PREDICTION OF PREECLAMPSIA IN TWIN PREGNANCY

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To Madalena, the best part of my life.

To Tiago, who is my constant strength and inspiration.

To my parents and my brother, who have made me what I am.

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ABSTRACT

Twin pregnancy is a high-risk condition obstetricians have to deal with frequently in clinical practice and its incidence has risen dramatically over the last decades. Additionally, hypertensive disorders including preeclampsia (PE), are amongst the most common medical complications of pregnancy and a major cause of maternal and perinatal morbidity and mortality. While it is unquestionable that multiple pregnancy has an increased risk of PE, it is still unclear what other risk factors are significant in the development of PE in twins.

The exact pathophysiology of PE is uncertain. Traditionally it was thought to be a condition of poor placentation, resulting in generalized vascular endothelial activation and vasospasm, with typical cardiovascular clinical manifestations. Recent published data support the hypothesis that an angiogenic/anti-angiogenic balance plays a causative role in endothelial cell injury. The impact of the maternal cardiac function in the development of PE has become more relevant in the last years.

The early detection of pregnancies at high-risk of PE could improve the maternal and neonatal outcome by a more individualized patient surveillance and the timely institution of prophylactic measures. In singleton pregnancies, screening for PE in the first trimester using a combined approach has a high detection rate. In twin pregnancies data related to PE screening is limited and inconsistent.

The objective of this thesis is to clarify some of the issues with regard to PE in twin pregnancy. We set out to determine the relative risk for total and preterm-PE in multiple pregnancy when compared with singletons. Additionally, the aim is to assess the effect of maternal characteristics and medical history in the prediction of PE in twin pregnancies. The final goal is to develop a screening model for PE in twins by a combination of maternal factors and biomarkers.

The research developed consists of three prospective screening studies for PE, with a population of 2219 twin pregnancies. In the first study, we found that there is a hidden high risk of PE in twin pregnancies. Although, as classically described, the relative risk of total PE is about three times higher in twins, the risk of preterm-PE is nine times higher than in singleton pregnancies. In the second study, we evaluated the effect of maternal and pregnancy characteristics on the risk of PE. Screening for preterm PE using maternal factors has a very high detection rate (99-100%) but at the expense of an extremely high screening positive rate (97-99%). In the third study, we developed a model combining maternal characteristics with biophysical and biochemical markers

to screen for PE in twin pregnancies. The performance of the screening model for preterm PE was very good with a detection rate for PE before 32 weeks of 100%, at any cut-off used. Once again the screen positive rate was high: 25%, 65% and 75%, using a cut-off 1:10, 1:50 or 1:75, respectively.

We believe that increasing the knowledge about PE in twin gestation may allow an improvement in patient's surveillance, the implementation of an effective screening method and the development of a preventive strategy.

RESUMO

A gravidez gemelar é uma condição de alto risco, com a qual os obstetras têm que lidar frequentemente na prática clínica e cuja incidência tem vindo a aumentar drasticamente nas últimas décadas. Por outro lado, a patologia hipertensiva incluindo a pré-eclâmpsia (PE), é uma das complicações médicas mais comuns na gravidez, sendo uma importante causa de morbilidade e mortalidade materna e perinatal. Embora seja inquestionável que a gravidez múltipla tem um aumento do risco de PE, existem ainda dúvidas sobre quais são os outros factores de risco relevantes para PE na gravidez gemelar.

A fisiopatologia exata da PE é desconhecida. Tradicionalmente é considerada uma condição resultante de uma placentação anómala, que causa uma ativação endotelial vascular generalizada e vasoespasmo, com manifestações clínicas cardiovasculares específicas. Estudos mais recentes defendem a hipótese de que um equilíbrio angiogênico/antiangiogênico desempenhe um papel causal na lesão das células endoteliais. O papel da função cardíaca materna no desenvolvimento da PE tornou-se mais pertinente nos últimos anos.

A identificação precoce de gestações com elevado risco de PE poderá melhorar o desfecho materno e neonatal, através de uma vigilância mais individualizada e da instituição atempada de medidas profiláticas. Nas gravidezes únicas, a utilização no primeiro trimestre de um método de rastreio combinado para a PE tem uma elevada taxa de detecção. Em gravidezes gemelares, os dados sobre o rastreio da PE são limitados e inconsistentes.

O objetivo desta tese é esclarecer alguns das dúvidas acerca da PE na gravidez múltipla. Em primeiro lugar pretendemos determinar o risco relativo para PE total e PE pré-termo em gestações gemelares quando comparadas com gestações únicas. Além disso, queremos avaliar o efeito das características maternas e da história clínica na predição de PE em gémeos. O objetivo final é desenvolver um modelo de rastreio para a PE na gravidez gemelar combinando fatores maternos, marcadores biofísicos e bioquímicos.

A investigação desenvolvida consiste em três estudos prospectivos de rastreio para PE, realizados em 2219 gravidezes gemelares. No primeiro estudo, constatou-se a existência de um elevado risco oculto de PE na gravidez gemelar. Embora, tal como classicamente descrito, o risco relativo de PE total seja cerca de três vezes superior nos gémeos, o risco de PE pré-termo é nove vezes maior do que em gestações únicas. No segundo estudo, avaliámos o efeito das características maternas e da história clínica no risco de PE. O rastreio da PE pré-termo, através de fatores

maternos, tem uma taxa de detecção muito elevada (99-100%), mas à custa de uma taxa extremamente alta de gravidezes com um rastreio positivo (97-99%). No terceiro estudo, desenvolvemos um modelo de rastreio para a PE nas gestações gemelares que combina as características maternas com marcadores biofísicos e bioquímicos. O desempenho do modelo de rastreio para a PE pré-termo foi notável, com uma taxa de detecção de PE antes de 32 semanas de 100%, em qualquer ponto de corte utilizado. Mais uma vez a percentagem de gravidezes com rastreio positivo foi alta: 25%, 65% e 75%, usando um ponto de corte 1:10, 1:50 ou 1:75, respectivamente.

Acreditamos que aprofundar o conhecimento sobre PE na gravidez gemelar pode permitir uma melhoria na vigilância destas gravidezes, a implementação de um método de rastreio eficaz e o desenvolvimento de uma estratégia preventiva.

PUBLISHED STUDIES

STUDY 1

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ETHICS COMMITTEE APPROVALS

According to Regulamento n^{o} 320/2015 (Artigo 19^{o} alínea e, from Diário da República, 2^{a} série, N^{o} 153, 7th August 2015), this PhD research project was approved by the United Kingdom National Health Service Research Ethics Committee (reference 02-03-033) and by the Ethics Research Committee NOVA Medical School/Faculdade de Ciências Médicas (n 51/2016/CEFCM).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ACOG: American College of Obstetricians and Gynaecologists

AMA: Advanced Maternal Age

APS: Antiphospholipid Syndrome

ART: Assisted Reproductive Technology

AT_1-AAs: Angiotensin II Type I Receptor Activating Autoantibodies

AUC: Area Under Curve

BC: Before Christ

BMI: Body Mass Index

CI: Confidence Interval

DC: Dichorionic

DELFIA: Dissociation-Enhanced Lanthanide Fluorescent Immunoassay

DNA: Deoxyribonucleic Acid

DR: Detection Rate

GA: Gestational Age

GH: Gestational Hypertension

ß-hCG: Human Chorionic Gonadotropin

HELLP: Hemolysis, Elevated Liver enzymes, Low Platelet

ISSHP: International Society for Study of Hypertension in Pregnancy

ISUOG: International Society of Ultrasound in Obstetrics & Gynaecology

IVF: In-vitro fertilisation

LV: Left Ventricle

MAP: Mean Arterial Pressure

MC: Monochorionic

MoM: Multiple of Median

NCCWCH: National Collaborating Centre for Women's and Children's Health

NHS: National Health Service

NK cells: Natural Killer cells

PAPP-A: Pregnancy Associated Plasma Protein-A

PE: Preeclampsia

PlGF: Placental Growth Factor

PP13: Placental Protein 13

ROC: Receiver–Operating Characteristics

SD: Standard Deviation

sEng: Soluble Endoglin

sFlt-1: Soluble Fms-like tyrosine kinase-1

SLE: Systemic Lupus Erythematosus

SOMANZ: Society of Obstetric Medicine of Australia and New Zealand

SPR: Screen-Positive Rate

TGF: Transforming Growth Factor

UK: United Kingdom

USA: United Sates of America

UtA-PI: Uterine Artery Pulsatility Index

VEGF: Vascular Endothelial Growth Factor

WHO: World Health Organisation

CHAPTER 1

INTRODUCTION

HISTORICAL PERSPECTIVE

The occurrence of seizures in pregnancy and its association with a poor obstetric prognosis was described as early as in the 5th century, BC. (Chesley, 1984) Throughout the following centuries numerous narratives and reports of cases suggestive of eclampsia can be found in medical and midwifery books. Nevertheless, only in 1739 did the word eclampsia was used for the first time to describe an acute form of convulsion by opposition to a chronic form of seizures (epilepsy). The association of proteinuria with eclamptic seizures was described in 1840 by Rayer, and the presence of high blood pressure soon after. In 1894, Vinay reported that high blood pressure and proteinuria could occur in pregnant women without eclamptic seizures and he defined the term preeclampsia (PE). (Chesley, 1984)

Nowadays preeclampsia is a global health problem of increasing significance and a leading cause of maternal and perinatal morbidity and mortality. (Duley, 2009; ACOG, 2013; Townsend *et al*., 2016)

On the other hand, with the increase in maternal age and the expanding use of assisted reproductive technology (ART), obstetricians face a growing number of multiple pregnancies. Twin pregnancy is a high-risk condition that differs from singletons in terms of monitoring, complications and outcomes. (Ananth & Chauhan, 2012; Fletcher, 2015; Chasen & Chervenak, 2017) Overall, it constitutes 2-5% of all pregnancies and has an increased risk of almost every pregnancy complication, with PE being one of the most significant. (ACOG, 2016)

DEFINITION

Preeclampsia is a pregnancy-specific hypertensive disorder, progressive and multi-systemic. The aetiology and pathophysiology are not fully understood, leading to discrepancies in the description of the disease. There is no consensus amongst the major scientific societies in terms of definition, diagnostic criteria and guidelines. (ISSHP, 2001; SOMANZ, 2009; NCCWCH, 2010; ACOG, 2013)

PE is characterized by the occurrence of a combination of symptoms and signs. Most definitions have a common base: the presence of hypertension and proteinuria in previously normotensive women, after 20 weeks of gestation.

The most commonly used diagnostic criteria for PE are from the International Society for the Study of

Hypertension in Pregnancy (ISSHP): development of hypertension (blood pressure of 140/90 mmHg on two separate occasions four hours apart after 20 weeks of gestation) in previously normotensive women with the presence of proteinuria (300 mg or more in 24 hours or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available). (ISSHP, 2001)

In 2014, the definition was adjusted by the ISSPH, further to which the clinical diagnosis of PE should be made in the presence of *de novo* hypertension after 20 weeks of gestation and new onset of one or more of the following criteria (table 1). (ISSHP, 2014)

Table 1.1 - Revised definition of PE (Adapted from ISSHP, 2014).

Hypertension developing after 20 weeks gestation and one or more of the following new onset conditions:
Proteinuria
Other maternal organ dysfunction: • renal insufficiency (creatinine ≥ 90 μmol/L) • liver involvement (elevated transaminases and/or severe right upper quadrant or epigastric pain) • neurological involvement (hyperreflexia accompanied by clonus, severe headaches accompanied by hyperreflexia, persistent visual scotomata, eclampsia, altered mental status, blindness, stroke) • haematological complications (thrombocytopenia, haemolysis, Disseminated Intravascular Coagulation)
Uteroplacental dysfunction - fetal growth restriction

The above clinical manifestations result from mild to severe microangiopathy of the respective organs (kidney, liver, brain and placenta) and should resolve completely within 12 weeks postpartum. (Tranquilli *et al*, 2014; ISSHP, 2014)

For the purposes of this thesis, we chose to use the ISSHP definition as it represents a consensus view of an appropriate definition for research. (ISSHP, 2001)

In terms of gestational age, PE can be classified as early-onset, when the diagnosis is at or before 34 weeks gestation, and as preterm or term according to the need for iatrogenic delivery before or after 37 weeks of gestation, respectively. (Von Dadelszen *et al*, 2003; August & Sibai, 2017)

INCIDENCE

Hypertensive disorders are amongst the most common medical complications of pregnancy with a described incidence varying from 5-10%. (Sibai, 2003)

Gestational hypertension (GH) occurs approximately in 8-10% of the pregnancies and it is associated with a small increase in the risk of maternal, fetal or neonatal complications. (Walker, 2000)

PE complicates 4-5% of pregnancies worldwide and according to different studies its incidence has increased by 25% in the past two decades. (Hutcheon *et al*., 2011; Abalos *et al*., 2013; Ananth *et al*., 2013; Lisonkova *et al*., 2014) The incidence rate significantly varies according to the studied population due to social, economic and racial differences. Consequently, while in developing countries PE or eclampsia affects around 5% of pregnancies, in some African countries the incidence may be as high as 20%. (WHO, 1988; WHO, 2003)

PE is a major cause of maternal and perinatal mortality and morbidity. Its severity ranges from a mild disorder with transient hypertension and proteinuria, to a life threatening illness with convulsions (eclampsia) or HELLP (Hemolysis, Elevated liver enzymes and Low platelet) syndrome. PE is estimated to be responsible for an average of 50000 maternal deaths every year, worldwide. Although the global burden of mortality attributed to PE is 12%, in some countries in Africa and South America this number can be as high as 35-40%. This emphasizes the disparity in incidence and severity in the different parts of the globe. (Walker, 2000; López-Jaramillo *et al*., 2001)

PREECLAMPSIA IN TWIN PREGNANCIES

Women with multiple gestations have an increased risk of hypertensive disorders. The reported incidence ranges from 13-36%. (McMullan, 1984; Krotz *et al*., 2002; Taguchi *et al*., 2014; ACOG, 2016)

This fact was first noticed by Hinselmann on the 18th century. Of the 7748 cases of eclampsia recorded in his work, 6.4% were in twin pregnancies, a rate 5.8 times higher than in singletons. (Chesley, 1984) During the last 20 years, numerous studies were published comparing single and multiple pregnancy in terms of rate of gestational hypertension, preeclampsia and eclampsia. According to those reports, the relative risk of gestational hypertension is 1.2 to 2.7, for PE is 2.8 to 4.4 and for eclampsia 3.4 to 5.1, when comparing twin to singleton gestations. (Sibai *et al*., 2000; Krotz *et al*., 2002; Duckitt, 2005)

RISK FACTORS IN TWIN PREGNANCIES

Multiple factors have been identified as potentially increasing the incidence and severity of hypertensive disorders in singleton pregnancies. The most important ones are nulliparity, advanced maternal age (AMA), black race, obesity, ART, gestational diabetes and antiphospholipid antibodies. (August & Sibai, 2017)

It is undeniable that multiple pregnancy is an important risk factor for gestational hypertensive disorders. Although it can be presumed that the risk factors for PE in twin pregnancies are the same as those for singleton pregnancies, due to the different maternal and pregnancy characteristics of twin pregnancies, this hypothesis is unproven. Data are limited, contradictory and most of the published studies have important bias, limiting the strength of the evidence available. (Fox *et al*., 2014a)

Parity

Similar to singleton pregnancies, parity appears to have a significant impact in the development of PE in multiple gestations. According to the literature, nulliparity increases the relative risk of PE approximately 3 times (1.6-5.2) in twin pregnancy. Additionally, the combination of nulliparity with a twin pregnancy increases the risk of PE around 14 times when compared to a multiparous with a singleton pregnancy. (Coonrod *et al*., 1995 ; Krotz *et al*., 2002 ; Fox *et al*., 2014a; Taguchi *et al*., 2014)

Age

When analysing maternal age, the risk of PE has a U-type distribution, with the lowest between 25 and 29 years and the highest in the group over 40 years. Young mothers (less than 17 years of age) with twin pregnancies have a relative risk 1.5 times higher for developing PE compared to those aged 17 to 25, and at an even higher risk compared to those older than 25 years. (Krotz *et al*., 2002)

Race

In twin pregnancies, race seems to play a less important role than in singletons, with black mothers having only a 1.8 times higher risk of PE than white mothers. (Krotz *et al*., 2002)

Pregestational Body Mass Index

Some studies showed that a higher pregestational Body Mass Index (BMI), especially a BMI superior to 30.0 kg/m2, significantly and independently increases the risk of PE in twins. (Lucovnik *et al*., 2012; Fox *et al*., 2014a)

Previous history of hypertensive disorder in pregnancy

As in singleton pregnancies, a past history of preeclampsia increases the risk of developing PE, when compared with women without this background. (Suzuki & Igarashi, 2009; Taguchi *et al*., 2014)

Zygosity

There are numerous studies investigating the incidence of PE in relation to zygosity in twin pregnancies. The results are conflicting and the incidence of PE in monozygotic twins has been reported as higher, similar or lower compared to dizygotic twin pregnancies. (Campbell, 1977; Savvidou *et al*., 2001) These contradictory results may be due to the changing definitions of PE over time, as well as to the determination of zygosity. The only accurate way to determine zygosity is analysing DNA markers, which can only be done prenatally by invasive testing. From a clinical point of view, the determination of zygosity is not easily available and it does not have the potential to be used as an element in screening large populations. (Sebire, 2002; Singh *et al*., 2014)

Chorionicity

Much more important than zygosity is chorionicity. Chorionicity can be reliably determined in the first trimester of pregnancy by ultrasonography and this information can be used prenatally to counsel these women. The association between PE and chorionicity is unclear and the existing data are inconsistent. There are studies showing a higher risk in dichorionic (DC) twins (Sparks *et al*., 2013; Sarno *et al*., 2014; Bartnik *et al*., 2016), others revealing a lower risk of PE (Campbell & Macgillivray, 1999; Campbell & Templeton, 2004) and some concluding lack of association. (Savvidou *et al*., 2001; Suzuki & Igarashi, 2009)

These incongruent findings can be explained by inconsistent diagnostic criteria for PE, small sample sizes and lack of adjusting the data to possible confounding factors. One important bias is the gestational age at delivery, as the incidence of PE could be influenced by the higher rate of preterm delivery in monochorionic (MC) twins. Another important confounder is maternal age: DC twins are more frequent in older mother. If corrections are not done, the data will be severally bias. More recent studies using multiple logistic regression revealed that chorionicity has no effect on the development of PE after adjusting for confounding factors like maternal age, race, parity and gestational age at delivery. (Fox *et al*., 2014a; Singh *et al*., 2014; Carter *et al*., 2015)

Assisted Reproductive Technology

ART role in the incidence of PE in multiple gestations has been explored in several studies but with inconsistent and controversial findings. Some authors found an increased risk of PE in the ART group (Daniel *et al*., 2000; Erez *et al*., 2006; Chaveeva *et al*., 2011; Pourali *et al*., 2016) while others found no difference between the two groups. (Suzuki & Igarashi, 2009; Lučovnik *et al*., 2012; Moini *et al.*, 2012; Anbazhagan, 2014; Geisler *et al.*, 2014)

Similar to what happens with chorionicity, these contrasting findings can be related to sample sizes and not taking in consideration possible confounding factors. When using a multiple logistic regression model the use of ART by itself seems to have no influence in the risk of PE. (Fan *et al*., 2013; Fox *et al*., 2014a) Nevertheless, some particularities of the technique used such as egg donation, seem to increase the rate of PE. (Fox *et al*., 2014a)

Other factors

Different studies have examined the significance of other traditional risk factors for PE. Gestational diabetes, family history of hypertension, maternal smoking, income level and genetic factors apparently have little or no effect on the development of PE in twins. (Krotz *et al*., 2002; Lučovnik *et al*., 2012; Fox *et al*., 2014a; Taguchi *et al*., 2014)

The latest studies support that the only risk factors independently associated with PE in twin pregnancy are pregestational high BMI, egg donation, previous history of hypertensive disorder in pregnancy and probably nulliparity. (Fox *et al*., 2014a; Taguchi *et al*., 2014) Additional studies are needed to confirm theses findings.

PATHOPHYSIOLOGY

The exact aetiology and pathogenesis of PE is largely unknown but it is likely to involve both maternal and placental factors. Abnormalities in the development of placental vasculature in early pregnancy may cause placental hypoxia and the release of factors leading to maternal endothelial dysfunction, causing hypertension and other manifestations of the disease. Considering there is no scientific evidence of a different pathogenesis of PE in twin pregnancy, it is logical to presume that the same mechanisms may be involved. (Karumanchi *et al*., 2017)

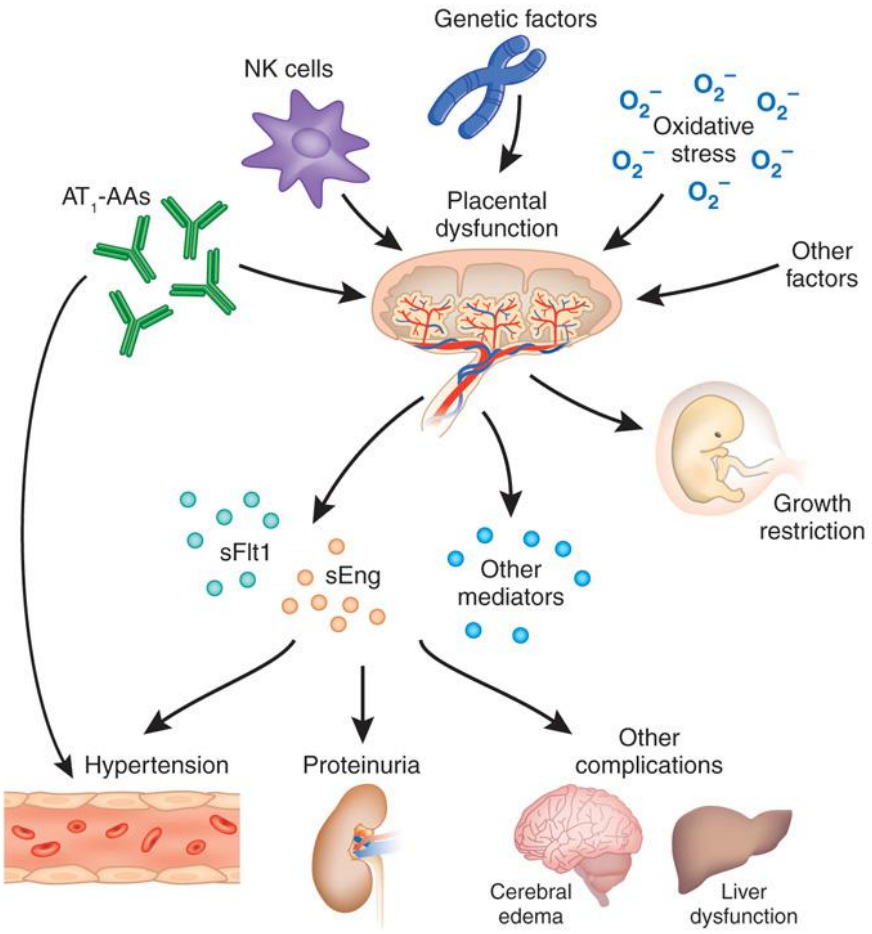

Figure 1.1 Pathogenesis of preeclampsia. (Power *et al.*, 2011) (Reprinted with permission from Wolters Kluwer Health, Inc.) AT_1-AAs: Angiotensin II Type I Receptor Activating Autoantibodies; NK cells: Natural Killer cells; sFlt1: soluble Fms-like tyrosine kinase-1; sEng: soluble Endoglin.

Immunological factors

The immune incompatibility theory supports that the immunological/genetic differences between mother and fetus contribute to the development of PE. This theory states that an immunologic event early in pregnancy activates a maladaptation of the maternal immune system to the fetal trophoblastic tissue. (Karumanchi *et al.*, 2017)

Various studies have proved the existence of increased levels of fetal nucleic acids and syncytiotrophoblast microparticles in maternal blood amongst women with PE. Others have studied immune mechanisms such as maternal natural killer cell interactions and cytotoxic T-cell responses as leading events to PE. If the immunological load is determinant in the development of PE, the

incidence of PE should be higher in dizygotic twins and similar in monozygotic twins and singleton pregnancies. Given most of the dichorionic twins are dizygotic it would be expected that the incidence of PE to be higher in DC compared to MC twins. The more recent studies have however demonstrated similar incidences in both types of twins, which does not support the immunological concept. (Maxwell *et al*., 2001 ; Savvidou *et al*., 2001 ; Sparks *et al*., 2013 ; Singh *et al*., 2014)

Placentation

One of the keys elements involved in PE is placentation. An impaired trophoblastic invasion of maternal spiral arteries can lead to high-pressure flow and damage of the placenta, resulting in placental hypoxia and release of factors leading to maternal endothelial dysfunction. The endothelial dysfunction is the main responsible factor for clinical manifestations like hypertension, proteinuria and end-organ damage. (Long & Oats, 1987; Moldenhauer *et al*., 2003; Rizzo *et al*., 2014; Ohkuchi *et al*., 2017)

One possible explanation for the increased rate of PE amongst women with twin gestations is the placental mass hypothesis. Twins have a larger placental volume, which may cause an increased release of molecules with anti-angiogenic activity, like soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng). However, some studies found no correlation between placental mass and circulation levels of anti-angiogenic factors, making the placental mass hypothesis unlikely. (Sparks *et al*., 2013; Fauple-Badger, 2015 ; Dröge *et al*., 2015 ; Bartnik *et al*., 2016)

Another possible justification for the increased of PE amongst twin gestation is the existence of a less efficient placentation, related with the total implantation site area. In twins is much more likely that at least one placenta is implanted in a hostile part of the uterus, potential resulting in some degree of utero-placental compromise and chronic hypo-perfusion. It is undoubted that the anti-angiogenic factors have a vasoconstrictor role and induce placental ischemia/hypoxia, but it is still not clear whether placental hypoxia is the effect or the cause of increased anti-angiogenic factors release. (Sebire, 2002; Fauple-Badger, 2015)

Angiogenic factors

The angiogenic/angiostatic balance theory supports that there is a variation in the balance of angiogenic proteins, inflammatory cytokines and other immune-modulating molecules in pregnancies with PE. These factors are thought to cause maternal endothelial dysfunction and a systemic inflammatory reaction. (Sargent *et al*., 2003; Karumanchi *et al*., 2017)

Placental growth factor (PlGF) is part of the vascular endothelial growth factor (VEGF) family. It is a protein mainly expressed in the placenta and is considered to be an angiogenic factor. It appears to be involved in mediating increased endothelial vascular permeability cells and angiogenesis. (Power *et al*., 2011; Karumanchi *et al*., 2017)

sFlt-1 is also protein produced by the syncytiotrophoblast but has an anti-angiogenic effect by inhibiting VEGF and PlGF activity. VEGF is important in maintaining endothelial function in fenestrated endothelium especially in the brain, liver and renal glomeruli. High levels of sFlt-1 have vasoconstrictor effects and, by interfering with VEGF function, cause endothelium dysfunction in main organs. (Karumanchi *et al*., 2017)

sEng is produced by the placenta and is an anti-angiogenic protein that appears to be another important mediator of PE. It acts by inhibiting transforming growth factor (TGF) signalling in endothelial cells, causing increased vascular permeability and hypertension. (Karumanchi *et al*., 2017)

Numerous studies in singletons showed an elevation in sFlt-1 and sEng, two anti-angiogenic proteins, previously to the clinical manifestation of PE. The circulating levels of PlGF decrease before the onset of PE and an increased of the sFlt-1/PlGF ratio has also been shown to precede the clinical symptoms of PE. (Ohkuchi *et al*., 2017)

In twin pregnancy angiogenic proteins levels were found to be higher than in singletons throughout all the pregnancy. (Fauple-Badger, 2015) These findings support the hypothesis that the angiogenic/angiostatic balance plays a relevant role in endothelial cell injury, in multiple as in singleton pregnancies. Although the placenta is central to the process, it remains unclear what causes sFlt-1 to increase and PlGF to decrease in women with PE, but it probably points to the multifactorial aspect of this disease. (Fauple-Badger, 2015)

Cardiovascular theory

Despite the fundamental role of the cardiovascular system in PE, data about the interaction between the placenta and the maternal heart are still limited.

It has been proposed that numerous cases of PE can be related to failure of the maternal heart muscle to remodel, especially in relation to diastolic blood pressure. Recent studies consistently show that women with placental failure and impaired left ventricle (LV) function were more likely to develop early-onset PE. These results originated the theory that the maternal cardiovascular response

to placental dysfunction may play an important role in the pathophysiology of PE. These cardiac changes may also play a part in the increased pre-disposition of women with PE to develop long-term cardiovascular disease. (Bellamy, 2007; Melchiorre *et al*., 2014; Osol & Bernstein, 2014)

In twins pregnancy complicated by PE it is recognized that the maternal cardiac function does not undergo the physiological changes that are required for adaptation to pregnancy. As in singletons, a failure in maternal cardiac adaptation, demonstrated by a lower cardiac output and increased total vascular resistance, has been associated with an increased risk of PE. (Ghi *et al*., 2011)

CLINICAL MANIFESTATIONS

The clinical manifestations of PE appeared to be similar in singleton and twins. (August & Sibai, 2017)

Table 1.2- Clinical manifestations of preeclampsia

Symptoms	Signs	Laboratory findings
Persistent and/or severe headache	Raised in BP	Proteinuria
Visual abnormalities	Peripheral edema	Elevated creatinine
Right upper abdominal and/or epigastric pain	Generalized hyperreflexia	Decreased platelet count
Nausea and/or vomiting	Seizure	Hemoconcentration
Oliguria / Anuria		Hyperuricemia
Altered mental status		Elevated transaminase levels

However, hypertensive disorders in twin pregnancies tend to occur earlier and to be more severe. Preterm PE occurs 2.8 - 3.7 times more frequently than in singleton pregnancies. This results in a higher probability of complications, such as preterm delivery: 34.5% in twin gestation versus 6.3% in singletons. (Long & Oats, 1987; Sibai *et al*., 2000 ; Krotz *et al*., 2002 ; Yuan et al., 2016) Twin pregnancies also have an increased risk (about 3 times higher) of eclampsia than singletons. *Abruptio placentae* was found to be 8.2 times more frequent in twin pregnancies with PE when compared to singleton pregnancies and 5.4 times when compared to normotensive twin pregnancies. (Long & Oats, 1987; Krotz *et al*., 2002; Svirsky *et al*., 2013; Henry *et al*., 2013; Taguchi *et al*., 2014)

The diagnosis and management of PE are similar to those in singleton pregnancies. (Chasen & Chervenak, 2017).

SCREENING

The early detection of pregnancies at high-risk of developing PE may improve the maternal and neonatal outcome. The definition of a high-risk population would allow a more intensive and tailored patient surveillance and the institution of prophylactic measures to prevent PE. (Poon *et al*., 2009; Nicolaides, 2011; ACOG, 2015)

In singleton pregnancies, screening for PE in the first trimester using prediction statistical models has a high detection rate. (Akolekar *et al*., 2011) When using a multifactor approach, combining maternal characteristics, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), pregnancy associated plasma protein-A (PAPP-A) and PlGF, the predicted detection rate for early PE is 93.4% for a 5% false-positive rate. (Akolekar *et al*., 2013)

In twin pregnancies, PE screening data are scarce. The importance of identifying twin pregnancies at higher risk of developing PE is high because of the association with an increased risk of perinatal morbidity and both short and long-term maternal complications. Several studies assessed the potential impact of the factors traditionally used in screening for PE in singletons, in screening for PE in twin pregnancies. (Fox *et al*., 2014a; Rizzo *et al*., 2014; Fauple-Badger, 2015; Svirsky *et al*., 2016)

Maternal characteristics

The most relevant maternal characteristics determining the risk of PE in twin pregnancies are not the same of singleton pregnancies. As stated before, the most important risk factors associated with PE in twin pregnancy are pregestational high BMI, egg donation, previous history of hypertensive disorder in pregnancy and nulliparity. (Fox *et al*., 2014a; Fox *et al*., 2014b; Taguchi *et al*., 2014)

Mean arterial pressure

The studies evaluating the distribution of MAP values found that the MAP measures, in unaffected pregnancies, were similar in twin and singleton pregnancies. Additionally, in twins with PE the levels of MAP were elevated parallel to what happens in singletons with PE. Another important finding was that MAP measurements were unrelated to chorionicity. (Svirsky *et al*., 2014) It seems that if screening of PE in twins is to be implemented, the curve used to express MAP levels in Multiple of Median (MoMs) in singletons can be use in twin pregnancy. (Svirsky *et al*., 2014)

Uterine artery pulsatility index

Measurement of uterine artery pulsatility index may be useful in prediction of uteroplacental dysfunction in twin pregnancy. Several studies confirmed an association between increased uterine artery resistance from the first trimester of pregnancy and subsequent PE. (Yu *et al*., 2002; Geipel *et al*., 2002; Yu *et al*., 2008; Rizzo *et al*., 2014)

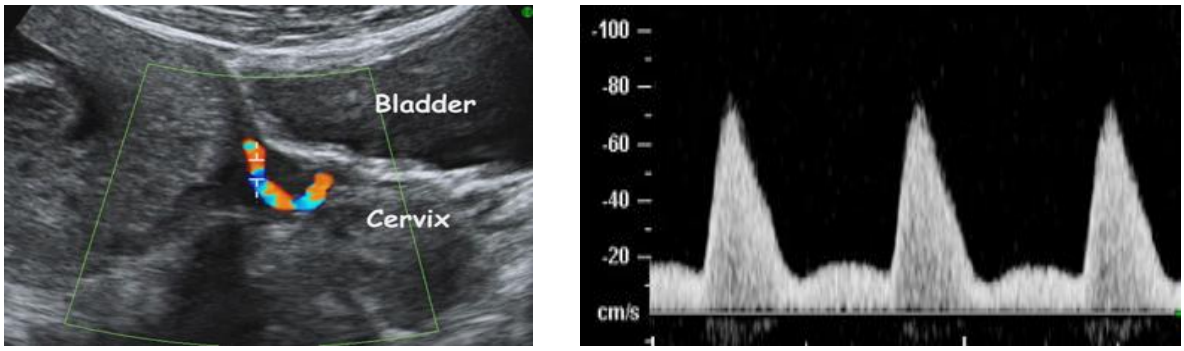

Figure 1.2 a) Transabdominal technique to obtain uterine artery waveform. B) Pulsed wave signal of uterine artery blood flow. (Adapted from Preeclampsia screening, FMF). (Fetal Medicine Foundation, 2018)

Several authors suggest that routine assessment of the uteroplacental circulation by measuring the UtA-PI may be of benefit in the clinical management of twin pregnancies by identifying an even higher-risk subgroup. (Svirsky *et al*., 2014; Ohkuchi *et al*., 2017; Townsend & Khalil, 2018)

It has been proved that, maybe due to the larger placental implantation size and the different hemodynamic maternal status, the mean uterine artery resistance is lower in twins than in singletons. (Yu *et al*., 2002; Klein *et al*., 2011; Geipel *et al*., 2011; Svirsky *et al*., 2014) Furthermore, there appears to be no significant difference in UtA-PI values between MC and DC pregnancies, which corroborates the current evidence that total placental size, rather than genetic or hormonal factors, is likely to play a central role in hemodynamic alterations related to implantation. (Yu *et al*., 2002; Klein *et al*., 2011; Rizzo *et al*., 2014)

In twin pregnancies, UtA-PI is increased in the first trimester in pregnancies that develop early-onset PE but not significantly in the cases of late-onset PE. These findings are consistent with those found in singleton pregnancies, showing that uterine artery Doppler is more efficient in identifying women who develop severe early-onset PE rather than the late form of the disease. (Svirsky *et al*., 2014; Rizzo *et al*., 2014) If the reference ranges used in singleton are applied to twin pregnancies, a higher rate of

false negative can be anticipated. (Papageorghiou *et al*., 2001; Sebire, 2002; Geipel *et al*., 2002) In recent studies the performance of uterine Doppler in the prediction of PE was slightly poorer for twins than for singletons. The application of twin reference curves results in sensitivity around 35% (for a screen-positive rates of 5%) and negative predictive values close to 95%. Despite the lower sensitivity, given the incidence of PE in twin pregnancies is higher than in singletons, the positive predictive value for this condition in twins is higher than in singletons. (Yu *et al*., 2002; Klein *et al*., 2011)

Biochemistry markers

Numerous findings support the hypothesis that an angiogenic imbalance is present in women that develop PE. Particularly, levels of the anti-angiogenic protein s-Flt1 are high and levels of pro-angiogenic proteins, such as PlGF are low in pregnancies with PE. (Maynard *et al*., 2003; Levine *et al*., 2004; Kusanovic *et al*., 2009; Chaiworapongsa *et al*., 2011)

When comparing singleton and twin pregnancies, studies demonstrated higher levels of PlGF, sFlt-1 and higher sFlt-1/PlGF ratio in twins. (Rana *et al*., 2012a; Dröge *et al*., 2015)

In twins with PE *versus* unaffected pregnancies, the serum levels of sFlt-1 and the sFlt-1/PlGF-ratio are significantly increased and PlGF is significantly decreased. (Bdolah *et al*., 2008; Rana *et al*., 2012b; Dröge *et al*., 2015; Svirsky *et al*., 2016) The deviations of these angiogenic markers appear to predict adverse outcomes, and the sFlt-1/PlGF-ratio is inversed correlated with the duration of pregnancy. These results are comparable to those found in singleton pregnancy. (Rana *et al*., 2012a; Rana *et al*., 2012b)

Another biochemistry marker assessed was PAPP-A. When comparing unaffected pregnancies, PAPP-A was significantly higher in twins versus singletons. In pregnancies complicated by PE the median PAPP-A level was significantly raised in twins, in contrast to what happens in singleton pregnancies. (Svirsky *et al*., 2016) One possible justification for the increased PAPP-A levels in twin pregnancies with PE is placental over-compensation. This could happen if, due to impaired blood flow in one placenta, blood flow is increased in the other placenta in order to compensate it but without adequate control. (Svirsky *et al*., 2016)

Screening algorithm

Similarly to aneuploidies, the most effective screening tool for PE in singleton pregnancies is achieved with a combined model. (O'Gorman *et al*., 2016) (O'Gorman *et al*., 2017) In these reports twin

pregnancies were excluded.

An earlier study aimed to create an effectively tool to predict PE in twins. (Maymon *et al*., 2017) The algorithm developed, combining maternal factors, biophysical (MAP and UtA-PI) and biochemistry markers (PAPP-A, PlGF and placental protein 13- PP13) reported a detection rate (DR) of 75% for a false positive rate (FPR) of 10%. The major limitations of this research are the small size of the cohort (n=105), the use of a contingent approach, with biomarkers being assessed in the first and second trimester and the introduction of a new marker (PP13) not routinely used in screening, which adds additional cost.

PREVENTION

Bearing in mind that the pathophysiology of PE is not fully understood, the search for a preventive strategy remains an unfulfilled goal. Many approaches to prevent PE have been studied, but only the use of low-dose dose aspirin has been found to be effective. (August, 2017; Norwitz, 2017)

In singletons pregnancies if screening is carried out before 16 weeks of pregnancy, the daily administration of a low-dose dose aspirin in the high-risk patient reduces the incidence of PE. According to the most recent studies a 10-fold reduction in the incidence of early-onset PE is expected. (Bujold *et al*., 2010; Roberge *et al*., 2012; Rolnik *et al*., 2017)

Similar to what happens in singletons, the identification during the first trimester of twin pregnancies at higher risk of developing PE could form the basis of prophylactic treatments started early in pregnancy. There is little data about the use of aspirin in multiple pregnancies. (Caspi *et al*., 1994; Euser *et al*., 2016) There are some health organizations that support the routine use of low-dose aspirin in all twin pregnancies to prevent PE. (WHO, 2011; U.S. Preventative Services Task Force, 2014) Nonetheless, if the adverse effects and compliance are considered, possibly not every twin pregnancy should receive aspirin. Screening strategies intended to select higher-risk twin pregnancies would be preferable to indiscriminate use.

Despite the extensive research publications in relation to PE in twins there are still several unanswered questions. One important problem is determining how high is the relative risk of PE in twins compared with singletons and what is the significance of the other risk factors for PE in twins. Furthermore is important to clarify the influence of the maternal characteristics and medical history in the risk of PE in twin pregnancy. It is also fundamental to determine the specific profile of different biomarkers in twins with PE and with unaffected pregnancies. These are the essential steps to create an effective algorithm to screen for PE, allowing the identification of an even high-risk group of twins that could benefit from more intensive surveillance and prophylactic measures.

AIMS OF THE THESIS

The aims of the studies in this thesis are:

1. To determine the relative risk for total and preterm-PE in twin pregnancies when compared with singleton pregnancies.

2. To access the influence of maternal characteristics and medical history in the prediction of PE in twin pregnancies.

3. To develop a model for the prediction of PE by a combination of maternal factors and biomarkers in twin pregnancies.

CHAPTER 2

STUDIES SUMMARY

This thesis involves three prospective studies for PE. The data were collected from twin pregnancies undergoing first-trimester combined screening for aneuploidy between January 2006 and December 2015 and subsequently delivering two phenotypically normal live or stillborn babies at or after 24 weeks' gestation. Data on pregnancy outcome were collected and the obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy. The study population included 1789 DC and 430 MC twin pregnancies. For comparison of data, a convenience cohort of 93297 singleton pregnancies that were examined in the same hospitals was analysed.

STUDY 1

The objective of this first study was to determine the gestational age at delivery in DC and MC twin pregnancies, with and without PE, and calculate the relative risk for total and preterm-PE compared to singleton pregnancies.

The distribution of gestational age at delivery of DC and MC twins was compared to singleton pregnancies from the same hospitals. The relative risk for total and preterm-PE in twins compared to singleton pregnancies was assessed. Kaplan Meier estimates of the cumulative incidence of PE in twin and singleton pregnancies, assuming no other cause for delivery, were determined and hazard ratios for twins relative to singletons were obtained from a Cox proportional hazards regression model.

Median gestational age at delivery was 40.0 weeks for singletons, 37.0 weeks for DC twins and 35.4 weeks for MC twins. The rate of PE in singletons was 2.3% (2162 of 93297), in DC twin pregnancies it was 8.1% (145 of 1789) and in MC twins it was 6.0% (26 of 430). Compared to singletons, the relative risk of total PE was 3.5 for DC twins and 2.6 for MC twins. Delivery before 37 weeks' gestation occurred in 5.5% of singletons, in 46.5% of DC twins and in 91.4% of MC twins. The rate of preterm-PE was 0.6%, 5.5%, 5.8% for singletons, DC twins and MC twins, respectively. Compared to singletons, the relative risk of preterm-PE was 8.7 for DC twins and 9.1 for MC twins. In the Cox proportional hazards model the hazard ratios for DC and MC twin pregnancies relative to singleton pregnancies were 14 and 23, respectively.

Our results showed that the relative risk for preterm-PE in DC and MC twins is similar and they are both substantially higher than in singleton pregnancies. In ongoing pregnancies the relative risk of PE

in the subsequent few days is much higher in twin than singleton pregnancies and thus the former may merit a closer monitoring.

STUDY 2

The objective of this study was to develop a survival-time regression model for the gestational age at delivery in DC and MC twin pregnancies with PE, using maternal demographic characteristics and medical history. The risk of PE with delivery before 37 and before 42 weeks' gestation in twin pregnancies was assessed and compared to singleton pregnancies.

A previous study showed that in singleton pregnancies the mean of the Gaussian distribution of gestational age for delivery with PE for a reference population (Caucasian, weight of 69 kg at 12 weeks' gestation, height of 164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome) is 55 weeks. (Wright et al., 2012) In DC twins with PE, the mean gestational age at delivery was shifted to the left by 8.2 (95% CI 7.2-9.1) weeks and in MC twins it was shifted to the left by 10.0 (95% CI 8.5-11.4) weeks. The estimated risk of PE before 37 weeks' gestation, assuming no other cause of delivery, was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins; the respective values for PE before 42 weeks were 3.6%, 27.0% and 36.5%. Screening for PE in twins, using maternal demographic characteristics and medical history, has a high DR but at the cost of a very high screen positive rate (SPR). At all risk cut-offs the screen-positive rate was much higher for twin than singleton pregnancies and, at a risk cut-off of 1 in 75, nearly all twin pregnancies were screened positive.

In conclusion, a model based on maternal characteristics and history has been developed for estimation of patient-specific risks for PE in DC and MC twin pregnancies. Such estimation of the a priori risk for PE is an essential first step in the use of Bayes theorem to combine maternal factors with biomarkers for the continuing development of more effective methods of screening for the disease.

STUDY 3

The objective of this study was to develop a model for screening for PE in twin pregnancies based on maternal demographic characteristics, medical history and biomarkers at 11-13 weeks' gestation.

In this study only patients with complete data on biophysical and biochemistry markers were included. The study population involved 1100 twin pregnancies and, for comparison of data, results from 35948 singleton pregnancies that were examined in the same hospitals were used. Bayes theorem was used to combine the *a priori* risk from maternal factors with various combinations of UtA-PI, MAP, PAPP-A and PlGF converted in MoM values. The performance of screening for PE requiring delivery before 32, 37 and 42 weeks' gestation was estimated.

In twin pregnancies that developed PE, the values of MAP and UtA-PI were increased and PlGF and PAPP-A were decreased. The distributions of MoM values of biomarkers with gestational age at delivery were similar to those that were previously reported in singleton pregnancies and it was therefore assumed that the same model can be used for both singleton and twin pregnancies. The performance of screening for PE by maternal factors was improved by the addition of MAP, UtA-PI and PlGF; there was no further improvement with the addition of PAPP-A. In a mixed population of singleton and twin pregnancies, combined screening by maternal factors, MAP, UtA-PI and PlGF and risk cut-off of 1 in 75 for PE before 37 weeks, the detection rate of PE before 32, 37 and 42 weeks in singleton pregnancies was 91%, 77% and 57%, respectively, at SPR of 13%; the respective rates for twin pregnancies were 100%, 99% and 97%, at SPR of 75%.

It was concluded that first-trimester combined screening for PE in singleton pregnancies can be adapted for screening in twins leading to detection of nearly all affected cases but at a high SPR.

CHAPTER 3

PUBLISHED STUDIES

STUDY 1

Francisco C, Wright D, Benkő Z, Syngelaki A, Nicolaides KH.

Hidden high rate of pre-eclampsia in twin compared with singleton pregnancy.

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Hidden high rate of pre-eclampsia in twin compared with singleton pregnancy

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KEYWORDS: first-trimester screening; pre-eclampsia; pyramid of pregnancy care; twin pregnancy

ABSTRACT

Objectives *To examine the gestational age at delivery in dichorionic (DC) and monochorionic (MC) twin pregnancies, with and without pre-eclampsia (PE), and to determine the relative risk of total and preterm PE compared with that in singleton pregnancies.*

Methods *This was a screening study for PE in twin pregnancies undergoing first-trimester combined screening for aneuploidy and subsequently delivering two phenotypically normal live or stillborn babies at ≥ 24 weeks' gestation. The distribution of gestational age at delivery in DC and MC twins was determined and compared with that in singleton pregnancies from the same population. The relative risk for total and preterm PE in twins compared with singleton pregnancies was determined. Kaplan–Meier estimates of the cumulative incidence of PE in twin and singleton pregnancies, assuming no other cause for delivery, were determined and hazard ratios for twins relative to singletons were obtained from a Cox proportional hazards regression model.*

Results *The incidence of PE in singletons was 2.3% (2162/93 297), in DC twin pregnancies was 8.1% (145/1789) and in MC twin pregnancies was 6.0% (26/430). Compared with singletons, the relative risk of total PE was 3.5 for DC twins and 2.6 for MC twins. Delivery < 37 weeks' gestation occurred in 5.5% of singletons, 46.5% of DC twins and 91.4% of MC twins. The incidence of preterm PE was 0.6%, 5.5% and 5.8% for singletons, DC twins and MC twins, respectively. Compared with singletons, the relative risk of preterm PE was 8.7 for DC twins and 9.1 for MC twins. In the Cox proportional hazards regression model, the hazard ratios for DC and MC twin pregnancies relative to singleton pregnancies were 14 and 23, respectively.*

Conclusions *The relative risk of preterm PE in DC and MC twins is similar and substantially higher than in singleton pregnancies. In ongoing twin pregnancies, the high relative risk of PE may merit a higher intensity of monitoring than is routine for singleton pregnancies.* *© 2017 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

In singleton pregnancies, the incidence of pre-eclampsia (PE) is 2–3%; in 25–30% of cases of PE, delivery occurs < 37 weeks' gestation (preterm PE) and in 70–75% delivery is at term[1]. In twin pregnancies, the rate of PE is higher than in singletons. In 10 studies reporting on 256 to 9998 twin pregnancies, the overall rate of PE was 9.5% (2069/21 817)[2–11]. Consequently, the relative risk of PE for twin compared with singleton pregnancies is about 3. Six studies examined the rate of PE in twins in relation to chorionicity; in five studies the rate was similar in dichorionic (DC) and monochorionic (MC) twins[3–5,7,9], but in one study the rate was twice as high in DC than in MC twins[6].

Twins are delivered at an earlier gestational age than are singletons and, consequently, comparison of the overall rates of PE between twin and singleton pregnancies may underestimate the relative risk of preterm PE in twins. The incidence of adverse fetal and maternal short-term and long-term consequences of PE is higher in preterm PE than in term PE[12–16].

The objective of this study was to examine the gestational age at delivery in DC and MC twin

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pregnancies, with and without PE, and to determine the relative risk of total and preterm PE compared with that in singleton pregnancies.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women with twin pregnancy attending their first routine hospital visit at 11 + 0 to 13 + 6 weeks' gestation at King's College Hospital and Medway Maritime Hospital, UK, between January 2006 and December 2015. In this visit, maternal characteristics and medical history were recorded and combined screening for aneuploidy was performed[17]. Gestational age was determined by measurement of fetal crown–rump length[18] of the larger twin. Chorionicity was determined by examining the intertwin membrane at its junction with the placenta[19]. Women gave written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee.

The inclusion criteria for this study on screening for PE were twin pregnancy undergoing first-trimester combined screening for aneuploidy and subsequently delivering two phenotypically normal live or stillborn babies at ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidy and major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of more than 3 days between death of one fetus and live birth of the second twin. For comparison of data from twin pregnancies, we obtained results from 93 297 singleton pregnancies that were examined in the same hospitals as the twin pregnancies and were included in a previous publication[1].

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[20]. PE was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women. There should also be proteinuria ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above), developing after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or presence of hypertension at booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

Statistical analysis

The distribution of gestational age at delivery in DC and MC twin pregnancies was determined and was compared with that in singleton pregnancies. The risks of total PE and PE < 37 weeks' gestation in twin pregnancies relative to those in singleton pregnancies were compared. Since the rate of preterm birth in twin pregnancies is higher than in singletons, we also compared the risk of PE < 37 weeks in twins *vs* singletons in pregnancies that were ongoing at 35 weeks' gestation. Kaplan–Meier estimates of the cumulative incidence of PE in twin and singleton pregnancies, assuming no other cause for delivery, were determined and hazard ratios for twins relative to singletons were obtained from a Cox proportional hazards regression model. The statistical software package R was used for data analyses[21]. The R package 'survival'[22] was used for Cox regression.

RESULTS

During the study period, 2554 twin pregnancies were examined, of which 335 (13.1%) cases were excluded because they had a major fetal defect ($n = 28$), pregnancy resulted in termination ($n = 61$) or miscarriage ($n = 156$) or there was no follow-up ($n = 90$). The study population included 1789 DC and 430 MC twin pregnancies. The maternal and pregnancy characteristics of the twin and singleton pregnancies included in the study are shown in Table 1. Compared with singletons, DC twin pregnancies had higher median maternal age and weight, a lower incidence of smokers and higher incidence of nulliparous women and conception by assisted reproductive techniques. Compared with singletons, MC twin pregnancies had a higher incidence of conception by *in-vitro* fertilization (IVF). Compared with singleton pregnancies that developed PE, DC twin pregnancies that developed PE had a higher median maternal age, a lower incidence of women of Afro-Caribbean racial origin and a much higher incidence of conception by IVF. Median gestational age at delivery was 40.0 weeks for singletons, 37.0 weeks for DC twins and 35.4 weeks for MC twins (Figure 1).

The incidence of PE was 2.3% (2162/93 297) in singletons, 8.1% (145/1789) in DC twins and 6.0% (26/430) in MC twins. Compared with singletons, the relative risk of total PE was 3.50 (95% CI, 2.97–4.11) for DC twins and 2.61 (95% CI, 1.79–3.77) for MC twins (Figure 2). Delivery < 37 weeks' gestation occurred in 5.5% of singletons, 46.5% of DC twins and 91.4% of MC twins. The incidence of preterm PE (< 37 weeks) was 0.6% (597/93 297) for singletons, 5.5% (99/1789) for DC twins and 5.8% (25/430) for MC twins. Compared with singletons, the relative risk of preterm PE < 37 weeks was 8.65 (95% CI, 7.02–10.63) for DC twins and 9.09 (95% CI, 6.15–13.30) for MC twins (Figure 2).

At 35 + 0 weeks' gestation, there were 91 196, 1373 and 243 ongoing singleton, DC twin and MC twin pregnancies, respectively. PE with delivery < 37 weeks

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Table 1 Maternal and pregnancy characteristics in screening population of singleton pregnancies and dichorionic and monochorionic twin pregnancies with and without pre-eclampsia (PE)

	Singletons		*Dichorionic twins*		*Monochorionic twins*	
Characteristic	*All* (n = *93 297*)	*PE* (n = *2162*)	*All* (n = *1789*)	*PE* (n = *145*)	*All* (n = *430*)	*PE* (n = *26*)
Maternal age (years)	31.0 (26.4–35.0)	31.1 (26.4–35.6)	33.2 (29.1–36.5)*	34.0 (30.3–37.3)†	31.5 (27.0–35.8)	31.0 (28.0–36.6)
Maternal weight (kg)	66.5 (59.0–77.0)	72.7 (63.0–86.5)	69.0 (60.5–79.0)*	72.0 (63.1–84.0)	65.3 (58.7–77.0)	71.9 (60.0–84.0)
Maternal height (cm)	164 (160–169)	163 (159–168)	165 (161–170)	165 (160–168)	164 (160–168)	163 (159–169)
GA at examination (weeks)	12.7 (12.3–13.1)	12.7 (12.3–13.1)	12.9 (12.5–13.3)	12.8 (12.4–13.2)	12.8 (12.5–13.3)	13.0 (12.5–13.5)
Racial origin						
Caucasian	70 380 (75.4)	1273 (58.9)	1390 (77.7)	104 (71.7)†	320 (74.4)	20 (76.9)
Afro-Caribbean	15 211 (16.3)	716 (33.1)	287 (16.0)	33 (22.8)†	66 (15.3)	4 (15.4)
South Asian	3761 (4.0)	97 (4.5)	57 (3.2)	4 (2.8)	23 (5.3)	2 (7.7)
East Asian	1790 (1.9)	31 (1.4)	22 (1.2)	3 (2.1)	11 (2.6)	0 (0)
Mixed	2155 (2.3)	45 (2.1)	33 (1.8)	1 (0.7)	10 (2.3)	0 (0)
Medical history						
Chronic hypertension	1203 (1.3)	245 (11.3)	27 (1.5)	13 (9.0)	3 (0.7)	0 (0)
Diabetes mellitus	799 (0.9)	46 (2.1)	18 (1.0)	4 (2.8)	5 (1.2)	0 (0)
APS/SLE	148 (0.2)	12 (0.6)	4 (0.2)	1 (0.7)	0 (0)	0 (0)
Cigarette smoker	10 087 (10.8)	166 (7.7)	160 (8.9)*	8 (5.5)	43 (10.0)	0 (0)
Family history of PE	4047 (4.3)	175 (8.1)	77 (4.3)	7 (4.8)	20 (4.7)	2 (7.7)
Obstetric history						
Nulliparous	44 145 (47.3)	1319 (61.0)	968 (54.1)*	101 (69.7)	216 (50.2)	13 (50.0)
Parous, previous PE	3143 (3.4)	300 (13.9)	52 (2.9)	11 (7.6)	16 (3.7)	1 (3.8)
Parous, no previous PE	46 009 (49.3)	543 (25.1)	769 (43.0)*	33 (22.8)	198 (46.0)	12 (46.2)
Interpregnancy interval (years)	3.0 (2.0–5.0)	4.1 (2.5–7.1)	3.1 (2.0–5.3)	4.1 (3.0–7.2)	3.0 (1.7–4.9)	3.4 (2.1–4.6)
GA at delivery of previous pregnancy (weeks)	40 (39–40)	39 (37–40)	40 (39–40)	40 (38–40)	40 (39–40)	40 (38–41)
Mode of conception						
Spontaneous	90 275 (96.8)	2048 (94.7)	1162 (65.0)*	90 (62.1)†	385 (89.5)*	24 (92.3)
Ovulation induction	1281 (1.4)	32 (1.5)	52 (2.9)*	5 (3.4)	3 (0.7)	0 (0)
In-vitro fertilization	1741 (1.9)	82 (3.8)	575 (32.1)*	50 (34.5)†	42 (9.8)*	2 (7.7)

Data are given as median (interquartile range) or *n* (%). *Post-hoc* Bonferroni correction used for multiple comparisons and significance defined by $P < 0.025$. Significant difference when compared with: *all singletons; †singletons with PE. APS, antiphospholipid syndrome; GA, gestational age; SLE, systemic lupus erythematosus.

in these pregnancies occurred in 249 (0.3%) of the singletons, 53 (3.9%) of the DC twins and 13 (5.3%) of the MC twins. Compared with singletons, the relative risk of PE < 37 weeks in these ongoing pregnancies was 14.14 (95% CI, 10.56–18.93) for DC twins and 19.59 (95% CI, 11.38–33.73) for MC twins (Figure 2).

Kaplan–Meier estimates of the cumulative incidence of PE in singleton and twin pregnancies, assuming no other cause for delivery, are shown in Figure 3. In the Cox proportional hazards regression model, the hazard ratios for DC and MC twin pregnancies relative to singleton pregnancies were 14.0 (95% CI, 9.5–20.6) and 23.3 (95% CI, 11.2–47.6), respectively.

DISCUSSION

The finding of this study, that, in twin pregnancies compared with singleton pregnancies, the overall incidence of PE is about three times higher, is consistent with the results of previous studies[2–11]. We also found that the rate of PE for DC and MC twin pregnancies is similar, which is also compatible with the results of previous studies[3–5,7,9].

A new finding of the study is that, in twin pregnancies, the rate of preterm PE, between 24 + 0 and 36 + 6 weeks' gestation, is nine times higher than in singleton pregnancies. Indeed, if we consider the risk for preterm PE in the subgroup of pregnancies that are ongoing at 35 weeks' gestation, the relative risk is 14 for DC twins and 20 for MC twins, and these rates were very similar to the hazard ratios of 14 for DC and 23 for MC twin pregnancies relative to singleton pregnancies. This hidden high risk of PE in twins relative to singletons is well illustrated in the Kaplan–Meier estimate of the cumulative incidence of PE, assuming no other cause for delivery (Figure 3).

The underestimate of the relative risk of PE in twins, by comparison with singletons, when reporting the total rate of PE from 24 to 43 weeks' gestation is the mere consequence of the lower gestational age at delivery in twin than in singleton pregnancies. In our study, the

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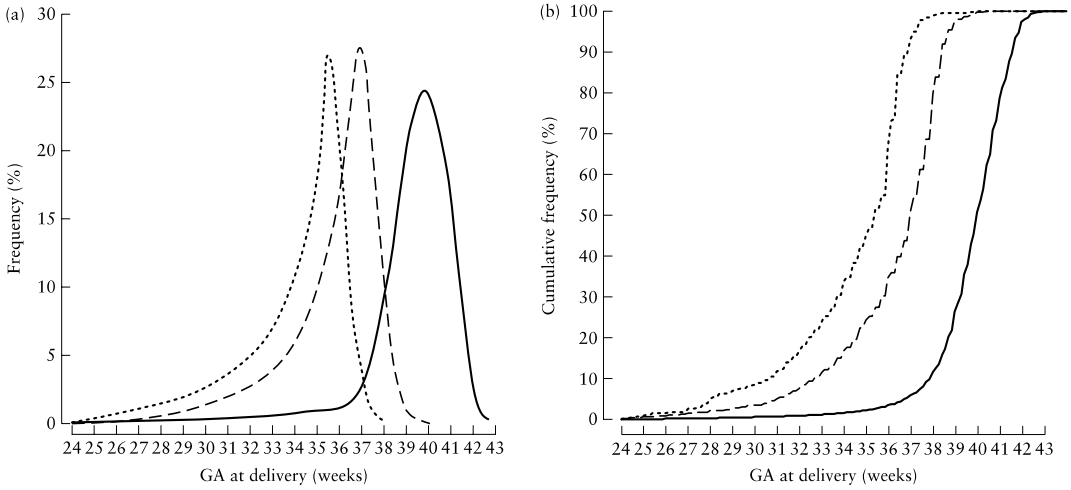

Figure 1 Frequency (a) and cumulative frequency (b) of gestational age (GA) at delivery in singleton (——), dichorionic twin (– – –) and monochorionic twin (·------) pregnancies.

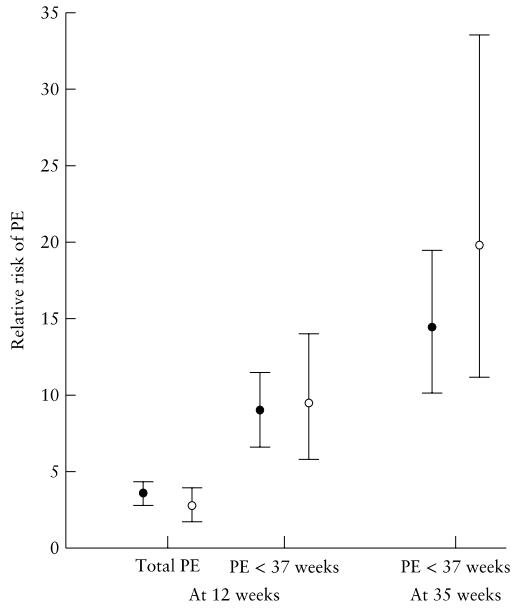

Figure 2 Relative risk, with 95% CI, of total pre-eclampsia (PE) and PE < 37 weeks at 12 and 35 weeks' gestation in dichorionic (●) and monochorionic (○) twin pregnancies, compared with singleton pregnancies.

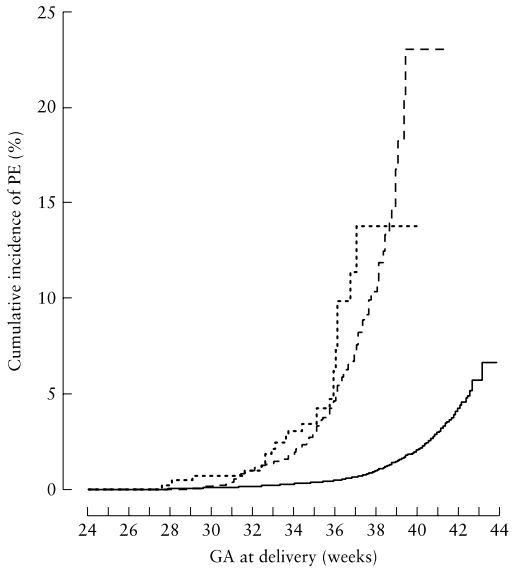

Figure 3 Kaplan–Meier estimates of cumulative incidence of pre-eclampsia (PE) in singleton (——), dichorionic twin (– – –) and monochorionic twin (·------) pregnancies, assuming no other cause for delivery. GA, gestational age.

median gestational age at delivery was 40 weeks for singletons, 37 weeks for DC twins and 35 weeks for MC twins and delivery < 37 weeks' gestation occurred in 6% of singletons, 47% of DC twins and 91% of MC twins. These rates are similar to those reported for all births in the USA in 2014 for which the rate of delivery < 37 weeks was 8% for singletons and 59% for twins[23]. Since a much higher proportion of twin compared with singleton pregnancies deliver before a given gestational age, in the ongoing pregnancies, the relative risk of PE in the subsequent few days is much higher than the value of 3 implied from the overall rate of PE; the true relative risk is about 14 for DC twins and > 20 for MC twins.

We have proposed previously the adoption of a survival-time model for the gestational age at delivery with PE[1,24]. This approach assumes that, if the pregnancy

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was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. It is therefore likely that if the duration of pregnancy in twins was as long as in singletons, the overall rate of PE in twins would be considerably higher than the observed rate.

The incidence of adverse fetal and maternal consequences of PE is higher in preterm PE than in term PE[12–16]. Clinicians managing twin pregnancies should be aware that the rate of preterm PE, relative to that in singleton pregnancies, is substantially higher than that implied by the overall rate of PE. Clinicians should also be aware that, during the third trimester in ongoing pregnancies, the relative risk of PE in the subsequent few days is much higher in twin than in singleton pregnancies and they, therefore, merit a higher intensity of monitoring for PE. Future studies will lead to development of an algorithm for screening for PE that, as in singleton pregnancies, would adopt a survival-time model for the gestational age at delivery with PE[1].

ACKNOWLEDGMENT

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STUDY 2

Francisco C, Wright D, Benkő Z, Syngelaki A, Nicolaides KH.

Competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history.

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Competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history

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KEYWORDS: Bayes' theorem; first-trimester screening; pre-eclampsia; pyramid of pregnancy care; survival model; twin pregnancy

ABSTRACT

Objective *A survival-time regression model for gestational age at delivery with pre-eclampsia (PE) in singleton pregnancy, using maternal demographic characteristics and medical history, was reported previously. The objective of this study was to extend this model to dichorionic (DC) and monochorionic (MC) twin pregnancy.*

Methods *The study population included 1789 DC and 430 MC twin pregnancies and 93 297 singleton pregnancies. A survival-time model for gestational age at delivery with PE was developed from variables of maternal characteristics and medical history. The risk of PE with delivery < 37 weeks and < 42 weeks in twin pregnancies was determined and compared with that in singleton pregnancies.*

Results *In singleton pregnancies comprising women of Caucasian racial origin, mean weight of 69 kg at 12 weeks' gestation, mean height of 164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, the mean of the Gaussian distribution of gestational age at delivery with PE was 55 weeks. In DC twins with PE, mean gestational age at delivery was shifted to the left by 8.2 (95% CI, 7.2–9.1) weeks and in MC twins it was shifted to the left by 10.0 (95% CI, 8.5–11.4) weeks. The risk of delivery with PE occurring at, or before, a specified gestational age is given by the area under the fitted distribution curve. For a reference population with the above characteristics, the estimated risk of PE < 37 weeks' gestation, assuming no other cause of delivery, was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins; the respective values for PE < 42 weeks were 3.6%, 27.0% and 36.5%.*

Conclusions *A model based on maternal characteristics and medical history has been developed for estimation of patient-specific risks for PE in DC and MC twin pregnancy. Such estimation of the* a-priori *risk for PE is an essential first step in the use of Bayes' theorem to combine maternal factors with biomarkers for the continuing development of more effective methods of screening for the disease. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

In singleton pregnancies, effective screening for pre-eclampsia (PE) can be achieved with the use of Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements[1–5]. A fundamental component of this approach is adoption of a survival-time model for the gestational age at delivery with PE. This approach assumes that, if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. In this model, the mean gestational age for delivery with PE for a reference population (Caucasian racial origin, weight 69 kg, height 164 cm, nulliparous, spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS)) is 55 weeks with estimated SD of 6.88 weeks (Figure 1)[3]. The effect of variables from maternal characteristics and history and biomarkers is to modify the mean of the

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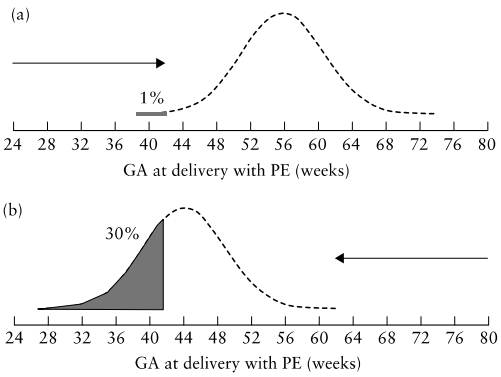

Figure 1 Distribution of gestational age (GA) at delivery with pre-eclampsia (PE) in pregnancy at low (a) or high (b) risk for PE. Distribution in low-risk pregnancies is shifted to right and, in most cases, delivery will occur before development of PE. Distribution in high-risk pregnancies is shifted to left. Risk of PE occurring at, or before, a specified gestational age is given by area under distribution curve (gray); risk of PE < 42 weeks' gestation is 1% for low-risk group and 30% for high-risk group.

distribution of gestational age at delivery with PE so that, in pregnancies at low risk for PE, the gestational-age distribution is shifted to the right, with the implication that, in most pregnancies, delivery will actually occur before development of PE. In high-risk pregnancies, the distribution is shifted to the left and the earlier the mean gestational age, the higher is the risk for PE. Variables from maternal factors that shift the Gaussian distribution of the gestational age at delivery with PE to the left include advancing maternal age, increasing maternal weight, Afro-Caribbean and South Asian racial origins, medical history of chronic hypertension, diabetes mellitus and SLE or APS, family history and personal history of PE and conception by *in-vitro* fertilization[1,3]. The risk for PE decreases with increasing maternal height and in parous women with no previous PE; in the latter, the protective effect, which is inversely related to the interpregnancy interval, persists beyond 15 years[1,3].

In twin pregnancy, the incidence of PE is about 10%[6–15], which is three-times higher than in singleton pregnancy. However, twins are delivered at an earlier gestational age than singletons and, consequently, comparison of the overall incidence of PE between twin and singleton pregnancies underestimates the relative risk of preterm PE in twins, which is nine-times higher; the rates of both total and preterm PE for dichorionic (DC) and monochorionic (MC) twins are similar[16].

The objective of this study was to modify the survival-time model for gestational age at delivery with PE, based on maternal demographic characteristics and medical history, that was developed from the study of singleton pregnancies to include adjustments for DC and MC twin pregnancies.

METHODS

Study population

Data for this study were derived from prospective screening for adverse obstetric outcomes in women attending their routine first hospital visit in pregnancy at King's College Hospital and Medway Maritime Hospital, UK. In this visit, at 11 + 0 to 13 + 6 weeks' gestation, maternal characteristics and medical history were recorded and combined screening for aneuploidy was performed[17]. Gestational age was determined by measurement of fetal crown–rump length[18] of the larger twin. Chorionicity was determined by examining the intertwin membrane at its junction with the placenta[19]. The women were screened between January 2006 and December 2015 and gave written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee.

Inclusion criteria for this study on screening for PE were twin pregnancy undergoing first-trimester combined screening for aneuploidy and subsequently delivering a phenotypically normal live birth or stillbirth at ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of more than 3 days between death of one fetus and live birth of the second fetus. For comparison of data from twin pregnancies, we obtained results from 93 297 singleton pregnancies that were examined in the same hospitals as the twins and were included in a previous publication[3].

Patient characteristics

Patient characteristics that were recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring *in-vitro* fertilization or use of ovulation drugs), cigarette smoking during pregnancy, history of chronic hypertension, diabetes mellitus, SLE or APS, family history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancy at ≥ 24 weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal weight and height were measured and body mass index was calculated.

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for

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the Study of Hypertension in Pregnancy[20]. PE was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women. Hypertension was defined as proteinuria ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as above) developing after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or presence of hypertension at booking visit before 20 weeks' gestation, in the absence of trophoblastic disease).

Statistical analysis

The previously developed model of a Gaussian distribution of gestational age (in weeks) at the time of delivery with PE based on maternal factors[3] was applied to DC and MC twin pregnancies. In this model, deliveries from causes other than PE were treated as censored observations. Established risk factors, including maternal age in years, weight in kg, height in cm, racial origin, interpregnancy interval in years, gestational age at delivery in weeks of previous pregnancy with and without PE, method of conception, chronic hypertension, diabetes mellitus, SLE or APS, DC and MC twins were included as covariates. The performance of screening for delivery with PE < 32 weeks, < 37 weeks and < 42 weeks' gestation in twin pregnancies was determined and compared with that in singleton pregnancies; the number of affected cases was too small to provide separate results for DC and MC twins.

The statistical software package R was used for data analyses[21]. The survival package was used for model fitting[22].

RESULTS

The study population included 1789 DC twin pregnancies, 430 MC twin pregnancies and 93 297 singleton pregnancies. Maternal and pregnancy characteristics are summarized in Table 1. The incidence of PE was 2.3% (2162/93 297) in singletons, 8.1% (145/1789) in DC twins and 6.0% (26/430) in MC twins.

The effect of maternal and pregnancy characteristics (estimate and 95% CI) on mean time to delivery with PE is shown in Figure 2. The effects are relative to a reference population comprising women of Caucasian racial origin, with weight of 69 kg at 12 weeks' gestation, height of

Table 1 Maternal and pregnancy characteristics in screening population of 93 297 singleton pregnancies, 1789 dichorionic twin pregnancies and 430 monochorionic twin pregnancies, and in the subgroups with pre-eclampsia (PE)

	Singletons		*Dichorionic twins*		*Monochorionic twins*	
Characteristic	*All* (n = *93 297*)	*PE* (n = *2162*)	*All* (n = *1789*)	*PE* (n = *145*)	*All* (n = *430*)	*PE* (n = *26*)
Maternal age (years)	31.0 (26.4–35.0)	31.1 (26.4–35.6)	33.2 (29.1–36.5)	34.0 (30.3–37.3)	31.5 (27.0–35.8)	31.0 (28.0–36.6)
Maternal weight (kg)	66.5 (59.0–77.0)	72.7 (63.0–86.5)	69.0 (60.5–79.0)	72.0 (63.1–84.0)	65.3 (58.7–77.0)	71.9 (60.0–84.0)
Maternal height (cm)	164 (160–169)	163 (159–168)	165 (161–170)	165 (160–168)	164 (160–168)	163 (159–169)
GA (weeks)	12.7 (12.3–13.1)	12.7 (12.3–13.1)	12.9 (12.5–13.3)	12.8 (12.4–13.2)	12.8 (12.5–13.3)	13.0 (12.5–13.5)
Racial origin						
Caucasian	70 380 (75.4)	1273 (58.9)	1390 (77.7)	104 (71.7)	320 (74.4)	20 (76.9)
Afro-Caribbean	15 211 (16.3)	716 (33.1)	287 (16.0)	33 (22.8)	66 (15.3)	4 (15.4)
South Asian	3761 (4.0)	97 (4.5)	57 (3.2)	4 (2.8)	23 (5.3)	2 (7.7)
East Asian	1790 (1.9)	31 (1.4)	22 (1.2)	3 (2.1)	11 (2.6)	0 (0)
Mixed	2155 (2.3)	45 (2.1)	33 (1.8)	1 (0.7)	10 (2.3)	0 (0)
Medical history						
Chronic hypertension	1203 (1.3)	245 (11.3)	27 (1.5)	13 (9.0)	3 (0.7)	0 (0)
Diabetes mellitus	799 (0.9)	46 (2.1)	18 (1.0)	4 (2.8)	5 (1.2)	0 (0)
APS/SLE	148 (0.2)	12 (0.6)	4 (0.2)	1 (0.7)	0 (0)	0 (0)
Cigarette smoker	10 087 (10.8)	166 (7.7)	160 (8.9)	8 (5.5)	43 (10.0)	0 (0)
Family history of PE	4047 (4.3)	175 (8.1)	77 (4.3)	7 (4.8)	20 (4.7)	2 (7.7)
Parity						
Nulliparous	44 145 (47.3)	1319 (61.0)	968 (54.1)	101 (69.7)	216 (50.2)	13 (50.0)
Parous, previous PE	3143 (3.4)	300 (13.9)	52 (2.9)	11 (7.6)	16 (3.7)	1 (3.8)
Parous, no previous PE	46 009 (49.3)	543 (25.1)	769 (43.0)	33 (22.8)	198 (46.0)	12 (46.2)
Interpregnancy interval (years)	3.0 (2.0–5.0)	4.1 (2.5–7.1)	3.1 (2.0–5.3)	4.1 (3.0–7.2)	3.0 (1.7–4.9)	3.4 (2.1–4.6)
GA at delivery of last birth (weeks)	40 (39–40)	39 (37–40)	40 (39–40)	40 (38–40)	40 (39–40)	40 (38–41)
Mode of conception						
Spontaneous	90 275 (96.8)	2048 (94.7)	1162 (65.0)	90 (62.1)	385 (89.5)	24 (92.3)
Ovulation induction	1281 (1.4)	32 (1.5)	52 (2.9)	5 (3.4)	3 (0.7)	0 (0)
In-vitro fertilization	1741 (1.9)	82 (3.8)	575 (32.1)	50 (34.5)	42 (9.8)	2 (7.7)

Data are given as mean (interquartile range) or *n* (%). APS, antiphospholipid syndrome; GA, gestational age; SLE, systemic lupus erythematosus.

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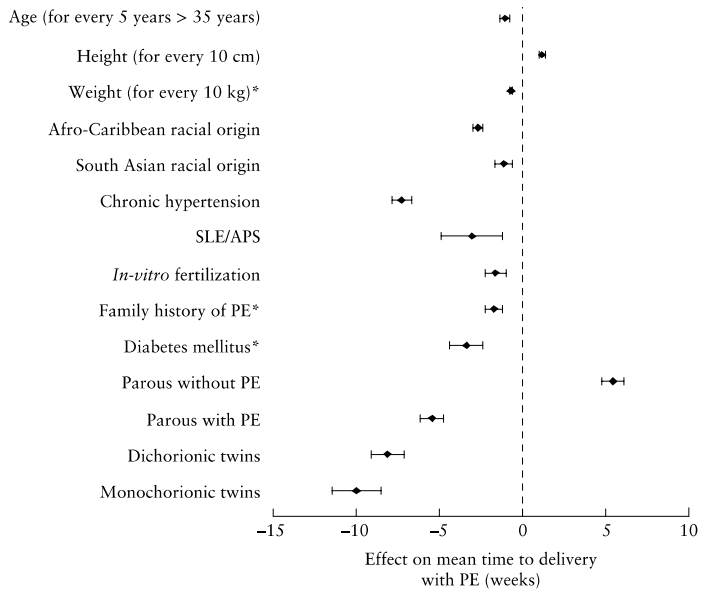

Figure 2 Effect of maternal and pregnancy characteristics (estimate and 95% CI) on mean time to delivery with pre-eclampsia (PE). Effects are relative to reference levels of singleton pregnancy, Caucasian racial origin, weight 69 kg, height 164 cm, nulliparous, spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS). For parous women without PE, effect is for interval of 1 year from birth of an unaffected pregnancy at 41 weeks' gestation and, for parous women with PE, effect is for previous affected pregnancy delivering with PE at 34 weeks' gestation. *In pregnancies without chronic hypertension.

164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, SLE or APS. Compared with singletons with the same maternal characteristics, DC twins delivered with PE on average 8.2 (95% CI, 7.2–9.1) weeks earlier and MC twins delivered with PE on average 10.0 (95% CI, 8.5–11.4) weeks earlier. The difference between the effects of DC and MC twins was significant ($P = 0.038$).

The Gaussian distribution of gestational age at delivery with PE in singletons, DC twins and MC twins is illustrated in Figure 3; the mean was 55 weeks for singletons, 47 weeks for DC twins and 45 for MC twins. The risk of delivery with PE occurring at, or before, a specified gestational age is given by the area under the distribution curve. For a reference population with the above characteristics, the estimated risk of PE < 32 weeks' gestation, assuming no other cause for delivery, was 0.06% for singletons, 1.9% for DC twins and 3.6% for MC twins; the respective values for PE < 37 weeks were 0.6%, 9.0% and 14.2% and for PE < 42 weeks were 3.6%, 27.0% and 36.5%.

Receiver–operating characteristics (ROC) curves for prediction of all PE and PE with delivery < 32 weeks and < 37 weeks' gestation for singleton and twin pregnancies are shown in Figure 4. Table 2 provides the screen-positive rate and detection rate in screening of a mixed population of singleton and twin pregnancies at risk cut-offs of 1 in 10, 1 in 50 and 1 in 75 for

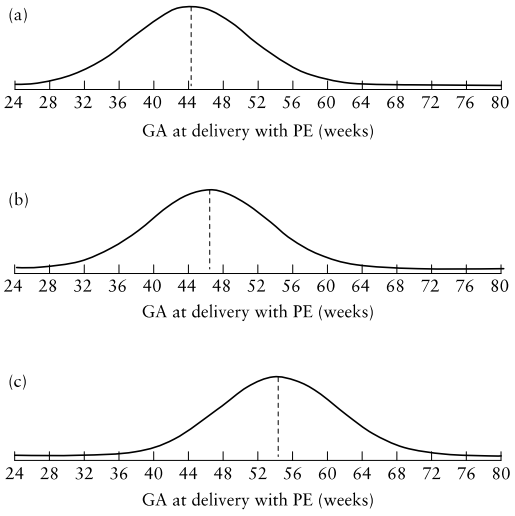

Figure 3 Distribution of gestational age (GA) at delivery with pre-eclampsia (PE) for monochorionic twin (a), dichorionic twin (b) and singleton (c) pregnancies, with reference characteristics of Caucasian racial origin, weight 69 kg, height 164 cm, nulliparous, spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome. Dashed line shows mean GA.

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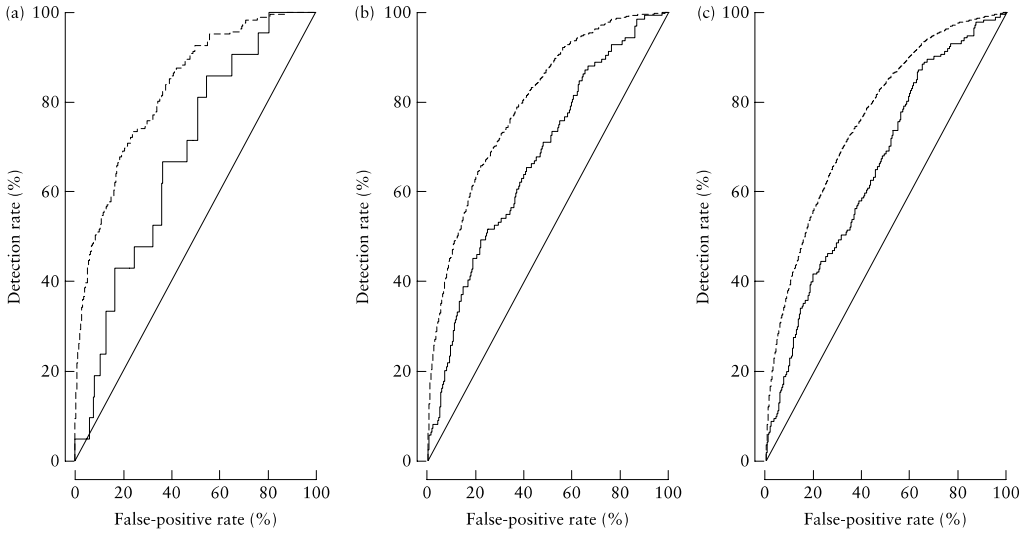

Figure 4 Receiver–operating characteristics curves for prediction of pre-eclampsia (PE) requiring delivery: (a) < 32 weeks; (b) < 37 weeks; and (c) in all cases of PE, in singleton (——) and twin (– – – –) pregnancies according to competing-risks model.

Table 2 Screen-positive rate and detection rate at different risk cut-offs in screening by maternal factors and medical history for pre-eclampsia (PE) in singleton and twin pregnancy

	Risk cut-off 1 in 10		*Risk cut-off 1 in 50*		*Risk cut-off 1 in 75*	
	Singletons (n = 93 297)	*Twins (n = 2219)*	*Singletons (n = 93 297)*	*Twins (n = 2219)*	*Singletons (n = 93 297)*	*Twins (n = 2219)*
Screen-positive rate	722 (0.8; 0.7–0.8)	1113 (50.2; 48.1–52.3)	6517 (7.0; 6.8–7.2)	2152 (97.0; 96.2–97.7)	12 512 (13.4; 13.2–13.6)	2211 (99.6; 99.3–99.8)
Detection rate						
PE < 32 weeks	26/161 (16.1; 10.8–22.8)	15/21 (71.4; 47.8–88.7)	74/161 (46.0; 38.1–54.0)	21/21 (100; 83.9–100)	90/161 (55.9; 47.9–63.7)	21/21 (100; 83.9–100)
PE < 37 weeks	76/597 (12.7; 10.2–15.7)	88/124 (71.0; 62.1–78.8)	222/597 (37.2; 33.3–41.2)	123/124 (99.2; 95.6–100)	299/597 (50.1; 46.0–54.2)	124/124 (100; 97.1–100)
PE < 42 weeks	175/2140 (8.2; 7.1–9.4)	116/171 (67.8; 60.3–74.8)	675/2140 (31.5; 29.6–33.6)	169/171 (98.8; 95.8–99.9)	935/2140 (43.7; 41.6–45.8)	171/171 (100; 97.9–100)

Data are given as *n*/*N* (%; 95% CI).

delivery with PE < 37 weeks' gestation. At all risk cut-offs the screen-positive rate was much higher for twin than singleton pregnancies and, at a risk cut-off of 1 in 75, nearly all twin pregnancies were screen positive.

DISCUSSION

Principal findings of this study

In twin pregnancy, the risk of PE is substantially higher than in singleton pregnancy and this is reflected in the distribution of gestational age at delivery with PE. In a population of women with singleton pregnancy, Caucasian racial origin, weight of 69 kg, height of 164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, SLE or APS, the mean gestational age at delivery with PE is 55 weeks. In DC and MC twin pregnancies with the same characteristics as the singleton pregnancies, the distribution of gestational age at delivery with PE is shifted to the left by 8 and 10 weeks, respectively. The estimated risk of PE < 37 weeks' gestation was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins.

In screening of a mixed population of singleton and twin pregnancies for PE < 37 weeks' gestation at a risk cut-off of 1 in 75, the screen-positive rate for singleton pregnancies was 13% whereas nearly all twin pregnancies were screen positive. This is not surprising because, in any screening program, certain maternal characteristics may be associated with such high risk that, irrespective of how favorable are all other factors, the posterior risk is above a cut-off used for stratification of the population into high- and low-risk groups. For example, in the context of first-trimester combined screening for trisomy

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21, the prior risk increases exponentially with maternal age and, for a woman aged 50 years, the posterior risk will remain above the screen-positive cut-off irrespective of how favorable are the measurements of fetal nuchal translucency thickness and serum free beta human chorionic gonadotropin and pregnancy-associated plasma protein-A.

Strengths and limitations

The major strengths of the study are, first, prospective examination of twin and singleton pregnancies in which specific questions were asked to identify known factors associated with PE, second, the use of multivariable survival analysis to identify the factors and define their contribution in the prediction of PE and third, the development of a survival-time model which allows estimation of individual patient-specific risks of PE requiring delivery before any specified gestational age.

A limitation of the study is that the number of twin pregnancies examined relative to that of singleton pregnancies is inevitably small. Another limitation is that the performance of screening by a model derived and tested using the same dataset is overestimated. External validation on independent data from different sources is required.

Comparison with previous studies

In previous studies, we established a competing-risks model for the prediction of PE in singleton pregnancy based on maternal factors which was expanded to include biomarkers[1–5]. In this study, the maternal factor-based model has been extended to include twin pregnancies. Previous studies in twin pregnancy merely reported that the rate of PE is about three-times higher than in singleton pregnancy[6–15].

Clinical implications of the study

The proposed competing-risks model allows estimation of patient-specific *a-priori* risk for PE in twins, which is an essential first step in the use of Bayes' theorem to combine maternal factors with biomarkers for the continuing development of more effective methods of screening for the disease. Bayes' theorem also provides a framework for updating the risk at different stages during pregnancy, forming the basis for stratified surveillance policies, as has been achieved for singleton pregnancies[23–25].

ACKNOWLEDGMENTS

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STUDY 3

Francisco C, Wright D, Benkő Z, Syngelaki A, Nicolaides KH.

Competing-risks model in screening for pre-eclampsia in twin pregnancy according to maternal factors and biomarkers at 11-13 weeks' gestation.

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Competing-risks model in screening for pre-eclampsia in twin pregnancy according to maternal factors and biomarkers at 11–13 weeks' gestation

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KEYWORDS: Bayes theorem; first-trimester screening; mean arterial pressure; placental growth factor; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; survival model; uterine artery Doppler

ABSTRACT

Objective *To develop a model for screening for pre-eclampsia (PE) in twin pregnancies based on maternal demographic characteristics and medical history and biomarkers at 11–13 weeks' gestation.*

Methods *This was a screening study in twin pregnancies at 11–13 weeks' gestation. Bayes theorem was used to combine the* a-priori *risk from maternal factors with various combinations of uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP), serum pregnancy-associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) multiples of the median (MoM) values. The performance of screening for PE requiring delivery at < 32, < 37 and < 42 weeks' gestation was estimated in 1100 twin pregnancies and 35 948 singleton pregnancies with complete data on UtA-PI, MAP, PlGF and PAPP-A.*

Results *In twin pregnancies that developed PE, the values of MAP and UtA-PI were increased and the values of PlGF and PAPP-A were decreased. The distributions of log_{10} MoM values of biomarkers with gestational age at delivery were similar to those that were previously reported in singleton pregnancies and it was therefore assumed that the same model could be used for both singleton and twin pregnancies. The performance of screening for PE by maternal factors was improved by the addition of MAP, UtA-PI and PlGF; there was no further improvement with the addition of PAPP-A. In a mixed population of singleton and twin pregnancies, combined screening by maternal factors, MAP, UtA-PI and PlGF and risk cut-off of 1 in 75 for PE at < 37 weeks, the detection rate of PE at < 32, < 37 and < 42 weeks in singleton pregnancies was 91%, 77% and 57%, respectively, at a screen-positive rate (SPR) of 13%; the respective rates for twin pregnancies were 100%, 99% and 97%, at a SPR of 75%.*

Conclusion *First-trimester combined screening for PE in singleton pregnancies can be adapted for screening in twins, leading to detection of nearly all affected cases but at a high SPR. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

In screening for pre-eclampsia (PE) we proposed the use of Bayes theorem to combine the *a-priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy[1–8]. We adopted this approach using a competing-risks model for the time to delivery with PE. This model assumes that if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. The effect of maternal factors is to modify the mean of the distribution of gestational age at delivery with PE so that in pregnancies at low risk for PE the gestational age distribution is shifted to the right with the implication that in most pregnancies delivery will actually occur before development of PE. In high-risk pregnancies the distribution is shifted to the left and the smaller the mean gestational age, the higher is the risk for PE. The distribution of biomarkers is specified conditionally on the gestational age at delivery with PE. For any woman with specific maternal factors and biomarker multiples of the median (MoM) values, the posterior distribution of the time to delivery with PE, assuming that there is no other cause of delivery,

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is obtained from the application of Bayes theorem. Studies in singleton pregnancies have demonstrated that effective screening for PE is achieved by a combination of maternal factors, uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP) and serum placental growth factor (PlGF) at 11–13 weeks' gestation; serum pregnancy-associated plasma protein-A (PAPP-A) did not provide significant improvement to any combination of biomarkers which included serum PlGF[4,5].

In twin pregnancy the risk of PE is substantially higher than in singleton pregnancy[9]. In a hypothetical population of singleton pregnancies in which women were of Caucasian racial origin, had weight of 69 kg and height of 164 cm, were nulliparous, had spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, the mean gestational age of delivery with PE was calculated to be 55 weeks[1,3]. In dichorionic (DC) and monochorionic (MC) twin pregnancies with the same characteristics as singleton pregnancies, the distribution of gestational age at delivery with PE was shifted to the left by 8 and 10 weeks, respectively[10].

The objective of this study of twin pregnancies with data on MAP, UtA-PI, PlGF and PAPP-A at 11–13 weeks' gestation, was to examine the potential improvement in performance of screening for pre-eclampsia by maternal factors alone with the addition of each biomarker and combinations of biomarkers.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women with twin pregnancy attending their first routine hospital visit at King's College Hospital and Medway Maritime Hospital, UK, between January 2006 and December 2015. This visit, which was held at 11 + 0 to 13 + 6 weeks' gestation, included: first, recording of maternal characteristics and medical history[3,9]; second, measurement of MAP by validated automated devices and standardized protocol[11]; third, measurement of the left and right UtA-PI by transabdominal color Doppler ultrasound and calculation of the mean UtA-PI[12]; and, fourth, measurement of serum concentration of PlGF and PAPP-A (DELFIA Xpress system; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). Gestational age was determined by the measurement of fetal crown–rump length[13] of the larger twin. Chorionicity was determined by examining the intertwin membrane at its junction with the placenta[14]. Measurements of biomarkers were carried out for only some of the patients (UtA-PI, $n = 1764$; MAP, $n = 1179$; PlGF, $n = 1366$; PAPP-A, $n = 1999$: measurements of all four biomarkers were obtained from 1100 pregnancies). The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

The inclusion criteria for this study on screening for PE were twin pregnancy undergoing first-trimester combined screening for aneuploidy and subsequently delivering a phenotypically normal live birth or stillbirth at ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of more than 3 days between the death of one fetus and the live birth of the second twin. For comparison of data from twin pregnancies we obtained results from 35 948 singleton pregnancies, including 1058 (2.9%) that developed PE, with complete data on UtA-PI, MAP, PlGF and PAPP-A that were included in a previous publication[4].

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[15]. Outcome measures were delivery with PE at < 32, < 37 and < 42 weeks' gestation.

Statistical analyses

Our model for gestational age at delivery with PE was defined by two components: first, the prior distribution based on maternal factors; and, second, the conditional distribution of biomarker values given the gestational age with PE and maternal factors. We have previously reported the prior distribution for twins by modifying the one for singletons[10]. In this paper we extend the singleton model for the distribution of biomarker values to include twins. Our aim was to do this as simply as possible whilst achieving good screening performance. For the distribution of biomarkers in unaffected twin pregnancies, we assumed the same relationships with gestational age, maternal weight and other factors at the time of measurement as for singleton pregnancies[16–18] and estimated twin effects that can be applied to produce MoM values for twins. In the case of PAPP-A, MoM values were obtained using our previously published algorithm which provides MoM values for both singleton and twin pregnancies[19]. In twin pregnancies with PE, the distributions of MoM values, conditionally on gestational age at delivery, were compared with those previously reported for singleton pregnancies[4]. The performance of screening for delivery with PE at < 32, < 37 and < 42 weeks' gestation in a mixed population of singleton and twin pregnancies was determined; the number of affected cases was too small to provide separate results for DC and MC twins.

The statistical software package R was used for data analyses[20]. The survival package[21] was used for fitting the maternal factors model, and the package pROC[22] was used for the receiver–operating characteristics (ROC) curve analysis.

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Table 1 Maternal and pregnancy characteristics in the screening population

	Singleton pregnancy		*Twin pregnancy*	
Variable	*All (n = 35 948)*	*PE (n = 1058)*	*All (n = 1100)*	*PE (n = 93)*
Maternal age (years)	31.3 (26.8–35.0)	31.5 (27.0–35.6)	33.1 (28.8–36.6)	34.2 (29.0–37.3)
Maternal weight (kg)	66.7 (59.0–77.2)	72.1 (63.0–86.7)	69.0 (60.3–80.0)	73.4 (62.6–84.0)
Maternal height (cm)	165 (160–169)	163 (159–168)	165 (161–170)	164 (160–168)
Gestational age (weeks)	12.7 (12.3–13.1)	12.7 (12.3–13.1)	12.8 (12.5–13.2)	12.8 (12.4–13.2)
Racial origin				
Caucasian	25 879 (72.0)	564 (53.3)	812 (73.8)	61 (65.6)
Afro-Caribbean	6681 (18.6)	394 (37.2)	199 (18.1)	25 (26.9)
South Asian	1623 (4.5)	56 (5.3)	43 (3.9)	4 (4.3)
East Asian	846 (2.4)	17 (1.6)	18 (1.6)	2 (2.2)
Mixed	919 (2.6)	27 (2.6)	28 (2.5)	1 (1.1)
Medical history				
Chronic hypertension	561 (1.6)	140 (13.2)	19 (1.7)	9 (9.7)
Diabetes mellitus	325 (0.9)	22 (2.1)	12 (1.1)	2 (2.2)
SLE/APS	53 (0.1)	5 (0.5)	2 (0.2)	1 (1.1)
Cigarette smoker	3263 (9.1)	68 (6.4)	85 (7.7)	2 (2.2)
Family history of PE	1518 (4.2)	90 (8.5)	46 (4.2)	5 (5.4)
Parity				
Nulliparous	17 361 (48.3)	622 (58.8)	538 (48.9)	60 (64.5)
Parous: previous PE	1276 (3.5)	153 (14.5)	35 (3.2)	7 (7.5)
Parous: no previous PE	17 311 (48.2)	283 (26.7)	527 (47.9)	26 (28.0)
Interpregnancy interval (years)	3 (2–5)	4.1 (2.4–7.4)	3.1 (2.0–5.4)	4.1 (2.4–6.4)
Gestational age of last birth (weeks)	40 (39–40)	39 (37–40)	40 (39–40)	40 (38–40)
Conception				
Spontaneous	34 743 (96.6)	998 (94.3)	779 (70.8)	65 (69.9)
Ovulation induction	349 (1.0)	19 (1.8)	20 (1.8)	0 (0)
In-vitro fertilization	856 (2.4)	41 (3.9)	301 (27.4)	28 (30.1)
Chorionicity of twin pregnancy				
Dichorionic			885 (80.5)	77 (82.8)
Monochorionic			215 (19.5)	16 (17.2)

Data given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; PE, pre-eclampsia; SLE, systemic lupus erythematosus.

RESULTS

Maternal and pregnancy characteristics of the population of 1100 twin and 35 948 singleton pregnancies with available data for all four biomarkers are summarized in Table 1.

Twin pregnancies unaffected by PE, compared with singleton pregnancies, had lower UtA-PI and higher MAP, PlGF and PAPP-A (Table 2, Figure 1). In twin pregnancies that developed PE the dependencies of $\log_{10}$ MoM values of UtA-PI, MAP and PlGF on gestational age at delivery were similar to those previously described for singleton pregnancies (Figure 2)[4] and they all showed significant associations with gestational age at delivery ($P < 0.05$). In the case of PAPP-A, in twins there was little evidence of any substantive difference between PE and unaffected pregnancies and no evidence of association with gestational age at delivery.

Estimates of standard deviation of $\log_{10}$ MoM values were similar to those previously described for singletons[4]. The largest discrepancy occurred with PlGF, for which the standard deviation of $\log_{10}$ MoM was some 25% higher in twins than in singletons; 0.2222 (95% CI, 0.2142–0.2309) in twins compared with 0.1772 (95% CI, 0.1760–0.1785) in singletons[4]. Correlations between $\log_{10}$ MoM values in twins were consistent with those from singletons. With the exception of PAPP-A, the similarity between the distributional characteristics of marker MoM values in twins and in singletons suggests that the distributional parameters for singleton pregnancies could also be applied in twin pregnancies. We therefore propose, as an initial model for twins, use of the twin-specific prior model[10] with the model for the distributional characteristics for singletons[4] applied to MoM values adjusted by the effects given in Table 2.

Table 2 Estimated median multiples of the median (MoM) values, with 95% confidence limits, for biomarkers in twin pregnancy derived from algorithms used for singleton pregnancy

Biomarker	*Median MoM value estimate*	P*
Placental growth factor		
Dichorionic twins	1.2326 (1.1926–1.2741)	< 0.0001
Monochorionic twins	0.9968 (0.9335–1.0644)	0.923
Uterine artery pulsatility index		
Dichorionic twins	0.8686 (0.8535–0.8839)	< 0.0001
Monochorionic twins	0.9647 (0.9320–0.9985)	0.041
Mean arterial pressure		
Dichorionic twins	1.0030 (0.9973–1.0088)	0.304
Monochorionic twins	1.0156 (1.0039–1.0275)	0.009

*Effect relative to singleton MoM, for which median for each biomarker is 1.0.

Performance of screening for PE in twins by maternal factors and biomarkers, using this initial model, is shown in the ROC curves in Figure 3 and the areas under the ROC curves in Table 3. The performance of screening for PE by maternal factors was improved by the addition of each biomarker, and the performance

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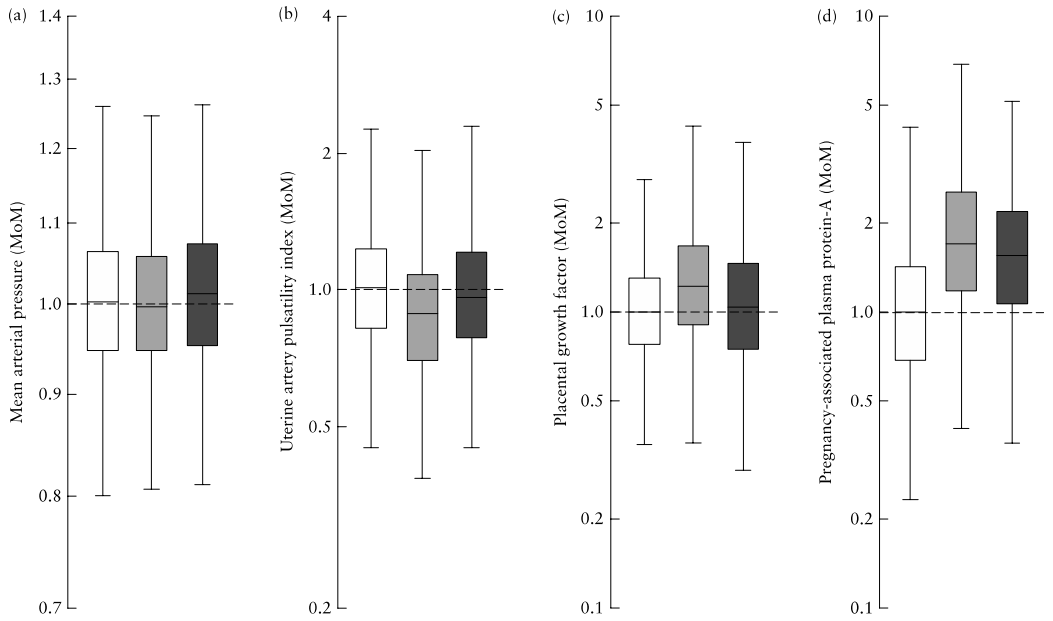

Figure 1 Box-and-whisker plots of mean arterial pressure (a), uterine artery pulsatility index (b), placental growth factor (c) and pregnancy-associated plasma protein-A (d) multiples of the median (MoM) values in singleton (□), dichorionic twin (▩) and monochorionic twin (■) pregnancies that did not develop pre-eclampsia.

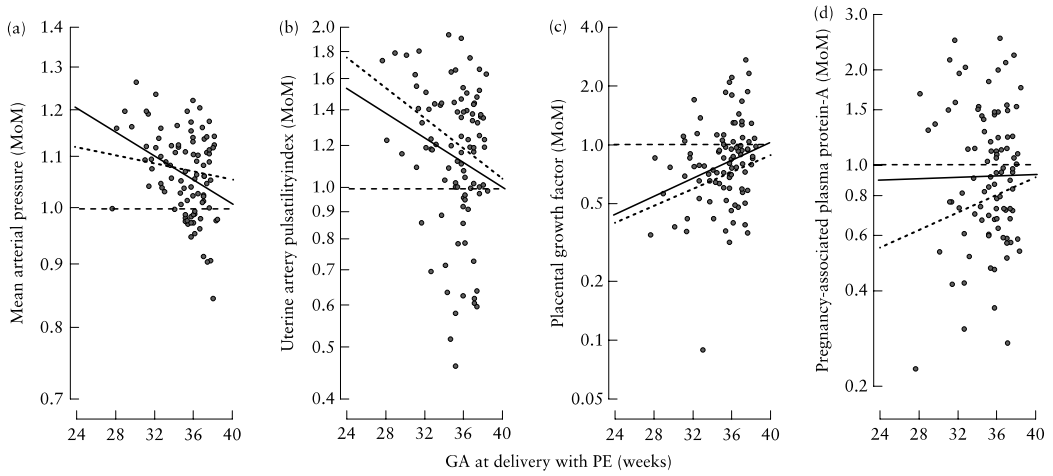

Figure 2 Scatterplot of mean arterial pressure (a), uterine artery pulsatility index (b), placental growth factor (c) and pregnancy-associated plasma protein-A (d) multiples of the median (MoM) values in twin pregnancies with pre-eclampsia and regression lines (——) with gestational age (GA) at delivery. Regression lines derived from a previous study in singleton pregnancies[4] are also shown (------).

was further improved by the combination of maternal factors, MAP, UtA-PI and PlGF (triple test); addition of PAPP-A did not improve the performance of the triple test. The performance of screening by the triple test using distributional parameters fitted to twins performed slightly better than the initial model (Table 3).

Table 4 provides the screen-positive rate (SPR) and detection rate (DR) in screening of a mixed population of singleton and twin pregnancies at risk cut-offs of 1 in 10, 1 in 50 and 1 in 75 for delivery with PE at < 37 weeks' gestation. At all risk cut-offs the SPR and DR were higher for twin pregnancies than for singleton pregnancies. In

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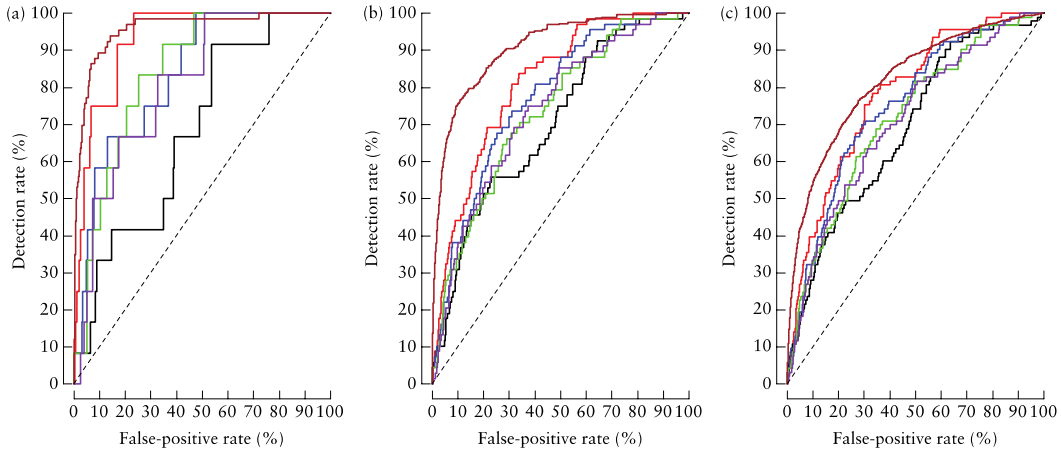

Figure 3 Receiver–operating characteristics curves for prediction of delivery with pre-eclampsia at < 32 weeks' (a), < 37 weeks' (b) and < 42 weeks' (c) gestation, according to maternal factors (——) and to combination of maternal factors with mean arterial pressure (——), uterine artery pulsatility index (——), placental growth factor (——) and their combination (——). Curves representing performance of screening according to combined test in singleton pregnancy are also shown (——).

Table 3 Areas under the receiver–operating characteristics curve (AUC) in screening for pre-eclampsia (PE) according to maternal factors and combination of maternal factors with biomarkers in twin pregnancy

	AUC (95% CI)		
Screening	*PE < 32 weeks*	*PE < 37 weeks*	*PE < 42 weeks*
Maternal factors	0.6805 (0.5436–0.8173)	0.7026 (0.6410–0.7642)	0.6880 (0.6337–0.7422)
Maternal factors with:			
MAP	0.8343 (0.7371–0.9314)	0.7725 (0.7198–0.8251)	0.7509 (0.7014–0.8004)
UtA-PI	0.8416 (0.7618–0.9214)	0.7354 (0.6768–0.7941)	0.7156 (0.6619–0.7693)
PlGF	0.8071 (0.7052–0.9089)	0.7395 (0.6810–0.7980)	0.7080 (0.6534–0.7625)
PAPP-A	0.6897 (0.5389–0.8406)	0.7149 (0.6556–0.7741)	0.7066 (0.6526–0.7607)
MAP, UtA-PI, PlGF	0.9293 (0.8851–0.9735)	0.8122 (0.7661–0.8583)	0.7796 (0.7342–0.8250)
MAP, UtA-PI, PlGF*	0.9400 (0.9077–0.9723)	0.8172 (0.7724–0.8621)	0.7857 (0.7409–0.8306)
MAP, UtA-PI, PlGF, PAPP-A	0.9227 (0.8727–0.9727)	0.8111 (0.7649–0.8572)	0.7804 (0.7353–0.8254)

*Results from regressions fitted to twin data. MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

combined screening by maternal factors, MAP, UtA-PI and PlGF and risk cut-off of 1 in 75, the DR of PE at < 32, < 37 and < 42 weeks in singleton pregnancies was 91%, 77% and 57%, respectively, at an SPR of 13%; the respective rates for twin pregnancies were 100%, 99% and 97% at an SPR of 75%.

DISCUSSION

Principal findings of this study

In DC twin pregnancies that did not develop PE, compared with singleton pregnancies, UtA-PI was lower, PlGF was higher and MAP was not significantly different. In MC twins, UtA-PI was lower, MAP was higher and PlGF was not significantly different. In a previous study we found that at 11–13 weeks' gestation the estimated median MoM of PAPP-A was about 2.0 for DC twins and 1.6 for MC twins[19].

In twin pregnancies that developed PE, MAP and UtA-PI at 11–13 weeks' gestation were increased and serum PlGF was decreased. The distribution of biomarkers with gestational age at delivery was similar to the previously reported fitted regression relationships for singleton pregnancies with PE[4] and it was therefore assumed that the same model could be used for both singleton and twin pregnancies. We propose an initial model using a twin-specific prior[10] with biomarker MoM values adjusted for twins, but singleton distributional parameters for biomarkers. The results obtained using this model are marginally worse than those using twin-specific parameters. However, this may be the consequence of the optimistic bias introduced from fitting and testing on the same data.

The performance of screening for PE by maternal factors was improved by the addition of MAP, UtA-PI and PlGF. In screening for PE by the combined test and use of risk cut-off of 1 in 75 to define the high-risk group in a mixed population of singleton and twin pregnancies, the estimated SPR and DR of preterm PE for singletons were 13% and 77%, respectively, and the values for twins were 75% and 99%.

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Table 4 Screen-positive and detection rates with 95% CI in screening for pre-eclampsia (PE) in singleton and twin pregnancies according to a combination of maternal factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and placental growth factor (PlGF) at risk cut-offs for PE at <37 weeks of 1 in 10, 1 in 50 and 1 in 75

	Risk cut-off 1 in 10		*Risk cut-off 1 in 50*		*Risk cut-off 1 in 75*	
	Singletons (n = *35 948*)	*Twins* (n = *1100*)	*Singletons* (n = *35 948*)	*Twins* (n = *1100*)	*Singletons* (n = *35 948*)	*Twins* (n = *1100*)
Screen-positive rate						
Maternal factors	344 (1.0, 0.9–1.1)	559 (50.8, 47.8–53.8)	2794 (7.8, 7.5–8.1)	1069 (97.2, 96.0–98.1)	5231 (14.6, 14.2–14.9)	1095 (99.5, 98.9–99.9)
+ MAP, UtA-PI, PlGF	560 (1.6, 1.4–1.7)	278 (25.3, 22.7–28.0)	3140 (8.7, 8.4–9.0)	724 (65.8, 62.9–68.6)	4541 (12.6, 12.3–13.0)	825 (75.0, 72.3–77.5)
Detection rate						
PE <32 weeks						
Maternal factors	11/66 (16.7, 8.6–27.9)	9/12 (75.0, 42.8–94.5)	32/66 (48.5, 36.0–61.1)	12/12 (100, 73.5–100)	60/66 (90.9, 81.3–96.6)	12/12 (100, 73.5–100)
+ MAP, UtA-PI, PlGF	36/66 (54.5, 41.8–66.9)	12/12 (100, 73.5–100)	58/66 (87.9, 77.5–94.6)	12/12 (100, 73.5–100)	60/66 (90.9, 81.3–96.6)	12/12 (100, 73.5–100)
PE <37 weeks						
Maternal factors	37/292 (12.7, 9.1–17.0)	51/68 (75.0, 63.0–84.7)	116/292 (39.7, 34.1–45.6)	67/68 (98.5, 92.1–100)	158/292 (54.1, 48.2–59.9)	68/68 (100, 94.7–100)
+ MAP, UtA-PI, PlGF	104/292 (35.6, 30.1–41.4)	47/68 (69.1, 56.7–79.8)	210/292 (71.9, 66.4–77.0)	67/68 (98.5, 92.1–100)	226/292 (77.4, 72.2–82.1)	67/68 (98.5, 92.1–100)
PE <42 weeks						
Maternal factors	92/1044 (8.8, 7.2–10.7)	68/93 (73.1, 62.9–81.8)	355/1044 (34.0, 31.1–37.0)	91/93 (97.8, 92.4–99.7)	474/1044 (45.4, 42.4–48.5)	93/93 (100, 96.1–100)
+ MAP, UtA-PI, PlGF	191/1044 (18.3, 16.0–20.8)	59/93 (63.4, 52.8–73.2)	502/1044 (48.1, 45.0–51.2)	88/93 (94.6, 87.9–98.2)	590/1044 (56.5, 53.4–59.5)	90/93 (96.8, 90.9–99.3)

Values are given as *n*/*N* (%, 95% CI).

Strengths and limitations

The strengths of the study are: first, prospective examination of twin pregnancies attending for routine care in a gestational-age range which is widely used for assessment of gestational age, determination of chorionicity, diagnosis of major fetal defects and screening for trisomies; second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE and use of a multivariable logistic model to define the prior risk; third, use of a specific methodology and appropriately trained doctors to measure UtA-PI and MAP; fourth, use of automated machines to provide accurate measurement within 40 min of sampling of maternal serum concentration of PlGF and PAPP-A; fifth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements; and, sixth, use of Bayes theorem to combine the prior risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

A limitation of the study is that the number of twin pregnancies examined relative to that of singleton pregnancies was inevitably small and we adopted the pragmatic view of using the model that was previously described for singleton pregnancies to examine both singleton and twin pregnancies. This model requires validation and possible adjustments from the study of larger datasets of twins.

Comparison with previous studies

In previous studies we established a competing-risks model for the prediction of PE in singleton pregnancies based on maternal factors and biomarkers[2,4]. In this study the model has been extended to include twin pregnancies.

Studies on uterine artery Doppler during the second and third trimesters reported that UtA-PI in twin pregnancies is significantly lower than in singleton pregnancies, but the value is increased in pregnancies that develop PE[23–26]. A study of 421 twin and 500 singleton pregnancies at 11–13 weeks' gestation, in which UtA-PI was measured and values were converted to MoM after correction for maternal body mass index, ethnicity and gestational age, showed that: first, median UtA-PI MoM was similar between MC and DC twins; second, UtA-PI in twins was lower than in singletons; and, third, in twin pregnancies that developed PE at < 34 weeks' gestation, but not in those with PE at ≥ 34 weeks, UtA-PI was increased[27]. Another first-trimester study in 147 twin and 110 singleton pregnancies converted the values to MoM after correction for maternal weight and gestational age; the study reported that: first, UtA-PI in twins was lower than in singletons; second, UtA-PI was higher in MC twins than in DC twins; and, third, in 12 twins that developed PE, median UtA-PI was significantly reduced[28]. This study also measured MAP, converted the values to MoM after correction for maternal weight and gestational age and reported that: first, MAP in twins was not significantly

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different from that in singletons; second, MAP was unrelated to chorionicity; and, third, in the twin pregnancies that developed PE, median MAP was increased[28].

A study in 144 twin and 109 singleton pregnancies at 11–13 weeks' gestation measured serum PAPP-A and PlGF, converted the values to MoM and reported that: first, in twin pregnancies PAPP-A and PlGF were approximately twice as high compared with singletons; and, second, in 12 twin pregnancies that developed PE, median PlGF was decreased but PAPP-A was increased[29]. A study in 74 twin and triplet pregnancies and 698 singleton pregnancies measured serum PlGF at 12–18 weeks' gestation and reported that: first, in multiple pregnancies the levels were higher than in singletons; and, second, in five multiple pregnancies that developed PE median PlGF was decreased[30].

Clinical implications of the study

In singleton pregnancies, effective screening for preterm PE can be provided at 11–13 weeks' gestation by a combination of maternal factors, MAP, UtA-PI and PlGF[4,5]. Such early identification of high-risk pregnancies is useful because the rate of PE may be reduced by the prophylactic use of low-dose aspirin and/or pravastatin[31–33]. This study has demonstrated that the same model of screening can be adapted for use in mixed populations of singleton and twin pregnancies.

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CHAPTER 4

DISCUSSION

PRINCIPAL FINDINGS AND COMPARISON WITH PREVIOUS STUDIES

The results of our first study showed that in twin pregnancies, compared to singleton pregnancies, the overall rate of PE is about 3 times higher, consistent with the results of previous reports. (Savvidou *et al*., 2001; Yu *et al*., 2002; Geipel *et al*., 2002; Klein *et al*., 2011; Sparks *et al*., 2013; Rizzo *et al*., 2014; Bensdorp *et al*., 2016; Lučovnik *et al*., 2016; Wang *et al*., 2016; Barda *et al*., 2017) We also found that the rate of PE for DC and MC twins is similar. This is also compatible with the results of other studies. (Savvidou *et al*., 2001; Yu *et al*., 2002; Klein *et al*., 2011; Rizzo *et al*., 2014; Lučovnik *et al*., 2016)

Additionally, our findings revealed that in twin pregnancies the rate of preterm-PE, between 24+0 and 36+6 weeks' gestation, is nine times higher than in singletons. In fact, if we consider the risk for preterm-PE in the subgroup of pregnancies that are ongoing at 35 weeks' gestation the relative risk is 14 for DC twins and 20 for MC twins and these rates were very similar to the hazard ratios of 14 for DC and 23 for MC twin pregnancies relative to singleton pregnancies.

The underestimate relative risk of PE in twins, by comparison with singletons, when reporting the total rate of PE from 24 to 42 weeks' gestation is a mere consequence of the lower gestational age at delivery in twin pregnancy. In our study the median gestational age at delivery was 40 weeks for singletons, 37 weeks for DC twins and 35 weeks for MC twins. Delivery before 37 weeks' gestation occurred in 6% of singletons, 47% of DC twins and 91% of MC twins. These rates are similar to those reported for all births in the USA in 2014, where the rate of delivery before 37 weeks was 8% for singletons and 59% for twins. (Therneau, 2015) Since a much higher proportion of twin compared to singleton pregnancies deliver before a given gestational age, in the ongoing pregnancies the relative risk of PE in the subsequent few days is much higher than the value of 3 implied from the overall rate of PE. The true relative risk is about 14 for DC twins and more than 20 for MC twins.

The adoption of a survival-time model for the gestational age at delivery with PE has previously been proposed. (Wright *et al*., 2012; Wright *et al*., 2015) This approach assumes that if the pregnancy was to continue indefinitely all women would develop PE. Whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. It is therefore likely that if the pregnancy in twins was as long as in singletons the overall rate of PE in twins would be considerably higher than observed.

Former studies established a competing risk model for the prediction of PE in singleton pregnancies based on maternal factors. (Wright *et al*., 2012; Akolekar *et al*., 2013; Wright *et al*., 2015; O'Gorman *et al*., 2016; O'Gorman *et al*., 2017) In our second study, the maternal factor based model has been extended to include twin pregnancies.

We confirmed that in twin pregnancies the risk of PE is substantially higher than in singleton pregnancies and this is reflected in the distribution of gestational age of delivery with PE. In a reference population (Caucasian women, weight of 69 kg, height of 164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome), the mean gestational age of delivery with PE is 55 weeks. In DC and MC twin pregnancies with the same characteristics the distribution of gestational age of delivery with PE is shifted to the left by 8 and 10 weeks, respectively. The estimated risk of PE before 37 weeks' gestation was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins.

In screening for PE before 37 weeks' gestation at a risk cut-off of 1 in 75, in a mixed population of singleton and twin pregnancies, using maternal demographic characteristics and medical history, when the SPR for singleton pregnancies was 13% virtually all twins were screened positive. This is not surprising because in any screening programme certain maternal characteristics may be associated with such high-risk that irrespective of how favourable all other factors are, the posterior risk is above a cut-off used for stratification of the population into high and low-risk groups. For example, in the context of first-trimester combined screening for trisomy 21, the prior risk increases exponentially with maternal age and for a woman aged 50 years the posterior risk will remain above the screen positive cut-off, irrespective of how favourable the measurements of fetal nuchal translucency thickness and serum free ß-hCG (human Chorionic Gonadotropin) and PAPP-A are. As far as we known, this is the first study were the maternal factor based model has been extended to include twin pregnancies.

In our last study the competing risk model for the prediction of PE in twin pregnancies based on maternal factors has been extended to include biomarkers.

Previous studies on uterine artery Doppler during the second and third trimesters reported that UtA-PI in twin pregnancies is significantly lower than in singleton pregnancies, but the value is increased in pregnancies that develop PE. (Yu *et al*., 2002; Geipel *et al*., 2002; Geipel *et al*., 2011; Klein *et al*., 2011) A study in 421 twins and 500 singleton pregnancies at 11-13 weeks' gestation measured UtA-PI and converted the values to MoM after correction for maternal body mass index, ethnicity and

gestational age. (Rizzo *et al*., 2014) First, the study reported that median UtA-PI MoM was similar between MC and DC twins. Second they showed that UtA-PI in twins was lower than in singletons and third, in twin pregnancies that developed PE before 34 weeks' gestation, but not in those with PE at or after 34 weeks, UtA-PI was increased. (Rizzo *et al*., 2014) Another first-trimester study in 147 twin and 110 singleton pregnancies assessed UtA-PI and converted the values to MoM after correction for maternal weight and gestational age. (Svirsky *et al*., 2014) The study reported that first, UtA-PI in twins was lower than in singletons, second, UtA-PI was higher in MC than DC twins and third, in 12 twins that developed PE median UtA-PI was significantly lower. (Svirsky *et al*., 2014) This study also measured MAP and converted the values to MoM after correction for maternal weight and gestational age. They determined that first, MAP in twins was not significantly different from that in singletons, second, MAP was unrelated to chorionicity and third, in the twin pregnancies that developed PE median MAP was higher. (Svirsky *et al*., 2014)

A study in 144 twin and 109 singleton pregnancies at 11-13 weeks' gestation measured serum PAPP-A and PlGF and converted the values to MoM. (Svirsky *et al*., 2016) They reported that first, in twin pregnancies PAPP-A and PlGF were approximately twice as high compared to singletons, and second, in the 12 twin pregnancies that developed PE PlGF values were decreased but PAPP-A was increased. (Svirsky *et al*., 2016) A study in 74 twin and triplet pregnancies and 698 singleton pregnancies measured serum PlGF at 12-18 weeks' gestation and showed that first, in multiple pregnancies the levels were higher than in singletons and second, in the 5 multiple pregnancies that developed PE PlGF was lower. (Boucoiran *et al*., 2013)

In our study, DC twin pregnancies that did not develop PE, compared to singleton pregnancies, UtA-PI was lower, PlGF was higher and MAP was not significantly different. In MC twins, UtA-PI was lower, MAP was higher and PlGF was not significantly different. A previous study found that at 11-13 weeks' gestation the estimated median MoM of PAPP-A was about 2.0 for DC twins and 1.6 for MC twins. (Madsen *et al*., 2011)

In twin pregnancies that developed PE, MAP and UtA-PI at 11-13 weeks' gestation were increased and serum PlGF was decreased. The distribution of biomarkers with gestational age at delivery was similar to the previously reported fitted regression relationships for singleton pregnancies with PE (O'Gorman *et al*., 2016) and it was therefore assumed that the same model can be used for both singleton and twin pregnancies. We propose an initial model using a twin specific *prior* risk with biomarker MoM values adjusted for twins, but singleton distributional parameters for biomarkers.

The results obtained using this model are marginally worse than using twin specific parameters. However, this may be the consequence of the optimistic bias introduced from fitting and testing on the same data.

The performance of screening for PE by maternal factors was improved by the addition of MAP, UtA-PI and PlGF. In screening for PE using a combined test in a mixed population of singleton and twin pregnancies, at a risk cut-off of 1 in 75, the estimated DR and SPR of preterm PE for singletons were 77% and 13%, respectively, and the values for twins were 99% and 75%. If we use the risk cut-off of 1 in 10 for twins, the DR of preterm PE and PE before 32 weeks is 69% and 100%, respectively, at an estimated SPR of 25%.

STRENGTHS AND LIMITATIONS

The major strength of our studies is the prospective examination of twin and singleton pregnancies attending for routine care, with identification of known factors associated with PE early in pregnancy. The assessment of pregnancies at this stage is already widely used to determine gestational age, chorionicity, diagnosis of major fetal defects and screening for trisomies.

One other strong point is the fact that in study 1 the gestational age at delivery was taken into consideration and that the rate of preterm-PE was analysed instead of just the overall incidence of PE.

In both studies 1 and 2 we used a survival-time model, which allows estimation of an individual patient-specific risk of PE requiring delivery before any specified gestation. In study 2 and 3, the application of a multivariable survival analysis to identify the factors and define their contribution in the prediction of PE was also a central point of the investigation. In study 3, the use of a specific methodology and appropriately trained doctors to measure UtA-PI and MAP combined with the utilization of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of PlGF and PAPP-A were vital. The expression of the values of the biomarkers as MoMs and the use of Bayes theorem to combine the *a priori* risk from maternal factors with biomarkers, to estimate patient-specific risks, are also essential features of the study. The final strength of this research is the possibility to assess the performance of screening for PE at different stages of pregnancy.

Overall, the major limitation of the studies is the inevitably small number of twin pregnancies examined relative to singleton ones. In the last two studies we adopted the pragmatic view of using the model

that was previously described for singleton pregnancies to examine both singleton and twin pregnancies. This model requires validation and possible adjustments from the study of larger datasets of twins. Additionally, both studies have the limitation of the performance of screening by a model derived and tested using the same dataset being overestimated. We believe, external validation on independent data from different sources is required.

CLINICAL IMPLICATIONS

Clinicians managing twin pregnancies should be aware that the rate of preterm-PE, relative to that in singleton pregnancies, is substantially higher than what is implied by the overall rate of PE. They should also be attentive that, during the third trimester in ongoing pregnancies, the relative risk of PE in the subsequent few days is much higher in twin than singleton pregnancies which should merit a higher intensity of monitoring for PE.

The proposed competing risk model allows an estimation of patient-specific *a priori* risk for PE in twins, which is an essential first step in the use of Bayes theorem to combine maternal factors with biomarkers and for the continuing development of more effective methods of screening. Bayes theorem also provides a framework for updating the risk at different stages during pregnancy, forming the basis for stratified surveillance policies as achieved for singleton pregnancies.

The same model of screening can be adapted and used in mixed populations of singleton and twin pregnancies. More studies, with independent data from different sources, are required to validate this model.

FINAL REMARKS

PE is one of the most common medical complications of pregnancy and is a major cause of maternal and perinatal morbidity and mortality. Nevertheless, its physiopathology is still enfolded in plenty of uncertainty, which generates several unanswered questions.

Obstetricians face a growing number of multiple pregnancies, which have an increased risk of PE. Therefore, PE in twin pregnancies is a pertinent obstetric problem of increasing importance, requiring more research to elucidate the particularities of this pathology.

The aim of this thesis was to clarify some of the issues of PE in twin pregnancy. The studies enclosed can be the starting point of more research required to determine the best approach in screening for PE in twin pregnancy. One fundamental question that still needs to be addressed is whether there are effective prophylactic measures in twin pregnancy and what those are. One other important issue is whether it is more cost-effective to screen these pregnancies or to offer prophylactic measures universally. If screening is the way forward, it is imperative to improve the used screening method.

Given all the above, this is definitely an area that deserves further attention by the Obstetric community.

CHAPTER 5

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Note: Additional bibliography for each study is included on their respective *References*.

CHAPTER 6

SUPPLEMENTS

NHS
National Research Ethics Service
NRES Committee London - Dulwich
Room 4W/12, 4th Floor
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London
W6 8RF

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07 October 2011

Professor Kypros H Nicolaides
Director, Fetal Medicine Unit
King's College Hospital NHS Trust
Harris Birthright Research Centre for Fetal Medicine
Suite 9, Golden Jubilee Wing
Denmark Hill, London
SE5 9RS

Dear Professor Nicolaides,

Study title:	**Early prediction of pregnancy complications**
REC reference:	**02-03-033**
Amendment number:	**Amendment 9**
Amendment date:	**06 September 2011**

The above amendment was reviewed at the meeting of the Sub-Committee held on 30 September 2011 by the Sub-Committee in correspondence.

Ethical opinion

There were no ethical issues.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Consent Form	6	31 August 2011
Participant Information Sheet	8	28 February 2009
Protocol	8	31 August 2011
Notice of Substantial Amendment (non-CTIMPs)	Amendment 9	06 September 2011
Covering Letter	Amendment 9	06 September 2011

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

02-03-033:	**Please quote this number on all correspondence**

Yours sincerely,

Dr David Jewitt
Chair

E-mail: Alene.Pointon@imperial.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Annie Atherton*
King's College Hospital NHS Trust
annie.atherton@kingsch.nhs.uk

Decisão final sobre o projeto "Prediction of preeclampsia in twin pregnancy"

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar a adenda ao projeto de investigação intitulado "Prediction of preeclampsia in twin pregnancy" (nº51/2016/CEFCM), submetido pela Dra. Carla Ferreira Francisco Rodrigues.

Lisboa, 11 de Abril de 2017

O Presidente da Comissão de Ética,

(Prof. Doutor Diogo Pais)

TO WHOM IT MAY CONCERN

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously approved the addendum to Project entitled **"**Prediction of preeclampsia in twin pregnancy**"** (nr.51/2016/CEFCM), submitted by Dr. Carla Ferreira Francisco Rodrigues.

Lisbon, April 11th, 2017

The Chairman of the Ethics Research Committee,

(Diogo Pais, MD, PhD)

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