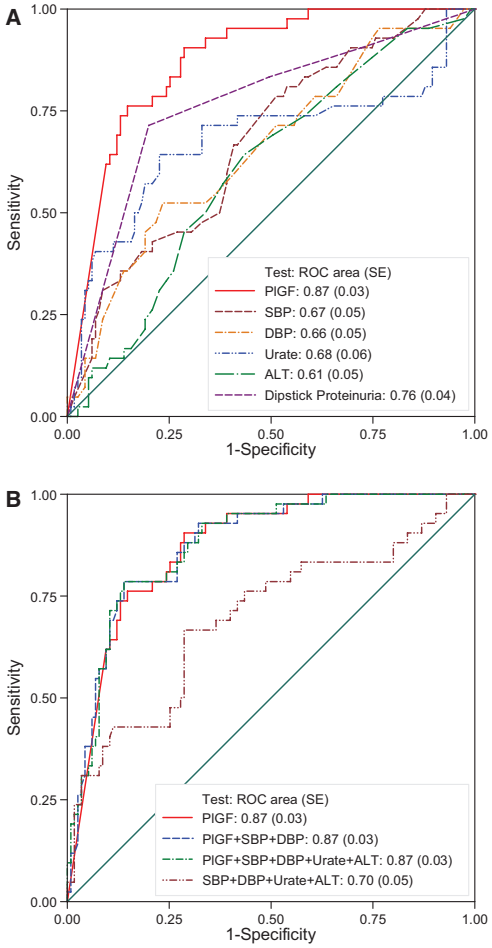


Figure 3. ROC areas (standard error) for PlGF compared with 5 other signs/tests (systolic and diastolic blood pressure, uric acid, alanine transaminase, and proteinuria) in determining preeclampsia requiring delivery within 14 days in 176 women presenting before 35+0 weeks gestation with all tests measured using parameters singly (**A**) or in combination (**B**). ALT indicates alanine transaminase; DBP, diastolic blood pressure; PlGF, placental growth factor; ROC, receiver operating characteristics; and SBP, systolic blood pressure.

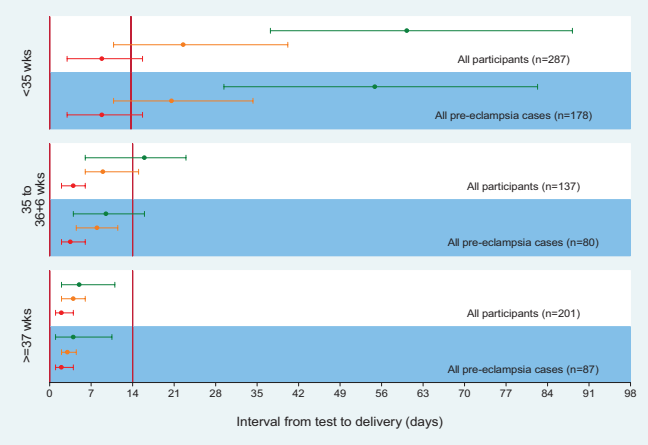
For test reproducibility, 595 paired samples were measured both at the study site and at the central laboratory (30 results were unevaluable owing to error codes, or mismatched sample identifications). Four hundred twenty-five were in-range (between 12 and 3000 pg/mL) on both evaluations and had a Pearson correlation coefficient of 0.950. For all 595 PlGF test pairs (including out-of-range values), the Spearman (rank) correlation coefficient was 0.948. A Bland-Altman plot is presented in Figure II in the online-only Data Supplement. Of women evaluated, 85.4% would receive the same classification in both laboratories; 11.1% moved between low and very low (in either direction), and a further 3.5% moved between low and normal. No woman moved between very low and normal. The sensitivity and specificity of the test in predicting the primary outcome were changed by <1% when 29 twin pregnancies were excluded. There were no adverse events associated with the collection of the blood necessary for performing PlGF testing.

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 ≤100 pg/mL) has high sensitivity and negative predictive value in determining which women presenting with suspected disease at <35 weeks' gestation are likely to need delivery for preeclampsia within 14 days. A previous review has highlighted the need for a test with high sensitivity in this setting, because there is greater preference for minimizing false negatives when considering overall benefits and harms and in ensuring appropriate resource use.[15] 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 the need for delivery than other commonly used signs and tests, either singly or in combination, in current clinical practice. Sensitivity and negative predictive values were also high for delivery of an small for gestational age infant <1st centile; this indicator is most likely to equate to fetal growth restriction of placental origin and to 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 <100 pg/mL) for whom stratified surveillance is also advantageous and the risks/benefits of delivery remain uncertain.

The strengths of this study include the use of multiple centers encompassing a wide demographic and ethnic profile and a pragmatic approach to enrollment with minimal exclusion criteria, enabling generalizability. The main research question was chosen to be clinically relevant, with the use of a primary outcome where delivery was indicated for the mother or infant, despite being preterm. Final diagnoses were independently adjudicated by 2 senior clinicians following database record review with the use of 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 prespecified methods and outcomes, with subsequent transparent evaluation of a single PlGF 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[16]; components of this composite (other than blood transfusion) are reported in our study. Maternal plasma PlGF normally declines in the latter part of the third trimester, reducing test performance at >35 weeks' gestation; an ideal test would maintain separation between preeclamptic cases and other women, which is probably unachievable by using a single biomarker at



Gestational age at testing (weeks, days)	< 35+0	35+0 to 36+6	≥37+0
Total number of women	N=287	N=137	N=201
Delivery for all diagnoses	29 (11 to 59) (n=287)	10 (5 to 19) (n=137)	4 (2 to 9) (n=201)
Very low PlGF (<12pg/ml)	9 (3 to 16) (n=69)	4 (2 to 9) (n=21)	2 (1 to 4) (n=35)
Low PlGF (<5th centile)	23 (11 to 40) (n=100)	9 (6 to 15) (n=51)	4 (2 to 7) (n=41)
Normal PlGF (≥5th centile)	62 (38 to 90) (n=118)	16 (6 to 23) (n=65)	5 (2 to 11) (n=125)
Delivery for preeclampsia	19 (7 to 33) (n=176)	7 (3 to 12) (n=81)	3 (1 to 5) (n=89)
Very low PlGF (<12pg/ml)	9 (3 to 16) (n=67)	4 (2 to 8) (n=18)	2 (1 to 4) (n=23)
Low PlGF (<5th centile)	21 (11 to 35) (n=74)	8 (4 to 12) (n=36)	3 (2 to 5) (n=27)
Normal PlGF (≥5th centile)	49 (29 to 84) (n=35)	9 (4 to 16) (n=27)	4 (1 to 10) (n=39)

Figure 4. Time to delivery (median, IQR) stratified by PlGF concentration for all participants and for preeclampsia cases. Red line indicates very low PlGF (<12 pg/mL); orange line, low PlGF (<5th centile); green line, normal PlGF (≥5th centile). The numbers in the table below relate to the figure bars. IQR indicates interquartile range; and PlGF, placental growth factor

all gestations. More accurate determination of very low PlGF 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 prespecified threshold of <5th centile (low PlGF, or PlGF <100 pg/m:) rather than very low PlGF.

This is the largest, and the first prospective multicenter, study to evaluate PlGF in women with suspected preeclampsia. Other studies have evaluated PlGF and other factors including soluble Flt-1 (sFlt-1; soluble fms-like tyrosine kinase-1), a trophoblast derived antiangiogenic factor that is increased in plasma from preeclamptic women. A case-control study[7] and a small prospective observational study[17] using the Triage assay reported promising test performance. A more recent study using a different assay for sflt-1/PlGF 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 <34 weeks' gestation,[18] a level of sensitivity that is unlikely to be useful in clinical practice. Direct comparison of assays in 128 pregnant women (44 with preeclampsia) confirmed higher sensitivity of the Triage test than the sflt-1/PlGF ratio (Elecsys) in diagnosing early-onset preeclampsia,[19] which may relate to different target epitopes of PlGF used in the Triage test. Other studies have not reported sensitivity and specificity (recommended measures of diagnostic accuracy), making direct comparison difficult,[20,21] have compared assays in women with established disease[22] or have tested at a fixed time point rather than at presentation.[23,24]

Suspected preeclampsia is the most frequent clinical presentation to obstetric day care assessment units, and those with early-onset disease are at the greatest risk. Current signs and tests do not perform well in predicting need for delivery or adverse outcomes.[25–27] We hypothesize that adding PlGF 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 on underlying pathophysiology, enable individualized management of women with the disease, with the potential to reduce associated maternal morbidity and unnecessary health service usage. There may be a double benefit: targeting of resources to those at highest risk, while minimizing excessive assessment and intervention in women at lower risk. One decision-analytic modeling analysis has estimated $1400 cost saving associated with the introduction of PlGF testing (based on a sensitivity of 0.82) for the management of pregnant women in a UK setting.[28] Cost savings may be greater when the Triage platform has been adapted to test whole blood at the point-of-care. We would propose that further assessment of PlGF should be undertaken in the context of a randomized, controlled trial, as recommended for all new diagnostic tests, to measure the impact on the health of mother and infant through changing diagnostic/ treatment decisions, time to treatment, and potential harms, as well.[29]

Hypertensive disorders of pregnancy remain a challenge worldwide, as indicated by the recent Global Burden of Disease Study[30] ;improved detection and management have also been strongly recommended for reduction of stillbirths.[31] Although 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.[32]

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Disclosures

Drs Myer and Kenny have received honoraria for speaking at an Alere-sponsored symposium at an international conference. Dr Kenny has a minority shareholding in Metabolomic Diagnostics, licensed from University College Cork. Dr Simpson, C. Redman, and Dr Shennan have been paid as a consultant for and received honoraria from Alere; Dr Shennan has also been paid as a consultant for Roche and Perkin Elmer. The other authors report no conflicts.

References

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*. 2010;376:631–644.
2. Menzies J, Magee LA, Macnab YC, Ansermino JM, Li J, Douglas MJ, Gruslin A, Kyle P, Lee SK, Moore MP, Moutquin JM, Smith GN, Walker JJ, Walley KR, Russell JA, von Dadelszen P. Current CHS and NHBPEP criteria for severe preeclampsia do not uniformly predict adverse maternal or perinatal outcomes. *Hypertens Pregnancy*. 2007;26:447–462.
3. Meis PJ, Goldenberg RL, Mercer BM, Iams JD, Moawad AH, Miodovnik M, Menard MK, Caritis SN, Thurnau GR, Bottoms SF, Das A, Roberts JM, McNellis D. The preterm prediction study: risk factors for indicated preterm births. Maternal-Fetal Medicine Units Network of the National Institute of Child Health and Human Development. *Am J Obstet Gynecol*. 1998;178:562–567.
4. Meads CA, Cnossen JS, Meher S, Juarez-Garcia A, ter Riet G, Duley L, Roberts TE, Mol BW, van der Post JA, Leeflang MM, Barton PM, Hyde CJ, Gupta JK, Khan KS. Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol Assess*. 2008;12:iii–iv, 1.
5. Maynard SE, Karumanchi SA. Angiogenic factors and preeclampsia. *Semin Nephrol*. 2011;31:33–46.
6. Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. *Placenta*. 1997;18:657–665.
7. Knudsen UB, Kronborg CS, von Dadelszen, Kupfer K, Lee SW, Vittinghus E, Allen JG, Redman CW. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. *Pregnancy Hypertens*. 2011;2:8–15.
8. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004;350:672–683.
9. Robinson CJ, Johnson DD, Chang EY, Armstrong DM, Wang W. Evaluation of placenta growth factor and soluble Fms-like tyrosine kinase 1 receptor levels in mild and severe preeclampsia. *Am J Obstet Gynecol*. 2006;195:255–259.
10. Acog practice bulletin. Diagnosis and management of preeclampsia and eclampsia *Obstet Gynecol*. 2002;99:159–167.
11. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy*. 2001;20:IX–XIV.
12. Gardosi J, Francis A. Adverse pregnancy outcome and association with small for gestational age birthweight by customized and population-based percentiles. *Am J Obstet Gynecol*. 2009;201:28.e1–28.e8.
13. Gardosi J, Francis A. Software program for the calculation of customised birth weight percentiles. *Version* 6.4, 2000–2009.
14. Saffer C, Olson G, Boggess K, Beyerlein R, Eubank C, and the NORMALS Study Group. Determination of placental growth factor (plgf) levels in healthy pregnant women without signs or symptoms of preeclampsia. *Pregnancy Hypertension*. 2013;3:124–132.
15. Cnossen JS, ter Riet G, Mol BW, van der Post JA, Leeflang MM, Meads CA, Hyde C, Khan KS. Are tests for predicting pre-eclampsia good enough to make screening viable? A review of reviews and critical appraisal. *Acta Obstet Gynecol Scand*. 2009;88:758–765.
16. von Dadelszen P, Payne B, Li J, Ansermino JM, Broughton Pipkin F, Côté AM, Douglas MJ, Gruslin A, Hutcheon JA, Joseph KS, Kyle PM, Lee T, Loughna P, Menzies JM, Merialdi M, Millman AL, Moore MP, Moutquin JM, Ouellet AB, Smith GN, Walker JJ, Walley KR, Walters BN, Widmer M, Lee SK, Russell JA, Magee LA; PIERS Study Group. Prediction of adverse maternal outcomes in pre-eclampsia: development and validation of the fullPIERS model. *Lancet*. 2011;377:219–227.
17. Sibiude J, Guibourdenche J, Dionne MD, Le Ray C, Anselem O, Serreau R, Goffinet F, Tsatsaris V. Placental growth factor for the prediction of adverse outcomes in patients with suspected preeclampsia or intrauterine growth restriction. *PLoS ONE*. 2012;7:e50208.
18. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation*. 2012;125:911–919.
19. Benton SJ, Hu Y, Xie F, Kupfer K, Lee SW, Magee LA, von Dadelszen P. Angiogenic factors as diagnostic tests for preeclampsia: a performance comparison between two commercial immunoassays. *Am J Obstet Gynecol*. 2011;205:469.e1–469.e8.
20. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, Calda P, Holzgreve W, Galindo A, Engels T, Denk B, Stepan H. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol*. 2012;206:58.e1–58.e8.
21. Moore AG, Young H, Keller JM, Ojo LR, Yan J, Simas TA, Maynard SE. Angiogenic biomarkers for prediction of maternal and neonatal

complications in suspected preeclampsia. *J Matern Fetal Neonatal Med*. 2012;25:2651–2657.
22. Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, Hodges-Savola C. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. *Am J Obstet Gynecol*. 2010;202:40.e1–40.e7.
23. Lai J, Pinas A, Poon LC, Agathokleous M, Nicolaides KH. Maternal serum placental growth factor, pregnancy-associated plasma protein-a and free ß-human chorionic gonadotrophin at 30-33 weeks in the prediction of pre-eclampsia. *Fetal Diagn Ther*. 2013;33:164–172.
24. Chaiworapongsa T, Romero R, Korzeniewski SJ, Kusanovic JP, Soto E, Lam J, Dong Z, Than NG, Yeo L, Hernandez-Andrade E, Conde-Agudelo A, Hassan SS. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. *Am J Obstet Gynecol*. 2013;208:287.e1–287.e15.
25. Thangaratinam S, Ismail KM, Sharp S, Coomarasamy A, Khan KS; Tests in Prediction of Pre-eclampsia Severity review group. Accuracy of serum uric acid in predicting complications of pre-eclampsia: a systematic review. *BJOG*. 2006;113:369–378.
26. Thangaratinam S, Coomarasamy A, O'Mahony F, Sharp S, Zamora J, Khan KS, Ismail KM. Estimation of proteinuria as a predictor of complications of pre-eclampsia: a systematic review. *BMC Med*. 2009;7:10.
27. Thangaratinam S, Koopmans CM, Iyengar S, Zamora J, Ismail KM, Mol BW, Khan KS; TIPPS (Tests in Prediction of Preeclampsia's Severity) Review Group. Accuracy of liver function tests for predicting adverse maternal and fetal outcomes in women with preeclampsia: a systematic review. *Acta Obstet Gynecol Scand*. 2011;90:574–585.
28. Hadker N, Garg S, Costanzo C, Miller JD, Foster T, van der Helm W, Creeden J. Financial impact of a novel pre-eclampsia diagnostic test versus standard practice: a decision-analytic modeling analysis from a UK healthcare payer perspective. *J Med Econ*. 2010;13:728–737.
29. Ferrante di Ruffano L, Hyde CJ, McCaffery KJ, Bossuyt PM, Deeks JJ. Assessing the value of diagnostic tests: A framework for designing and evaluating trials. *Bmj*. 2012;344:e686.
30. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2095–2128.
31. Bhutta ZA, Yakoob MY, Lawn JE, Rizvi A, Friberg IK, Weissman E, Buchmann E, Goldenberg RL; Lancet's Stillbirths Series steering committee. Stillbirths: what difference can we make and at what cost? *Lancet*. 2011;377:1523–1538.
32. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, Charnock-Jones DS, Redman CW. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension*. 2013;61:932–942.

CLINICAL PERSPECTIVE

Current management of women who present with hypertension in pregnancy or other features of suspected preeclampsia is hampered by the use of signs (high blood pressure) or tests (proteinuria, abnormal platelets, uric acid, alanine transaminase) that reflect end-organ disease and are poorly predictive of subsequent adverse outcomes. In our study, we report the first prospective multicenter study of a biomarker in which we evaluated the diagnostic accuracy of placental growth factor (PlGF) in women presenting with suspected preeclampsia between 20 and 41 weeks' gestation. In women presenting before 35 weeks, low PlGF (<5th centile) had high sensitivity (0.96; 95% confidence interval, 0.89–0.99) and negative predictive value (0.98; 0.93–0.995) in determining delivery for confirmed preeclampsia within 14 days. The area under the receiver operating characteristics curve for low PlGF (0.87, standard error 0.03) was greater than all other commonly used tests, singly or in combination (range, 0.58–0.76). Suspected preeclampsia is the most frequent clinical presentation to obstetric assessment units, and those with early-onset disease are at greatest risk. We hypothesize that adding the PlGF measurement to the 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 on underlying pathophysiology, enable individualized management of women with the disease, with the potential to reduce associated maternal morbidity and unnecessary health service usage. There may be double benefit: targeting of resources to those at highest risk, while minimizing excessive assessment and intervention in women at lower risk.

Diagnostic accuracy of placental growth factor and ultrasound parameters to predict the small-for-gestational-age infant in women presenting with reduced symphysis-fundal height measurement

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Short title: PlGF to predict SGA

Key words: Small for gestation age (SGA), fetal growth restriction (FGR), placental growth factor (PlGF), estimated fetal weight (EFW), symphysis-fundal height (SFH).

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Abstract

Objectives: To assess the diagnostic accuracy of placental growth factor (PlGF) and ultrasound parameters to predict delivery of a small-for-gestational-age (SGA) infant in women presenting with reduced symphysis-fundal height (SFH).

Methods: Multicentre, prospective observational study recruiting 601 women with singleton pregnancies and reduced SFH between 24-37 weeks' gestation across 11 sites in UK and Canada. Plasma PlGF concentration <5th centile, estimated fetal weight (EFW) <10th centile, umbilical artery Doppler pulsatility index >95th centile and oligohydramnios (Amniotic Fluid Index <5cm) were compared as predictors for a SGA infant <3rd customised birth weight centile (SGA-3) and adverse perinatal outcome. Test performance statistics were calculated for all parameters in isolation and combination.

Results: 592 women were analysed. For predicting delivery SGA-3 (n=78), EFW <10th centile had 58% sensitivity (95%CI 46 to 69%) and 93% negative predictive value (NPV) (95%CI 90 to 95%), PlGF had 37% sensitivity (95%CI 27 to 49%) and 90% NPV (95%CI 87 to 93%); in combination, PlGF and EFW <10th centile had 69% sensitivity (95%CI 55 to 81%) and 93% NPV (95%CI 89 to 96%). The equivalent ROC areas were 0.79 (95%CI 0.74 to 0.84) for EFW <10th centile, 0.70 (95%CI 0.63 to 0.77) for low PlGF and 0.82 (95%CI 0.77 to 0.86) in combination.

Conclusions: In women presenting with reduced SFH, ultrasound parameters had modest test performance for predicting delivery of SGA-3. PlGF performed no better than EFW <10th centile in determining delivery of a SGA infant.

Introduction

Fetal growth restriction (FGR) is a failure to fulfill growth potential, associated with increased risk of stillbirth[1], neonatal morbidity[2, 3] and mortality[4-7]. Complications can extend into adult life, with greater risk of cardiovascular disease and type 2 diabetes mellitus[8]. Evaluation of birth weight centile identifies small for gestational age (SGA) infants (typically defined as birth weight below the 3rd or 10th birth weight centile); these include constitutionally small infants and those with FGR, and as a group these pregnancies are at increased risk of adverse neonatal outcome[9].

Identifying the SGA infant remains challenging in the low-risk population, relying on imprecise techniques such as SFH measurement[10]. If SGA is suspected, UK national guidance recommends ultrasound measured abdominal circumference (AC) or estimated fetal weight (EFW) <10th centile to diagnose an SGA fetus[11, 12]. However, a large proportion of SGA infants are not detected antenatally (32% of 215 high-risk women[1] and 82% of 195 stillbirths with SGA[13]).

UK national guidance[11] does not advocate routine ultrasound measurement in the third trimester as a screening tool for SGA, due to poor prediction (sensitivity 38-51%)[14-17] and no evidence of improved neonatal outcome[18]. However, preliminary results from a recent large prospective cohort study reported increased sensitivity of screening (79%) vs. selective (32%) sonography in the third trimester in an unselected nulliparous population for prediction of severe SGA[19].

Whilst the pathophysiology of FGR is multifactorial, placental insufficiency is causative in many cases. Markers of placental function could provide adjuncts to current techniques to identify high-risk pregnancies. Multiple biomarkers have been proposed to aid detection but none have sufficient accuracy for

incorporation into clinical practice[20]. However, low maternal Placental Growth Factor (PlGF) concentrations can distinguish placental SGA from constitutionally small fetuses (sensitivity 100%; specificity 86%)[21] and in a high-risk cohort with suspected preterm pre-eclampsia can predict pre-eclampsia and delivery of an SGA infant (birth weight <1st centile) with high sensitivity[22].

We performed a large, prospective, multicentre, cohort study in women with suspected SGA (reduced SFH measurement) with the aim of assessing the diagnostic accuracy of PlGF and ultrasound parameters to predict delivery of an SGA infant.

Methods

Participants and sampling:

Women were enrolled from eleven consultant-led units across the United Kingdom and Canada between December 2011 and July 2013 (approximate number of deliveries per year: St Thomas' Hospital London: 6650; St Marys' Hospital Manchester: 8200; Oxford: 6550; Leeds: 9550; Sheffield: 7000; St Georges' Hospital London: 4950; St Michael's Hospital Bristol: 5500; Lewisham: 4000; West Middlesex Hospital: 4700; Sunderland: 3200; Vancouver, Canada; 7000). Local audit data at St Thomas' Hospital London in the year prior to the study starting (2011) showed that approximately 1300 women were referred with reduced symphysis fundal height measurement; of these women, 8% delivered an SGA infant with customised birth weight less than the 3rd centile for gestational age. Ethical approval was granted by East London Research Ethics Committee (ref. 10/H0701/117).

Women were eligible if they were aged 16 years or over, with a singleton pregnancy, between 24^{+0} and 36^{+6} weeks' gestation and referred with suspected SGA by either: 1) SFH measuring more than 2 cm (i.e. 3 cm or more)

under that expected for any given gestational age in completed weeks (e.g. measuring 33cm or less at 36 weeks' gestation) or 2) SFH < 10th centile on customised SFH chart. Women with ~~with multiple pregnancies,~~ SGA already confirmed (EFW <10th customised centile), major fetal anomaly (fetal malformations that affect viability and/ or the quality of life of the fetus and require intervention[23]) or confirmed rupture of amniotic membranes were excluded.

Written informed consent was obtained from participants. A study specific database was designed and finalised prior to recruitment of the first participant. On the same day as the ultrasound scan, baseline demographic and pregnancy specific data were entered into the database and PlGF testing was performed. Blood was drawn into ethylenediamine tetra-acetic acid and labeled with a study-specific coded identifier. Samples were transported to the laboratory at the recruiting site and spun for 10 minutes at 3000 rpm. Plasma was extracted from each sample and stored at -80˚C until analysis. All samples were analysed for PlGF at the recruiting site using the Triage® PlGF Test (Alere, San Diego, CA) according to manufacturer's instructions. All laboratory staff received standardised training in sample processing delivered by the study monitor. All meters were programmed to produce a masked result, determining satisfactory test completion only, without revealing the value. All laboratory staff were masked to the clinical diagnosis. The assay uses fluorescently-labeled recombinant murine monoclonal antibodies and detects PlGF specifically and quantitatively, in the range of 12-3000 pg/mL, in approximately 15 minutes. The lower limit of detection of the assay is 12 pg/mL and PlGF results were classified as normal (PlGF ≥ 5th centile for gestational age), low (<5th centile) and very low (<12 pg/mL). To determine assay reproducibility, replicate

samples were also tested at a central laboratory. The Total Precision (coefficient of variation) on plasma controls at concentrations of 85 pg/mL and 1300 pg/mL was 12.8% and 13.2%, respectively.

All case outcomes were adjudicated by two independent senior physicians, without knowledge of PlGF concentrations. SGA was defined as delivery of an infant with a birth weight less than the 3rd (or 10th, as a secondary analysis) customised birth weight centile calculated using the Gestation Related Optimal Weight (GROW) method software[24]. A final maternal diagnosis was assigned using definitions from the American College of Obstetricians and Gynecologists' practice bulletin for maternal hypertensive disorders [25] and the International and Australasian Societies for the Study of Hypertension in Pregnancy for atypical pre-eclampsia, as predefined in the study protocol [26].

Any hospital attendances subsequent to enrolment were recorded in the study database, including repeat ultrasound assessments, details of delivery and adverse maternal and perinatal outcomes. Adverse maternal outcome was predefined as the 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 <50×10^{9}/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. Adverse perinatal outcome was 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. An independent data monitor conducted regular data monitoring at all sites.

Sample size and power:

The study was powered on the basis of the number of cases needed to reliably distinguish good (80%) from moderate (60%) sensitivity. 55 cases were needed for 90% power and 5% significance. This number was met for all endpoints by recruiting 601 women, giving 78 cases of SGA <3rd birth weight centile.

Statistical analysis:

The predefined primary outcome (reference standard) was delivery of a SGA infant < 3rd customised birth weight centile, calculated using version 6.7 of Gestation Related Optimal Weight (GROW) calculator. SGA < 10th centile, and adverse perinatal outcomes were considered as secondary outcomes.

Gestational-adjusted centiles for PlGF from a large low-risk antenatal population were used.[27] An abnormal result was taken as maternal PlGF concentration below the 5th centile, as this has previously been shown to offer a combination of high sensitivity and acceptable specificity for pre-eclampsia and SGA, with a high negative predictive value.[22] PlGF and three ultrasound parameters (EFW <10th centile, oligohydramnios, defined as an amniotic fluid index <5 cm and umbilical artery Doppler pulsatility index >95th centile) were compared, both in isolation and in combination, as predictors of delivery of an SGA infant <3rd and <10th customised centiles. Gestation adjusted centiles were calculated for each observed value of umbilical artery Doppler pulsatility index (UAPI) based on a mean value of 0.405 -0.0134 x gestational age in weeks', and a standard

deviation of 0.0794 for the $\log^{10}$ UAPI [28]. Sensitivity, specificity and positive and negative predictive values were calculated with 95% confidence intervals. Receiver operator characteristic (ROC) curve areas were also calculated for each individual parameter and combinations, and in a pre-defined subgroup who delivered within six weeks of PlGF sampling. Fisher's exact test was used to compare the event rate in women with normal and low PlGF measurements.

Statistical analysis was carried out in the Stata statistical package (version 11.2, StataCorp, College Station, Texas, USA).

This study is reported in accordance with the STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines (Table S1).

Results

601 women presenting with suspected SGA between 24^{+0} and 36^{+6} weeks' gestation were recruited across 11 sites between December 2011 and July 2013. We recruited all women who were approached, eligible and consented but did not document women who declined to participate. No outcome data were available for two participants and five women did not have PlGF results generated by the test meter. A further two women had no ultrasound data at enrolment available. After exclusion of these nine cases, 592 women were included in this analysis. Of these women, 192 delivered an SGA infant with birth weight <10th customised centile and 78 had a birth weight <3rd customised centile (Figure 1).

Characteristics of participants at booking are given in Table 1; higher rates of smoking were observed in women who delivered an SGA infant. Table 2 displays baseline characteristics at study enrolment. Details of maternal and neonatal outcomes and final adjudicated maternal diagnoses are shown in

Table 3. The majority of women (n=555) experienced no maternal complications during their pregnancy. Whilst the number of cases complicated by pre-eclampsia was small (n=16) most of these women delivered an SGA infant (n=12). Of 13 cases with adverse perinatal outcome there was one stillbirth, four cases of respiratory distress syndrome and nine infants admitted to the Neonatal Unit at term for greater than 48 hrs.

Induction of labour and caesarean section occurred more frequently in pregnancies delivering an SGA infant, compared to those with birth weights appropriate for gestational age. Maternal and perinatal adverse outcomes were reported in 3.2% and 2.2% of women and infants respectively. Both complications were higher in pregnancies with delivery of an SGA infant (4.7% and 3.1% respectively).

The median concentration of PlGF according to birth weight was 94.5 pg/ml (Interquartile Range (IQR) 36.3 to 324 pg/ml) for SGA <3rd centile, 253 pg/ml (IQR 125 to 631 pg/ml) for SGA <10th centile and 311 pg/ml (IQR 131 to 742 pg/ml) for birth weight ≥ 10th centile. The diagnostic accuracy of PlGF and ultrasound parameters to determine SGA <3rd and <10th centile are shown in Table 4, with EFW having the highest sensitivity and negative predictive value of all parameters assessed. Addition of PlGF to currently utilised ultrasound parameters altered test sensitivity from 58% to 69% (NPV 93 to 93%) in determining SGA <3rd centile and from 47% to 57% (NPV 77 to 78%) in determining SGA <10th centile. For women presenting prior to 37 weeks' gestation in whom EFW was measured as ≥10th centile, low PlGF concentrations at the time of scanning (<5th centile) would have detected an additional nine women with subsequent SGA <3rd centile. This difference in

SGA <3rd centile between those with normal PlGF (5.9%; 23/390) compared to those with low PlGF (20.5%; 9/44) is significant (p=0.002 by Fisher's exact test). In the whole cohort, the ROC area was higher for EFW <10th centile (0.79; 95% CI 0.74 to 0.84) than for low PlGF (0.70; 95% CI 0.63 to 0.77) for prediction of SGA < 3rd centile; in combination this increased to 0.82 (95% CI 0.77 to 0.86) (Figure 2A). In a planned subgroup analysis of 267 women where delivery occurred within six weeks of PlGF sampling (Table S2), ROC areas were 0.76 (95% CI 0.69 to 0.84) 0.74 (95% CI 0.66 to 0.83) and 0.81 (95% CI 0.72 to 0.88) for EFW <10th centile, low PlGF and a combination of both parameters respectively (Figure 2B).

The outcomes of 16 participants with a very low PlGF concentration (<12 pg/ml, lower level of assay detection) at enrolment, are shown in Table S3. Seven women had hypertensive complications of pregnancy (7/16; (44%) versus 17/577; (3%) in the rest of the cohort) and 11 women delivered an SGA infant with birth weight <10th customised centile.

There were no adverse events associated with blood sampling for PlGF measurement.

Discussion

In this multicentre, prospective cohort study of women presenting with reduced SFH measurement, currently utilised ultrasound parameters including EFW <10th centile had modest test performance for predicting delivery of an SGA infant. Maternal PlGF measurement performed no better than these ultrasound parameters and provided only minimal increments in overall test performance when used in combination. This contrasts with the findings of our previous study, assessing the diagnostic accuracy of PlGF in women with suspected pre-

eclampsia, which reported excellent performance of PlGF (sensitivity 93% and NPV 96%) in predicting SGA in women presenting before 35 weeks' gestation[22].

There are several possible explanations for differences observed in these studies. The majority of women recruited to this study reported here had no maternal complications of pregnancy (556/592; 93%) and only 24 (4%) had a hypertensive disorder. This contrasts with our previous high-risk cohort, where 61% of women enrolled before 35 weeks' gestation developed pre-eclampsia[22]. Differing pathological processes may occur in the placentas of pregnancies complicated by hypertensive disease, particularly if early onset, and those who remain normotensive but deliver an SGA infant[29]. The gestation at delivery of SGA infants <3rd centile in this study was 38.7 weeks (with 5% adverse perinatal outcome), compared to 33.8 weeks (with 39% adverse perinatal outcome) in the previous study, emphasizing the likely different placental pathophysiology. The average gestational age was 34 weeks at PlGF sampling and 40 weeks at delivery. PlGF appears to have limited clinical utility in women presenting late in pregnancy and delivering near term. This may reflect convergence of PlGF measurements between normal and pathological pregnancies with advancing gestation[27] and the heterogeneous aetiology of SGA even when categorised as birth weight <3rd customised centile. PlGF is an angiogenic factor produced principally by trophoblast cells. Low maternal plasma PlGF concentrations reflect placental dysfunction and have been described in early onset pre-eclampsia and SGA associated with abnormal placental pathology[21].

It is particularly notable that adverse perinatal outcome occurred infrequently (2.2%) in this study; this makes conclusions regarding the ability of PlGF to

determine adverse outcomes impossible. The single case of stillbirth had normal PlGF concentration and was not SGA, therefore placental insufficiency is an unlikely aetiology. The neonatal characteristics (Table 3) are markedly different to that described in the previous PlGF study, in which nine (2.1%) cases of stillbirth/neonatal death were reported with adverse perinatal outcome in 19%[22].

This is the largest reported prospective study evaluating the ability of third trimester PlGF to predict delivery of an SGA infant in women presenting with reduced SFH. Recruitment from 11 centres across the UK and Canada provided a diverse ethnic and geographical population. PlGF was measured at the recruiting site, as would occur if adopted into clinical practice. PlGF results were concealed until assignment of a final maternal diagnosis at study completion. The study entry criterion, reduced SFH, was selected for clinical relevance, reflecting current referral practice in the UK. A primary endpoint of delivering an infant < 3rd customised birth weight centile was selected as it includes fewer constitutionally small infants and has a stronger association with perinatal mortality[7].

This study only included PlGF measurement at study enrolment. Serial measurements to assess whether longitudinal changes in PlGF correlate with evolving placental dysfunction could be informative. Where routine antenatal third trimester ultrasound in low risk women is performed, the findings of this study may be less applicable.

A systematic review evaluating biomarkers for predicting FGR identified 13 studies that reported test performance for PlGF in predicting delivery of an SGA infant[20]. In a subgroup of studies recruiting women after 20 weeks' gestation, the pooled PlGF sensitivity (at various thresholds) for prediction of intrauterine

growth restriction (by differing definitions) was 49% (95% CI 44-53%). Comparisons were difficult due to heterogeneity between studies. The majority were case-control studies with only two cohort studies recruiting women over 20 weeks' gestation. Of these, one was in an abnormal population (abnormal uterine artery Doppler waveforms at 20 weeks' gestation) while in the other, delivery of an SGA infant was a secondary endpoint. No cohort studies recruiting in the third trimester were evaluated. A recent study evaluated maternal PlGF concentration at a fixed time point (30-34 weeks' gestation) and reported increased adjusted odds ratio for PlGF combined with other angiogenic factors in prediction of delivering an SGA infant but did not provide test performance statistics to enable comparison[30].

The capabilities of current standard ultrasound parameters to determine delivery of an SGA infant must also be considered. A large study published a sensitivity of 27% for SFH measurement to predict delivery of an SGA infant[10]. Reported test performance of EFW <10th centile to predict pregnancies delivering an SGA infant (sensitivity 21-46%; NPV 90-94%)[14, 17]. are similar to those published in this cohort (sensitivity 48%; NPV 77%). Three Cochrane systematic reviews evaluating SFH[31], routine ultrasound measurement (including EFW)[18] and fetal and umbilical artery doppler assessment in low-risk pregnancy[32] concluded that none of these techniques reduced adverse perinatal outcome. Use of customised SFH charts and EFW centiles, which adjust for maternal characteristics, may improve SGA detection[33], prediction of delivering an SGA infant[13, 34] and adverse outcome, including stillbirth[35] and neonatal death[36]. Implementation of customised charts in conjunction with accredited training is associated with reduction in stillbirth rates in areas of high uptake[37] but has not been validated in a randomised control trial.

A systematic review and meta-analysis assessing amniotic fluid index reported strong correlation between oligohydramnios and delivering an SGA infant (birth weight <10th centile) and mortality but the predictive accuracy for perinatal outcome was poor[38]. This agrees with our findings of high specificity for delivery of an SGA infant (99.6%, 95%CI 97.6-100%) but low sensitivity (3.4% 95% CI 0.9-8.5%), limiting clinical application without incorporating other clinical factors. Novel ultrasound parameters such as the cerebroplacental ratio have been reported as potentially useful in predicting neonatal unit admission and validation is awaited[39].

We previously suggested PlGF measurement as a useful adjunct to current clinical practice in women with suspected preterm pre-eclampsia, but the findings from this study cannot support its use in women with reduced SFH. Whilst EFW < 10th centile has only modest test performance for prediction of SGA, addition of PlGF measurement does not significantly improve test performance. This study highlights the need for caution in generalising findings from one population to another and alerts against overenthusiastic adoption of novel biomarkers without appropriate evaluation.

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References

1. Gardosi J, Madurasinghe V, Williams M, Malik A, Francis A. Maternal and fetal risk factors for stillbirth: population based study. *BMJ* 2013; **346**: f108.
2. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. *Am J Obstet Gynecol* 2013; **182**(1 Pt 1): 198-206.

3. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med* 1999; **340**(16): 1234-8.
4. Frøen JF, Gardosi JO, Thurmann A, Francis A, Stray-Pedersen B. Restricted fetal growth in sudden intrauterine unexplained death. *Acta Obstet Gynecol Scand* 2004; **83**(9): 801-7.
5. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet* 2005; **365**(9462): 891-900.
6. Lackman F, Capewell V, Richardson B, daSilva O, Gagnon R. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. *Am J Obstet Gynecol* 2001; **184**(5): 946-53.
7. Moraitis AA, Wood AM, Fleming M, Smith GC. Birth weight percentile and the risk of term perinatal death. *Obstet Gynecol* 2014; **124**(2 Pt 1): 274-83.
8. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. *Am J Clin Nutr* 2000; **71**(5 Suppl): 1344S-52S.
9. Malin GL, Morris RK, Riley R, Teune MJ, Khan KS. When is birthweight at term abnormally low? A systematic review and meta-analysis of the association and predictive ability of current birthweight standards for neonatal outcomes. *Br J Obstet Gynaecol* 2014; **121**(5): 515-26.
10. Persson B, Stangenberg M, Lunell NO, Brodin U, Holmberg NG, Vaclavinkova V. Prediction of size of infants at birth by measurement of symphysis fundus height. *Br J Obstet Gynaecol* 1986; **93**(3): 206-11.
11. Robson SC, Martin WL, Morris RK. The Investigation and Management of the Small–for–Gestational–Age Fetus. *RCOG - Green top guidelines* 2013: 1-34.
12. Chang TC, Robson SC, Boys RJ, Spencer JA. Prediction of the small for gestational age infant: which ultrasonic measurement is best? *Obstet Gynecol* 1992; **80**(6): 1030-8.
13. De Jong CL, Francis A, Van Geijn HP, Gardosi J. Customized fetal weight limits for antenatal detection of fetal growth restriction. *Ultrasound Obstet Gynecol* 2000; **15**(1): 36-40.
14. Ben-Haroush A, Yogev Y, Hod M, Bar J. Predictive value of a single early fetal weight estimate in normal pregnancies. *Eur J Obstet Gynecol and Reprod Biol* 2007; **130**(2): 187-92.
15. Secher NJ, Hansen PK, Lenstrup C, Eriksen PS. Controlled trial of ultrasound screening for light for gestational age (LGA) infants in late pregnancy. *European Journal of Obstetrics and Gynaecology* 1986; **23**(5): 307-13.
16. Souka AP, Papastefanou I, Pilalis A, Michalitsi V, Kassanos D. Performance of third-trimester ultrasound for prediction of small-for-gestational-age neonates and evaluation of contingency screening policies. *Ultrasound in Obstet Gynecol* 2012; **39**(5): 535-42.
17. David C, Tagliavini G, Pilu G, Rudenholz A, Bovicelli L. Receiver-operator characteristic curves for the ultrasonographic prediction of small-for-gestational-age fetuses in low-risk pregnancies. *Am J Obstet Gynecol* 1996; **174**(3): 1037-42.
18. Bricker L, Neilson JP, Dowswell T. Routine ultrasound in late pregnancy (after 24 weeks' gestation). *Cochrane Database Syst Rev* 2008; (4): CD001451.
19. Sovio U, Smith G, Dacey A. Level 1 evidence for the diagnostic effectiveness of routine sonography as a screening test for small for gestational age (SGA) infants. *Am J Obstet Gynecol* 2014; **210(S)**: S408.

20. Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. *Br J Obstet Gynaecol* 2013; **120**(6): 681-94.
21. Benton SJ, Hu Y, Xie F, Kupfer K, Lee SW, Magee LA, von Dadelszen P. Can placental growth factor in maternal circulation identify fetuses with placental intrauterine growth restriction? *Am J Obstet Gynecol* 2012; **206**(2): 163.e1-e7.
22. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Shennan AH. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation* 2013; **128**(19): 2121-31.
23. Addor MC, Pescia G, Schorderet DF. Registration of congenital anomalies in Switzerland by EUROCAT. *Schweiz Med Wochenschr* 2000; **130**(38): 1319-25.
24. Gardosi J, Francis A. Software program for the calculation of customised birth weight centiles. *Version 6 7* 2000-2009.
25. ACOG Committee on Practice Bulletins--Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002; **99**(1): 159-67.
26. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertension* 2001; **20**(1): IX-XIV.
27. Knudsen UB, Kronborg CS, von Dadelszen P, Kupfer, K, Lee SW, Vittinghus, E, Allen JG, Redman CW. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. *Pregnancy Hypertension* 2012; **2**(1): 8-15.
28. Parra-Cordero M, Lees C, Missfelder-Lobos H, Seed P, Harris C. Fetal arterial and venous Doppler pulsatility index and time averaged velocity ranges. *Prenat Diagn* 2007; **27**(13): 1251-7.
29. Redline RW. Placental Pathology: A Systematic Approach with Clinical Correlations. *Placenta* 2008; **29**: 86-91.
30. Chaiworapongsa T, Romero R, Korzeniewski SJ, Kusanovic JP, Soto E, Lam J, Dong Z, Than NG, Yeo L, Hernandez-Andrade E, Conde-Agudelo A, Hassan SS. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. *Am J Obstet Gynecol* 2013; **208**(4): 287.e1-e15.
31. Neilson JP. Symphysis-fundal height measurement in pregnancy. *Cochrane Database Syst Rev* 2000; (2): CD000944.
32. Alfirevic Z, Stampalija T, Gyte GM. Fetal and umbilical Doppler ultrasound in normal pregnancy. *Cochrane Database Syst Rev* 2010; (8): CD001450.
33. Gardosi J, Francis A. Controlled trial of fundal height measurement plotted on customised antenatal growth charts. *Br J Obstet Gynaecol* 1999; **106**(4): 309-17.
34. Gardosi J, Chang A, Kalyan B, Sahota D, Symonds EM. Customised antenatal growth charts. *Lancet* 1992; **339**(8788): 283-7.
35. Odibo AO, Cahill AG, Odibo L, Roehl K, Macones GA. Prediction of intrauterine fetal death in small-for gestational-age fetuses: impact of including

ultrasound biometry in customized models. *Ultrasound Obstet Gynecol* 2011; **39**(3): 288-92.
36. Clausson B, Gardosi J, Francis A, Cnattingius S. Perinatal outcome in SGA births defined by customised versus population-based birthweight standards. *Br J Obstet Gynaecol* 2001; **108**(8): 830-4.
37. Gardosi J, Giddings S, Clifford S, Wood L, Francis A. Association between reduced stillbirth rates in England and regional uptake of accreditation training in customised fetal growth assessment. *BMJ open* 2013; **3**(12): e003942.
38. Morris RK, Meller CH, Tamblyn J, Malin GM, Riley RD, Kilby MD, Robson SC, Khan KS. Association and prediction of amniotic fluid measurements for adverse pregnancy outcome: systematic review and meta-analysis. *Br J Obstet Gynaecol* 2014; **121**(6): 686-99.
39. Khalil AA, Morales-Rosello J, Elsadigg M, Khan N, Papageorghiou A, Bhide A, Thilaganathan B. The association between fetal Doppler and admission to neonatal unit at term. *Am J Obstet Gynecol* 2014; **212**: 1-7.

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Conflict of interest

LC, PS, MG, LM, DA, MLD, JG, AK, KH, AS have no conflict of interests. CR, AHS, NS and JM have been paid as consultants for Alere; AHS has also been paid as a consultant for Roche and Perkin Elmer.

Table 1: Characteristics of participants at booking. Values given are median (quartiles) or n (%) as appropriate.

	SGA <3rd centile (n=78)	SGA <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
General maternal				
Age (years)	29.1 (24.1 to 32.9)	29.6 (24.8 to 33.5)	30.0 (25.3 to 33.7)	29.9 (25.2 to 33.6)
Body Mass Index at booking (kg/m^2)	22.9 (20.3 to 25.2)	21.7 (20.1 to 24.1)	21.5 (20.0 to 23.4)	21.5 (20.0 to 23.6)
White ethnicity	52 (66.7)	122 (63.5)	266 (66.5)	388 (65.5)
Primiparity	65 (83.3)	163 (84.9)	344 (86.0)	507 (85.6)
Highest first trimester systolic BP (mmHg)	105 (100 to 114)	105 (100 to 114)	104 (100 to 112)	105 (100 to 112)
Highest first trimester diastolic BP (mmHg)	63 (60 to 70)	62 (60 to 70)	60 (60 to 69)	61 (60 to 70)
Smoking status				
Never smoked	46 (59)	128 (66.7)	306 (76.5)	434 (73.3)
Quit smoking before pregnancy	9 (11.5)	22 (11.5)	31 (7.8)	53 (8.9)
Quit smoking	10 (12.8)	16 (8.3)	24 (6.0)	40 (6.7)

during pregnancy				
Current smoker	13 (16.7)	26 (13.5)	39 (9.8)	65 (11.0)
Drug use				
History of drug use*	5 (6.4)	6 (3.1)	3 (0.8)	9 (1.5)
Current drug user †	1 (1.3)	2 (1.0)	0 (0.0)	2 (0.3)
Previous medical history				
Pre-eclampsia requiring delivery <34/40	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Chronic hypertension	0 (0)	1 (0.5)	1 (0.3)	2 (0.3)
SLE/ APS	1 (1.3)	1 (0.5)	0 (0)	1 (0.2)
Pre-existing diabetes mellitus	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Renal disease	0 (0)	0 (0)	0 (0)	0 (0)
Self-report of previous small baby	9 (11.5)	22 (11.5)	27 (6.8)	49 (8.3)

BP, blood pressure, SLE/ APS, Systemic lupus erythematosus/ anti-phospholipid syndrome

*Drugs used before pregnancy: include cannabis, cocaine, ecstasy, amphetamines (speed, crystal meth) and heroin.

†Drugs used during pregnancy: Cannabis only (rare or occasional)

Table 2: Baseline characteristics of participants at study enrolment. Values given are median (quartiles) or n (%) as appropriate.

	SGA <3rd centile (n=78)	SGA <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Gestational age at study enrolment (days)	238 (221 to 250)	235 (213 to 250)	236 (214 to 250)	236 (213 to 250)
Maternal BP				
Highest systolic BP (mmHg)	118 (109 to129)	115 (102 to 121)	110 (101 to 118)	110 (101 to120)
Highest diastolic BP (mmHg)	70 (60 to 81)	70 (60 to 80)	67 (60 to 73)	68 (60 to 74)
Dipstick proteinuria				
Not done	11 (14.1)	29 (15.1)	61 (15.3)	90 (15.2)
Negative	58 (74.4)	148 (77.1)	322 (80.5)	470 (79.4)
Present (+1 or greater)	9 (11.5)	15 (7.8)	17 (4.3)	32 (5.4)
Complications in current pregnancy				
Gestational hypertension	4 (5.1)	4 (2.1)	0 (0)	4 (0.7)
Pre-eclampsia	0 (0)	1 (0.5)	1 (0.3)	2 (0.3)
Gestational	1 (1.3)	3 (1.5)	4 (1.0)	7 (1.2)

diabetes				
Intrahepatic cholestasis of pregnancy	0 (0.0)	1 (0.5)	2 (0.5)	3 (0.5)
Fetal				
EFW < 10th centile	44 (57.9)	88 (47.1)	64 (16.3)	152 (25.9)
Oligohydramnios (AFI < 5 cm)	2 (3.6) (n=54)	4 (3.3) (n=118)	1 (0.4) (n=228)	5 (1.4) (n=346)
Absent/ reversed umbilical artery Doppler flow	1 (1.3) (n=76)	1 (0.6) (n=176)	1(0.3) (n= 358)	2 (0.4) (n=534)
Umbilical artery Doppler pulsatility index > 95th centile	10 (16.1) (n=61)	12 (8.2) (n=147)	14 (4.5) (n=312)	26 (5.7) (n=458)

BP, blood pressure, EFW, Estimated fetal weight <10th centile

Table 3: Characteristics of delivery and maternal and neonatal outcome. Values given are median (quartiles) or n (%) as appropriate.

	SGA <3rd centile (n=78)	SGA <10th centile (n=192)	Women with infant ≥10th centile (n=400)	All women (n=592)
Median gestation at delivery (weeks)	38.7 (37.1 to 40.1)	39.4 (38.0 to 40.4)	40.0 (39.0 to 40.9)	39.9 (38.9 to 40.7)
Maternal diagnosis				
No new maternal disease in pregnancy	68 (86.3)	173 (89.2)	382 (95.5)	555 (93.4)
Pre-eclampsia	8 (10.0)	12 (6.2)	4 (0.99)	16 (2.7)
Gestational hypertension	0 (0)	0 (0)	8 (1.9)	8 (1.3)
Chronic hypertension	0 (0)	2 (1.0)	0 (0)	2 (0.3)
Other diagnosis	2 (2.5)	5 (2.6)	6 (1.5)	11 (1.8)
Maternal medications				
Dexamethasone	5 (6.4)	7 (3.6)	4 (1.0)	11 (1.8)
Betamethasone	2 (2.6)	4 (2.1)	0 (0)	4 (0.7)
Methyldopa	2 (2.6)	2 (1.0)	0 (0)	2 (0.3)
Labetalol	6 (7.7)	9 (4.7)	2 (0.5)	11 (1.8)

Heparin	1 (1.3)	2 (1.0)	3 (0.8)	5 (0.8)
Nifedipine	1 (1.3)	2 (1.0)	1 (0.3)	3 (0.5)
Aspirin	3 (3.8)	4 (2.1)	8 (2.0)	12 (2.0)
Oral corticosteroids	0 (0)	3 (1.6)	2 (0.5)	5 (0.8)
Onset of labour				
Spontaneous	24 (30.8)	99 (51.6)	300 (75.0)	399 (67.4)
Induced	41 (52.6)	67 (34.9)	66 (16.5)	133 (22.5)
Pre-labour caesarean section	13 (16.7)	26 (13.5)	34 (8.5)	60 (10.1)
Mode of delivery				
Spontaneous	48 (61.5)	125 (65.1)	279 (69.8)	404 (68.2)
Assisted vaginal delivery	8 (10.3)	23 (12.0)	66 (16.5)	89 (15.0)
Caesarean section	22 (28.2)	44 (22.9)	55 (13.8)	99 (16.7)
Adverse maternal outcome ‡	5 (6.4)	9 (4.7)	10 (2.5)	19 (3.2)
Postpartum haemorrhage	2 (2.6)	5 (2.6)	7 (1.8)	12 (2.0)
Abruption	1 (1.3)	1 (0.5)	1 (0.3)	2 (0.3)
HELLP	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Fetal				
Fetal death	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Neonatal death	0 (0)	0 (0)	0 (0)	0 (0)
Median birth weight	2375 (2100 to	2660 (2360 to	3214 (3000 to	3050 (2740 to

	2610)	2854)	3470)	3329)
Adverse perinatal outcome §	4 (5.1)	6 (3.1)	7 (1.8)	13 (2.2)

HELLP, haemolysis, elevated liver enzymes, low platelets.

‡ 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 $<50\times10^9$/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.

§ 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: Test performance statistics for PlGF and ultrasound parameters (with 95 % confidence intervals) to predict SGA <3rd and <10th centiles (n = 592).

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N
SGA <3rd centile				
EFW <10th centile	57.9 (46.0 to 69.1) 44/76	78.8 (75.0 to 82.3) 402/510	28.9 (21.9 to 36.8) 44/152	92.6 (89.8 to 94.9) 402/434
Oligohydramnios (AFI < 5 cm)	3.7 (0.5 to 12.7) 2/54	99.0 (97.0 to 99.8) 289/292	40.0 (5.3 to 85.3) 2/5	84.8 (80.5 to 88.4) 289/341
Umbilical artery Doppler PI >95th centile	16.4 (8.2 to 28.1) 10/61	96.0 (93.5 to 97.7) 381/395	38.5 (20.2 to 59.4) 10/26	88.2 (84.8 to 91.1) 381/432
PlGF <5th centile	37.2 (26.5 to 48.9) 29/78	88.7 (85.7 to 91.3) 456/514	33.3 (23.6 to 44.3) 29/87	90.3 (87.4 to 92.7) 456/505
Combinations				
Abnormal AFI or EFW	57.7 (43.2 to 71.3) 30/52	79.0 (73.9 to 83.6) 230/291	33.0 (23.5 to 43.6) 30/91	91.3 (87.1 to 94.4) 230/252
Abnormal PlGF or AFI or EFW	69.2 (54.9 to 81.3) 36/52	72.2 (66.6 to 77.2) 210/291	30.8 (22.6 to 40.0) 36/117	92.9 (88.8 to 95.9) 210/226
SGA <10th centile				

EFW <10th centile	47.1	84.0	57.9	77.2
	(39.7 to 54.5)	(80.0 to 87.4)	(49.6 to 65.8)	(72.9 to 81.1)
	88/187	335/399	88/152	335/434
Oligohydramnios	3.4	99.6	80.0	66.6
(AFI <5 cm)	(0.9 to 8.5)	(97.6 to 100)	(28.4 to 99.5)	(61.3 to 71.6)
	4/118	227/228	4/5	227/341
Umbilical artery	8.2	95.5	46.2	68.8
Doppler PI >95th	(4.3 to 13.8)	(92.6 to 97.5)	(26.6 to 66.6)	(64.1 to 73.1)
centile §§	12/147	297/311	12/26	297/432
PlGF <5th centile	24.5	90.0	54.0	71.3
	(18.6 to 31.2)	(86.6 to 92.8)	(43.0 to 64.8)	(67.1 to 75.2)
	47/192	360/400	47/87	360/505
Combinations				
Abnormal AFI or	48.7	84.6	61.5	76.6
EFW	(39.3 to 58.2)	(79.3 to 89.1)	(50.8 to 71.6)	(70.9 to 81.7)
	56/115	193/228	56/91	193/252
Abnormal PlGF or	57.4	77.6	56.4	78.3
AFI or EFW	(47.8 to 66.6)	(71.7 to 82.9)	(46.9 to 65.6)	(72.4 to 83.5)
	66/115	177/228	66/117	177/226

EFW, Estimated fetal weight <10th centile; PI, pulsatility index; PlGF, placental growth factor

Footnote: AFI and umbilical artery Doppler were not recorded for all subjects

Table S1: STARD checklist for reporting of studies of diagnostic accuracy

Section and Topic	**Item #**		**On page #**
TITLE/ ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	Title page 1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	Page 7
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	Page 7&8
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	Page 5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	Results page 8

	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	Page 5
Test methods	7	The reference standard and its rationale.	Pages 6 & 7
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	Pages 8&9
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	Pages 8&9
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	Page 8
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	Page 8
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	Page 10
	13	Methods for calculating test reproducibility, if done.	Page 9

RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	Page 12
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	Table 1
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	Figure 1
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	Tables 2 & 3
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	Table 3
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	Table 4 Results page 9
	20	Any adverse events from performing the index tests or the reference standard.	There were no adverse events

Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	Table 4 and figure 2
	22	How indeterminate results, missing data and outliers of the index tests were handled.	Figure 1
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	Not applicable
	24	Estimates of test reproducibility, if done.	Page 9
DISCUSSION	25	Discuss the clinical applicability of the study findings.	Page 18

Table S2: Test performance statistics for PlGF and ultrasound parameters (with 95% confidence intervals) to predict SGA <3rd centile when PlGF sampled within 6 weeks of delivery (n=267).

Biomarker/ clinical indicator	Sensitivity % (95% CI) n/N	Specificity % (95% CI) n/N	PPV % (95% CI) n/N	NPV % (95% CI) n/N
EFW <10th centile	62.2 (46.5 to 76.2) 28/45	73.0 (66.6 to 78.7) 162/221	31.8 (22.3 to 42.6) 28/88	90.5 (85.2 to 94.4) 162/179
Oligohydramnios (AFI < 5 cm)	5.9 (0.7 to 19.7) 2/34	97.7 (93.4 to 99.5) 126/129	40.0 (5.3 to 85.3) 2/5	79.7 (72.6 to 85.7) 126/158
Umbilical artery Doppler PI >95th centile	22.2 (10.1 to 39.2) 8/36	96.0 (91.9 to 98.4) 167/174	53.3 (26.6 to 78.7) 8/15	85.6 (79.9 to 90.2) 167/195
PlGF < 5th centile	42.2 (27.7 to 57.8) 19/45	86.6 (81.4 to 90.8) 194/224	38.8 (25.2 to 53.8) 19/49	88.2 (83.2 to 92.1) 194/220
Combinations				
Abnormal AFI or EFW	62.5 (43.7 to 78.9) 20/32	67.2 (58.3 to 75.2) 86/128	32.3 (20.9 to 45.3) 20/62	87.8 (79.6 to 93.5) 86/98
Abnormal PlGF or AFI or EFW	70.0 (50.6 to 85.3) 21/30	58.3 (49.2 to 67.0) 74/127	28.4 (18.5 to 40.1) 21/74	89.2 (80.4 to 94.9) 74/83

EFW, Estimated fetal weight <10th centile; PI, pulsatility index; PlGF, placental growth factor

Table S3: Maternal outcomes for women with very low PlGF (<12 pg/mL) at sampling. There were no fetal or neonatal complications in this group.

Subject ID	Gestation at sampling	Gestation at delivery	Birth weight (g)	Customised birth weight centile	Maternal complications
A	30+0	33+6	1935	10.1	HELLP Syndrome
B	31+4	33+3	1305	0	Severe pre-eclampsia
C	31+4	35+4	1825	1.2	Gestational diabetes mellitus
D	34+2	39+3	2530	2.7	Atypical pre-eclampsia
E	35+2	37+0	2225	0.6	Severe pre-eclampsia
F	35+5	36+6	2905	38.5	None
G	35+5	38+0	2330	2.6	None
H	35+6	38+0	2260	9.5	Severe pre-eclampsia
I	36+0	36+4	2525	38.4	Mild pre-eclampsia
J	36+0	37+6	1958	0.1	None
K	36+1	39+0	2765	8.8	None

L	36+3	38+3	2600	17.4	None
M	36+3	41+1	2710	1.3	None
N	36+4	37+1	2000	0.3	None
O	36+4	37+3	2398	6.0	Chronic hypertension
P	36+6	40+2	3720	63.2	Gestational hypertension

HELLP, haemolysis, elevated liver enzymes, low platelets.