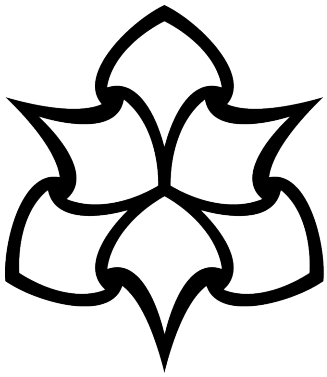

PREDICTION OF PREECLAMPSIA

AND ITS PREVENTION WITH ASPIRIN

D L ROLNIK

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PREDICTION OF PREECLAMPSIA AND ITS PREVENTION WITH ASPIRIN

DANIEL LORBER ROLNIK

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Department of Nursing

Faculty of Health, Psychology and Social Care

The Manchester Metropolitan University

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ABSTRACT

Background: Preeclampsia (PE) affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. The current approach to screening for PE is based on the identification of risk factors from maternal characteristics and medical history. This approach, however, fails to identify a high proportion of cases of PE and does not provide individualised, patient-specific results. An alternative approach is to combine maternal factors with biophysical and biochemical markers to estimate the individual probability of developing PE with higher detection rates. To date, no intervention is proven to reduce the risk of the disease, and several studies evaluating the use of aspirin for prevention of PE led to inconclusive results.

Objectives: The aims of the studies included in this thesis are, first, to prospectively validate in a large European population a first-trimester algorithm for prediction of PE that combines maternal demographic characteristics and medical history with biophysical and biochemical markers; second, to compare this method of screening to the performance of currently used guidelines; third, to evaluate a possible beneficial effect of aspirin initiated at 11 to 14 weeks of gestation and at a dose of 150 mg in the prevention of PE in a multicentre, double-blind, placebo-controlled randomised trial; and fourth, to analyse a potential role of cell-free DNA testing in the prediction of PE.

Methods: Combined screening for PE was applied in the first or second trimester, and women found to be at high-risk in the first trimester were offered participation in a double-blind trial of aspirin against placebo in six European countries. We have recorded maternal characteristics and history, measured the uterine artery pulsatility index (UtPI) on ultrasound, the mean arterial pressure (MAP), serum concentration of pregnancy-associated plasma protein A (PAPP-A) and placental growth factor (PLGF). Pregnancy outcomes were obtained from the hospital maternity records. Bayes theorem was used to combine the *a priori* risk from maternal factors with the results of biomarker measurements and estimate individual probabilities. In the randomised trial, the analysis was performed in an intention-to-treat basis and the treatment effect on the primary outcome (the development of PE with delivery before 37 weeks of gestation) was reported with 95% confidence interval (CI), and on

secondary outcomes with 99% CI. Cell-free DNA fetal fraction was compared with other first trimester markers for PE and in a case-control study.

Results: Detection rates of combined screening, for a false-positive rate (FPR) of 10%, were 89% (95% CI 79-96%), 75% (95% CI 70-80%) and 47% (95% CI 44-51%) for PE <32 weeks, preterm PE and term disease, respectively. The performance of combined screening was superior to methods based on risk factors alone, both in the first and second trimesters. The use of aspirin by high-risk women reduced the incidence of preterm PE by 62% (adjusted odds ratio 0.38, 95% CI 0.20-0.74). Secondary analyses have shown that the effect of aspirin was influenced by the level of compliance to treatment and was consistent in different subgroups according to maternal characteristics and obstetric history, but there was no evidence of beneficial effect of aspirin in women with chronic hypertension. Aspirin reduces the length of stay in NICU and costs through a reduction in premature births before 32 weeks due to PE. Fetal fraction on cell-free DNA testing correlates with other first trimester markers, but its role in screening for PE is uncertain.

Conclusions: This thesis has demonstrated that combined screening for PE is superior to current guidelines based on maternal characteristics and history alone, and that aspirin, at a daily dose of 150 mg and given to high-risk patients from 11 to 14 weeks until 36 weeks of gestation, reduces the incidence preterm PE and the length of stay in NICU. The effect of the medication depends on good adherence to treatment and is questionable in patients with chronic hypertension.

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Most of the studies included in this thesis derive from the work performed at the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, where I was a research fellow in Fetal Medicine for three years. It would not have been possible to complete these studies and this thesis without the help and hard work from all research fellows, midwives, consultant obstetricians and Fetal Medicine specialists from all the hospitals involved in the ASPRE study. In particular, I need to mention Dr Liona Poon and Dr Argyro Syngelaki, whose input and advice were key to the completion of this work. I also want to thank Professor David Wright, for his patience, hard work and guidance in all statistical aspects of our studies.

I would like to thank Dr Neil O'Gorman, my friend in joyful and in difficult moments, whom I worked with very closely during my time at King's College Hospital, for all his kindness, friendship and support. After so many hours spent together and delivering my son, Neil has become part of my family.

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ABBREVIATIONS

ACOG: American College of Obstetricians and Gynecologists

ART: Assisted reproduction technologies

ASPRE: Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention trial

AT1-AA: Angiotensin II type 1-Receptor Autoantibody

BCE: Before the current era

BMI: Body mass index

BP: Blood pressure

CCTU-UCL: Comprehensive Clinical Trials Unit, University College London

CI: Confidence interval

CLASP: Collaborative Low-dose Aspirin Study in Pregnancy

DNA: Deoxyribonucleic acid

DR: Detection rate

FGR: Fetal growth restriction

FMF: Fetal Medicine Foundation

FPR: False-positive rate

GBP or £: British pound

GH: Gestational hypertension

HLA: Human leukocyte antigen

ISSHP: International Society for the Study of Hypertension in Pregnancy

IVF: *In vitro* fertilisation

kg: Kilograms

m: Metres

MAP: Mean arterial pressure

mg: Milligrams

MHC: Major histocompatibility complex

mmHg: Millimetres of mercury

mmol: Millimole

MoM: Multiples of the median

NICE: National Institute for health and Clinical Excellence

NK: Natural killer cells

NNT: Number needed to treat

OR: Odds ratio

PAPP-A: Pregnancy-associated plasma protein A

PARIS: Perinatal Antiplatelet Review of International Studies

PE: Preeclampsia

PIH: Pregnancy-induced hypertension (same as GH)

PLGF: Placental growth factor

sFLT-1: Soluble fms-like tyrosine kinase-1

SPREE: Screening Program for Preeclampsia study

TNF-α: Tumour necrosis factor alpha

UK: United Kingdom

US$: American Dollars

USA: United States of America

UtPI: Uterine artery pulsatility index

VEGF: Vascular endothelial growth factor

µm: Micrometres

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1. Introduction

1.1. Overview

The first description of eclampsia, Greek word that means lightning, comes from ancient times, between the 5th and 4th centuries before the current era (BCE), when Hippocrates used the theory of imbalances in the four humours of the body (blood, phlegm, yellow bile, and black bile) to describe the cause of illness and disease (Bell, 2010). In 1739, François Boissier de Sauvages de Lacroix, a French physician and botanist, differentiated for the first time what is now known as epilepsy from the condition that causes convulsions in pregnancy, calling it *eclampsia parturientum* (Chesley, 1984). The finding that these seizures were accompanied by proteinuria came to light in 1840, shortly followed by recordings of high blood pressure (BP). The term preeclampsia (PE) was coined in 1894, describing the occurrence of hypertension and proteinuria in pregnancy, without eclamptic seizures (Chesley, 1984).

PE is a serious complication that typically affects 2-3% of the pregnancies and remains one of the leading causes of maternal and perinatal morbidity and mortality (Duley, 2009). Over half a million women die each year due to preeclampsia-related causes, and more than 99% of these deaths occur in developing countries (Koonin *et al.*, 1997; World Health Organization, 2005). The economic burden of the disease is a major public health issue in most countries. In the United States of America (USA), preeclampsia accounts for 18% of all maternal deaths and 15% of all premature births (Koonin *et al.*, 1997; Kuklina *et al.*, 2009). The cost of the disease in the first 12 months has been estimated at US$ 2.18 billion, and is disproportionally borne by premature births (Stevens *et al.*, 2017).

The disease is characterised by elevated BP and involvement of the kidneys with increased levels of protein in the urine (proteinuria) after 20 weeks of gestational age. Complications of PE include symptoms, signs and laboratory manifestations of maternal organ dysfunction, involving: the kidneys, with increased serum creatinine concentration; the liver, with elevated transaminases; the haematological system, with haemolysis and low platelet count; and neurological complications, including hyperreflexia, headache, visual disturbance, reduction of the visual sight, seizures

(eclampsia) and stroke, which constitutes the main cause of maternal mortality (Crovetto *et al.*, 2013).

A recent large meta-analysis has also confirmed that the occurrence of PE significantly increases the risk of adverse cardiovascular outcomes in the long-term. There is a four-fold increase in the risk of heart failure and a two-fold increase in coronary heart disease, cardiovascular disease-related death and stroke in women who experienced PE compared to those who did not (Wu *et al.*, 2017).

The aetiology of PE is still unclear. Previous studies have shown that abnormal development of the placenta is implicated, with an impaired trophoblastic invasion of the spiral arteries (Khong *et al.*, 1986; Robertson *et al.*, 1986; Lyall, 2002). In healthy pregnancies, the muscular layer of these arteries, mainly in their myometrial portion, is remodelled to increase their diameter and capacitance, thus reducing the resistance to blood flow (Hamilton and Boyd, 1960; Brosens *et al.*, 1967). Due to unknown reasons, this process does not seem to happen in full in pregnancies affected by PE, leading to increased vascular resistance. This triggers the release of placental and endothelial substances with an imbalance in angiogenic factors (such as placental growth factor – PLGF – and vascular endothelial growth factor – VEGF) and anti-angiogenic factors (such as soluble fms-like tyrosine kinase-1 – sFLT-1) that ultimately lead to increased BP, endothelial dysfunction and kidney injury (Robertson *et al.*, 1967).

Different entities have proposed various methods to identify women at high risk for the development of PE, mostly based on maternal characteristics (such as age and body mass index - BMI) and medical history. Frequently used guidelines are the ones suggested by the American College of Obstetricians and Gynecologists (ACOG) in the USA and by the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom (UK) (Visintin *et al.*, 2010; American College of Obstetricians and Gynecologists, 2013; American College of Obstetricians and Gynecologists, 2015). Nevertheless, these recommendations are primarily based on experts' opinion and were rarely tested prospectively. Hence, their clinical performances are unknown.

In the last few decades, authors have proposed an alternative Bayesian approach for individual-risk calculation based on logistic regression and competing

risk models that combine maternal characteristics and history with biophysical and biochemical markers shown to be different in women with PE in pre-clinical stages when compared to those that have an uncomplicated pregnancy, with higher reported predictive accuracy (Onwudiwe *et al.*, 2008; Poon *et al.*, 2009a; Odibo *et al.*, 2015; Gabbay-Benziv *et al.*, 2016; O'Gorman *et al.*, 2016b). The most widely employed of these models are the ones published by the Fetal Medicine Foundation (FMF), from the UK (Poon *et al.*, 2009a; O'Gorman *et al.*, 2016b). These models, however, have been criticised due to the lack of studies of prospective external validation (Oliveira *et al.*, 2014; Brunelli and Prefumo, 2015).

As of prevention of PE in women at high risk, several pharmacological and non-pharmacological measures have been studied. The vast majority of the studies evaluated a possible beneficial effect of antiplatelet agents in the prevention of PE, commonly low-dose aspirin (Beaufils *et al.*, 1985; CLASP Collaborative Group, 1994; Askie *et al.*, 2007; Bujold *et al.*, 2010; Roberge *et al.*, 2012a; Roberge *et al.*, 2012b; Bujold *et al.*, 2014; Meher *et al.*, 2017). However, their results are controversial, possibly due to heterogeneous methodologies applied regarding the method for identification of a high-risk population, different doses of aspirin prescribed and initiation of pharmacological prophylaxis at different gestational ages. Recent evidence suggests that aspirin is only beneficial in reducing the incidence of preterm and early-onset PE (but not term PE) and if started before 16 weeks of gestation at doses higher than 100 mg (Roberge *et al.*, 2012a; Roberge *et al.*, 2013a; Roberge *et al.*, 2014; Roberge *et al.*, 2017b).

1.2. Definition of preeclampsia

Preeclampsia is traditionally defined as the presence of hypertension and proteinuria in a previously normotensive woman, after 20 weeks of gestational age.

Several diagnostic criteria have been proposed in the last few decades, but a widely accepted definition is the one published by the International Society for the Study of Hypertension in Pregnancy (ISSHP) (Davey and MacGillivray, 1988; Brown *et al.*, 2001). According to the ISSHP, preeclampsia is defined by increased blood pressure with systolic measurement ≥ 140 mmHg and/or diastolic measurement ≥ 90

mmHg on at least two occasions, four hours apart and after 20 weeks of gestational age, accompanied by proteinuria ≥ 300 mg in 24 hours or two positive dipstick analyses of at least ++ of protein on midstream or catheter urine samples, in the absence of a 24-hour collection. More recently, a spot urine protein/creatinine ratio ≥ 30 mg per mmol was also added to the diagnostic criteria (Tranquilli *et al.*, 2014).

The occurrence of hypertension after 20 weeks of gestation without significant proteinuria or other signs of PE is classified as pregnancy-induced or gestational hypertension (PIH or GH). Other differential diagnoses include chronic or essential hypertension, usually manifested before 20 weeks of gestation, or white-coat hypertension (Tranquilli *et al.*, 2014).

Since hypertension is a necessary finding in PE, monitoring BP during antenatal care is fundamental. The use of validated automated devices following a standardised technique has been recommended (Poon *et al.*, 2012).

Recent definitions of PE have been used, and recent publications have disregarded the need for significant proteinuria, including amongst the diagnostic criteria any clinical or laboratory sign of end-organ damage, such as elevated liver enzymes, impaired renal function, neurological symptoms or fetal growth restriction (FGR) (Tranquilli *et al.*, 2014). The proponents of this classification, however, acknowledge that the inclusion of proteinuria would ensure more specificity around the diagnosis when reporting clinical criteria for patients enrolled in scientific research (Tranquilli *et al.*, 2014). For this research, the ISSHP classification and diagnostic criteria, including the need for proteinuria to be present, were chosen, as they represent a consensus of an appropriate and specific definition for use in research (Brown *et al.*, 2001; Tranquilli *et al.*, 2014).

The contradictory findings of the literature regarding the accuracy of prediction and effectiveness of prevention of PE may be, to some extent, due to different definitions of PE used in various studies. An individual-patient data meta-analysis evaluating the use of anti-platelet agents for the prevention of PE, for example, found a very modest effect of aspirin in the prevention of PE but included 31 studies that comprised 14 different definitions of the disease (Askie *et al.*, 2007).

1.3. Future cardiovascular risk

Although signs and symptoms of PE usually resolve after delivery, its occurrence impacts significantly on future maternal cardiovascular health. A recent systematic review and meta-analysis including over six million pregnancies and more than 258,000 cases of PE found a four-fold increase in the risk of heart failure and a two-fold increase in coronary heart disease, stroke and cardiovascular-related death among women who experienced PE compared to those who did not (Wu *et al.*, 2017). These risks seem to be closely related to the severity and earlier gestational age at the onset of the disease (Cirillo and Cohn, 2015; Christensen *et al.*, 2017).

It is unknown whether preeclampsia is the cause, predisposing women to cardiovascular complications in the future, or the consequence, being an early clinical manifestation of a suboptimal cardiovascular performance triggered by pregnancy. Nonetheless, pregnancy possibly constitutes a good window of opportunity to identify women at high-risk for cardiovascular disease. It would be reasonable to assume that preventing the onset of preeclampsia would impact in the cardiovascular profile later in life. Some authors even argue that the cardiovascular system may be the cause behind PE, mainly in late-onset forms of the disease, triggering placental hypoperfusion and the release of placental and endothelial mediators (Kalafat and Thilaganathan, 2017).

1.4. Pathogenesis of preeclampsia – mechanism of disease

Although the exact mechanism of disease remains uncertain, the pathogenesis of PE is thought to occur in two different stages: a symptomless placental phase in which an abnormal trophoblastic invasion occurs between eight and 18 weeks of gestation, followed by an abnormal maternal vascular response that results in the release of inflammatory mediators (reason why the condition used to be called toxaemia), endothelial injury and dysfunction, eventually resulting in clinical manifestations including hypertension, proteinuria and oedema.

1.4.1. Placental factors

In humans, placentation involves remodelling of the maternal blood vessels to provide the fetus with sufficient nutrients from the mother's blood supply. PE is thought to be caused by an impaired trophoblastic invasion of the spiral arteries, which are branches of the arcuate artery and ultimately of the uterine artery within the uterine wall (Figure 1).

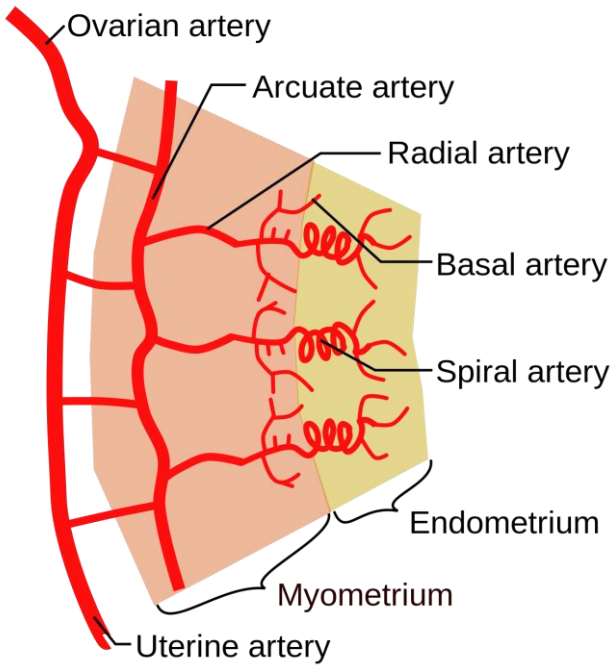

Figure 1 – Arterial blood supply to the uterus from the uterine and ovarian arteries, giving origin to the arcuate artery, the radial arteries and the spiral arteries in the myometrium and the endometrium; taken from Robertson (1976).

The human placenta originates from cells denominated trophoblasts, which are subdivided into syncytiotrophoblasts and cytotrophoblasts. Approximately 11 to 12 days after conception, the syncytiotrophoblasts infiltrate in the endothelium of the maternal capillaries to establish the uteroplacental circulation, under the influence of angiogenic growth factors, including VEGF and PLGF. About four to six weeks post-conception, the cytotrophoblasts disrupt the maternal vascular integrity by invading the spiral arteries. This process leads to remodelling of the spiral arteries through the

destruction of the media layer muscular and elastic fibres, in a process known as trophoblastic invasion, which is thought to take place in two different stages of pregnancy: one initial wave of trophoblastic invasion, at approximately eight weeks of gestation and involving the decidual portion of the spiral arteries; and a second wave at the myometrial portion of the spiral arteries at 14 to 24 weeks of pregnancy (Redman, 1992; Lyall, 2002). The small-diameter (15-20 µm), high-resistance spiral arteries then become grossly dilated (300-500 µm), funnel-shaped and low-resistance vessels (Figure 2), increasing the blood flow by about ten times to ensure sufficient blood supply to the developing fetus (Brosens *et al.*, 1972).

In healthy pregnancies, the uteroplacental circulation shows decreasing vascular resistance, markedly in the second trimester and mainly due to the remodelling process resulting in an increased capacitance of these vessels. Previous studies in which placental bed biopsies were performed have shown significantly reduced diameter of the spiral arteries in their myometrial portion in patients affected by PE when compared to healthy controls (Brosens *et al.*, 1972; Pijnenborg *et al.*, 1991; Redman, 1992; Meekins *et al.*, 1994; Lyall, 2002) (Figure 2).

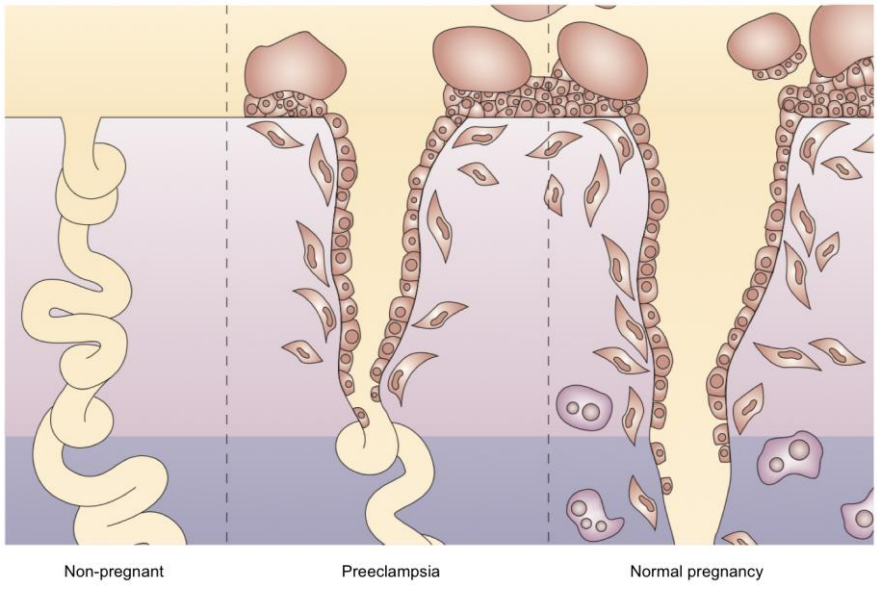

Figure 2 – Differences in diameter of the spiral arteries in non-pregnant women (left), in normal pregnancies after the remodelling of these vessels (right) and in pregnancies affected by PE (centre); adapted from Moffett-King (2002).

The impedance to blood flow in the uteroplacental circulation can be measured with ultrasound Doppler velocimetry techniques through the evaluation of the resistance on the uterine arteries and expressed as the uterine artery pulsatility index (UtPI), which shows a clear positive association with severity of the disease and histological changes (Olofsson *et al.*, 1993).

Not all pregnancies affected by PE, however, show signs of abnormal placentation. In PE that occurs at term, often histological studies of the placenta resemble those of normotensive pregnancies, and the diagnosis of PE is more likely to be confirmed before 32 weeks of gestation in those cases with abnormalities in the uteroplacental circulation (Ghidini *et al.*, 1997). This highlights that high resistance in the uterine arteries not always predicts the development of PE but is rather a predisposing factor.

Histopathology studies have also shown that acute atherosclerosis is a common feature in placentas from pregnancies affected by PE (Sheppard and Bonnar, 1981). However, this finding is not unique to PE, being also present in pregnancies complicated by fetal growth restriction, thrombophilia or miscarriage (Frusca *et al.*, 1989).

1.4.2. Immunologic and genetic factors

The cause of inadequate remodelling of the spiral during the placentation process in pregnancies affected by PE is unknown. Immunological and genetic theories have been implicated in the pathogenesis of the disease (Redman, 1992).

The development of immunological tolerance to the semi-allogenic fetus, whose genes are half paternal, is facilitated by its reduced expression of the major histocompatibility complex (MHC) and the human leukocyte antigen (HLA) system, a mechanism that endeavours to avoid fetal cells from being innately rejected. Additionally, uterine natural killer (NK) cells initiate smooth muscle remodelling of the spiral arteries and regulate the trophoblastic invasion through the secretion of angiogenic growth factors and cytokines (Lash et al., 2011). These changes allow the innate immune system to adapt and thus prevent a pregnancy loss. A possible altered maternal immune response against the paternally-derived antigens on the

trophoblast is hypothesised as a potential aetiological factor (Sargent *et al.*, 2006). Although identifying the exact immunological mechanism of origin is difficult while the immune system changes in early pregnancy are not entirely understood, this suggestion is corroborated by epidemiological studies that found a higher incidence of PE in pregnancies conceived with the use of donor egg (Martinez-Varea *et al.*, 2014). The immunological theories implicated in PE pathogenesis are also supported by previous studies that reported that higher intervals since the first coitus and co-habitation to conception result in lower incidence of PE, which could be a consequence of development of tolerance to the HLA present in the semen (Marti and Herrmann, 1977; Dekker *et al.*, 1998). Similarly, PE is more common in the first pregnancy, less common in subsequent pregnancies when the partner is the same; and changing partner or prolonged inter-pregnancy interval above ten years increase the risk of PE (Robillard *et al.*, 1993; Dekker *et al.*, 1998; Harutyunyan *et al.*, 2013; Cormick *et al.*, 2016).

It seems that both maternal and fetal genotypes are implicated in the induction of immunological tolerance. Hence, a genetic predisposition may also be involved, and previous studies have shown that women with family history of PE are three times more likely to develop this condition (Arngrimsson *et al.*, 1990; Duckitt and Harrington, 2005). Different genes were reported to be associated with increased risk of PE by regulating either blood pressure or trophoblastic invasion (Cooper and Liston, 1979; Chesley and Cooper, 1986). Hiby *et al.* (2008), for instance, found that a specific combination of maternal KIR (killer-cell immunoglobulin-like receptor) and fetal HLA-C genotypes is associated with an increased risk of PE. Other polymorphisms in various genes, such as tumour necrosis factor alpha (TNF-α), angiotensinogen, 5,10-methylenetetrahydrofolate reductase and factor V Leiden, have also been associated with increased risk of PE (Morgan and Ward, 1999; Lachmeijer *et al.*, 2001a; Lachmeijer *et al.*, 2001b; Lachmeijer *et al.*, 2001c; Benedetto *et al.*, 2002; Heiskanen *et al.*, 2002).

A recent large meta-analyses found a higher risk of severe PE when the following gene mutations were found: coagulation factor V (proaccelerin) gene polymorphism rs6025, coagulation factor II (thrombin) gene mutation G20210A, leptin receptor (LEPR) gene polymorphism rs1137100 and the thrombophilic gene group (Fong *et al.*, 2014). Another meta-analysis reported that the presence of a

prothrombin 20210A polymorphism increases by three times a patient's risk of developing severe PE (Wang *et al.*, 2014). However, the causality of these associations and the clinical utility of identifying such changes are yet to be examined in larger studies.

1.4.3. Endothelial dysfunction

Endothelial dysfunction seems to be a significant contributor to the pathogenesis of PE. Endothelial cell activation or suppression are hypothesised to be secondary to placental hypoperfusion, ischaemia and infarction with release of inflammatory mediators, cytokines and reactive oxygen species, leading to significant intravascular inflammatory response (Gervasi *et al.*, 2001; Tannetta *et al.*, 2003; Cindrova-Davies *et al.*, 2007; Myatt and Webster, 2009).

Infarcted placental cells also release receptor proteins such as sFLT-1, a protein produced by the syncytiotrophoblasts that is responsible for antagonising angiogenic growth factors, such as VEGF and PLGF (Kendall and Thomas, 1993; Huppertz, 2008). Previous studies have utilised the measurements of sFLT-1 and PLGF, or the ratio between the two, to increase the diagnostic accuracy in patients with suspected PE (Rana *et al.*, 2012; Zeisler *et al.*, 2016).

The vascular injury leads to protein leakage and fluid retention in different organs and systems, causing, for example, proteinuria and generalised oedema. These abnormalities in the vascular system also cause an imbalance on levels of angiogenic proteins (i.e. VEGF and PLGF), and anti-angiogenic proteins (i.e. sFLT-1 and soluble endoglin), further exacerbating the endothelial dysfunction (Maynard *et al.*, 2003; Widmer *et al.*, 2007; Murphy *et al.*, 2013).

Endothelial dysfunction also affects vascular tone and platelet activation. These changes are responsible for platelet dysfunction and thrombocytopaenia that can be present even before the onset of hypertension. Levels of prostacyclin, a vasodilator that inhibits platelet aggregation, are reduced in PE, while levels and thromboxane A2, a vasoconstrictor that promotes platelet aggregation, are increased (Bussolino and Benveniste, 1980; Bussolino and Camussi, 1980; Romero and Duffy, 1980; Walsh, 1985; Romero *et al.*, 1988).

In summary, it appears that poor placentation in the first half of the pregnancy is triggered by genetic factors that lead to poor immunological tolerance to the semi-allogenic fetus. The suboptimal remodelling of the spiral arteries leads to the release inflammatory mediators, an imbalance in angiogenic and anti-angiogenic factors, and oxidative stress. This pre-symptomatic phase is responsible, in the second half of the pregnancy, for endothelial dysfunction that will exacerbate the production of inflammatory mediators and eventually lead to clinical manifestations of PE in different organs and systems (Figure 3).

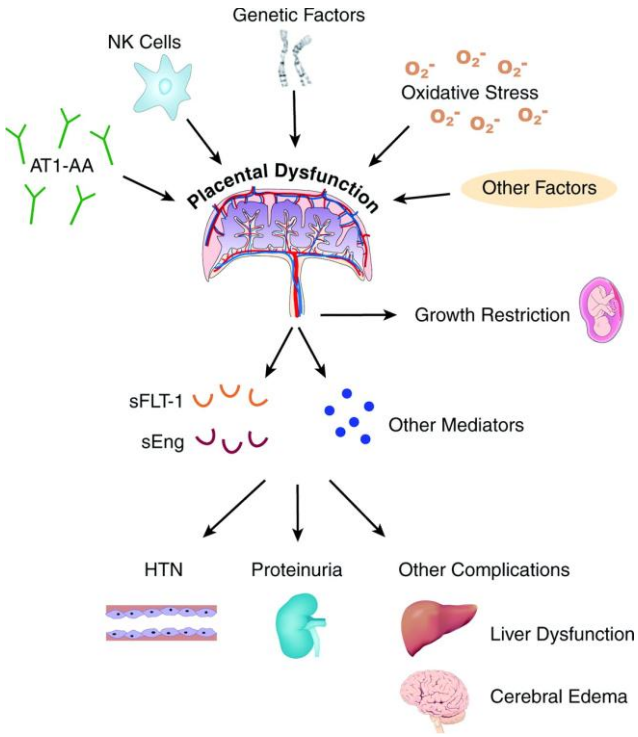

Figure 3 – A summary of the pathogenesis of preeclampsia; taken from Powe et al. (2011). HTN: hypertension.

1.5. Screening for preeclampsia

The current approach to screen for PE is by identifying risk factors from maternal characteristics and medical history and by measuring BP in antenatal clinics serially. The criteria to classify patients as high-risk and the management of such high-risk patients vary according to different protocols. In the UK, for example, the NICE guidelines are the recommended method of screening and patients with at least one high-risk factor or at least two moderate-risk factors should be reviewed by a healthcare professional every three weeks from 24 to 32 weeks of gestation and at least fortnightly from 32 weeks of gestation until delivery, as well as receive aspirin 75 mg daily (Visintin *et al.*, 2010). In the USA, in a statement published in 2017, the ACOG concludes that currently there are no accurate predictive tests for identifying women at high risk for PE and continues to recommend against other methods of screening, stating that a detailed medical history and serial BP measurements are the best tools to identify patients at high risk of PE.

1.5.1. Maternal risk factors

The vast majority of the studies conducted on prediction and prevention of PE and the protocols aiming to identify women at risk are based on maternal characteristics and medical history. Traditional risk factors are:

- *Maternal age:* a wealth of studies published have suggested that the risk of PE increases with maternal age. In women older than 35 years of age, the risk of PE is doubled when compared to younger controls (Bianco *et al.*, 1996; Lawoyin and Ani, 1996). Mittendorf *et al.* (1996) reported that the risk of PE increases by 30-40% for every additional year after the age of 34 years. Further studies also support this finding, even after adjustment for confounders and interactions (Poon *et al.*, 2010).
- *Parity, change of partner and inter-pregnancy interval:* as previously mentioned, primigravidas seem to have a three-fold increase in the likelihood of developing PE (Duckitt and Harrington, 2005), even after adjustment for other risk factors such as maternal age, ethnic origin and body mass index (Luo *et al.*, 2007), whereas women who have previously conceived appear to have

some degree of protection against PE even after a miscarriage (Dekker *et al.*, 1998; Dekker and Sibai, 1998). Multiparity reduces the risk of PE, but this protective effect is lost when the new pregnancy happens after a change of partner (Robillard *et al.*, 1993). Similarly, epidemiological studies have shown an increase in the risk of PE with increasing inter-pregnancy interval, being equivalent of that of a primigravida when the interval is superior to ten years (Conde-Agudelo and Belizan, 2000a; Skjaerven *et al.*, 2002).

- *Previous history of PE:* experiencing PE in a previous pregnancy increases the risk of recurrence in subsequent pregnancies by seven to ten times (Campbell *et al.*, 1985; Sibai *et al.*, 1986; Lee *et al.*, 2000; Mostello *et al.*, 2002). A large meta-analysis including data from 94 studies reported a risk of recurrence of 13.8%, being inversely related to the gestational age at delivery in the previous pregnancy affected by PE (van Oostwaard *et al.*, 2015).
- *Ethnic origin:* there is clear evidence showing an increased risk of PE in specific ethnic backgrounds. Afro-Caribbean ethnicity is associated with a 20-50% increase in the risk of PE (Mittendorf *et al.*, 1996; Sibai *et al.*, 1997; Knuist *et al.*, 1998; Mostello *et al.*, 2002; Caughey *et al.*, 2005). Women of South Asian origin are also at increased risk of PE. These findings may be mirroring a different cardiovascular profile, since these are groups also at increased risk of chronic hypertension and cardiovascular disease (Cappuccio, 1997; Cappuccio *et al.*, 1997; Ramaraj and Chellappa, 2008; Khalil *et al.*, 2013). East Asian origin, in contrast, is associated with lower risk of PE, possibly due to differences in BMI and nutritional factors (Xiao *et al.*, 2014).
- *Obesity:* obesity has become a significant public health issue in the last few decades (The G. B. D. Obesity Collaborators *et al.*, 2017) and is an important risk factor for PE. Increased BMI confers a two to four-fold increase in the risk of PE (Mittendorf *et al.*, 1996; Mostello *et al.*, 2002; Duckitt and Harrington, 2005), and this increase in risk is mainly due to a higher prevalence of late-onset PE among overweight and obese women (Syngelaki *et al.*, 2011; Durst *et al.*, 2016).
- *Assisted reproduction technologies (ART):* various studies show an increased risk of PE following conception with ART (Maman *et al.*, 1998; Jackson *et al.*, 2004; Shevell *et al.*, 2005; Trogstad *et al.*, 2009). It is unclear whether it is the

in vitro fertilisation (IVF) or the ovulation induction process that increases the risk of PE. A study including more than 47 thousand pregnancies has shown that conception by IVF increases the risk of PE and the risk is even higher in frozen-thawed cycles compared to fresh cycles (Opdahl *et al.*, 2015). Women who conceived by IVF with donor egg are also at significantly higher risk compared to those who undergo IVF with autologous egg (Masoudian *et al.*, 2016; Simeone *et al.*, 2016).

- *Family history of PE:* the risk of PE is increased three to four-fold in women with a family history of the disease occurring in the mother or the sister (Arngrimsson *et al.*, 1990; Cincotta and Brennecke, 1998).
- *Diabetes mellitus:* the risk of developing PE in women with type I diabetes appears to be more than three times higher than in the general population (Garner *et al.*, 1990; Ros *et al.*, 1998; Lee *et al.*, 2000).
- *Chronic hypertension:* pre-existing hypertension (or chronic hypertension diagnosed before 20 weeks of gestation) doubles the risk of PE (Conde-Agudelo and Belizan, 2000b) and women with this condition are more likely to develop early-onset and severe forms of the disease (Catov *et al.*, 2007).
- *Renal disease:* women with renal disease are more likely to develop PE with a three-fold increase in risk (Martinell *et al.*, 1990; Maruotti *et al.*, 2012).
- *Autoimmune disease:* the presence of anti-cardiolipin antibodies or lupus anticoagulant has been shown to significantly increase the risk of PE (Branch *et al.*, 1989; Sletnes *et al.*, 1992; Pattison *et al.*, 1993; Dreyfus *et al.*, 2001). A case-control study has also found women who experienced PE to be six times more likely to have an autoimmune disease such as systemic lupus erythematosus (Stamilio *et al.*, 2000).

1.5.2. Current guidelines

In the UK, the NICE guidelines were published in 2008 and revised in 2010 (Visintin *et al.*, 2010). These guidelines state that women should be considered at high risk for PE if they have any high-risk factor or two or more moderate-risk factors.

The high-risk factors are history of hypertensive disease in a previous pregnancy, chronic kidney disease, autoimmune disease, diabetes mellitus and

chronic hypertension; the moderate-risk factors are first pregnancy, maternal age of 40 years or more, inter-pregnancy interval of more than ten years, BMI at first visit of 35 kg/m^2 or more, and family history of PE. According to NICE, all high-risk women should be offered low-dose aspirin (75 mg daily) (Visintin *et al.*, 2010).

In the USA, according to the ACOG, taking a medical history to evaluate for risk factors is currently the best and only recommended screening approach for PE; the risk factors are nulliparity, age above 40 years, BMI higher than 30 kg/m^2, conception by IVF, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus, systemic lupus erythematosus or thrombophilia (American College of Obstetricians and Gynecologists, 2015). According to ACOG use of aspirin should be reserved for women with a history of PE in two or more previous pregnancies or at least one previous pregnancy affected by PE requiring delivery before 34 weeks of gestation (American College of Obstetricians and Gynecologists, 2013).

These national guidelines were rarely tested in prospective cohorts and do not seem to perform well in studies that evaluated their accuracies (Verghese *et al.*, 2012). The approaches recommended by NICE and ACOG essentially attribute similar weights to each risk factor and does not take in account protective factors, such as multiparity without previous history of PE. In the recently published "Screening Program for Preeclampsia" (SPREE) study, only 40.8% of the cases of preterm PE were identified in the first trimester by the NICE guidelines and less than a quarter (23%) of the patients at high risk were prescribed aspirin, suggesting low compliance to the protocol currently used in the UK (Tan *et al.*, 2018).

1.5.3. Biomarkers

Several markers of preeclampsia were described in the last few decades and could potentially be used in screening for PE. However, no marker has shown to be accurate enough when used isolated. Instead, a combination of markers in a predictive model that also takes into account the interactions between them can increase accuracy and reduce false-positive results. The most commonly used and with higher performance are biophysical markers such as BP and uterine artery Doppler, and biochemical markers such as pregnancy-associated plasma protein

(PAPP-A), placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFLT-1).

1.5.3.1. Biophysical markers

Blood pressure

Previous studies have consistently shown that women destined to develop PE have elevated BP already in the first and second trimesters before other clinical manifestations of the disease appear (Moutquin *et al.*, 1985; Higgins *et al.*, 1997; Poon *et al.*, 2008). The difference between BP levels in women who will later present with PE and unaffected pregnancies is more prominent in cases of severe and early-onset disease than in cases of PE at term (O'Gorman *et al.*, 2016b).

Mercury sphygmomanometers have traditionally been used to measure BP in antenatal care, but concerns regarding their accuracy and safety have been raised (Markandu *et al.*, 2000). The use of automated devices may be more accurate, and a standardised technique has been described for reliable measurement of MAP in pregnancy (Poon *et al.*, 2012), which involves two readings in each arm at the level of the heart, with adequate cuff size and after five minutes of rest. These readings are then used to calculate the MAP and its value, just like all other biomarkers, is then expressed in multiples of the median (MoM) after adjustment for gestational age and maternal factors that influence the MAP.

Uterine artery Doppler

Assessment of the resistance to blood flow in the uterine arteries was first described in 1983 (Campbell *et al.*, 1983). The literature consistently shows that higher resistance levels, reported as high pulsatility index (PI) or resistance index (RI), correlate with both histological studies and clinical severity of PE. As with MAP, the earlier and more severe PE develops, the more pronounced the differences in mean UtPI from cases to controls, being poorly predictive of term disease (O'Gorman *et al.*, 2016b). The use of indices is preferred over the reporting of subjective markers of high resistance like early diastolic notch (Figure 4), which are

poorly predictive of adverse pregnancy outcomes (Kaminopetros *et al.*, 1991; Olofsson *et al.*, 1993).

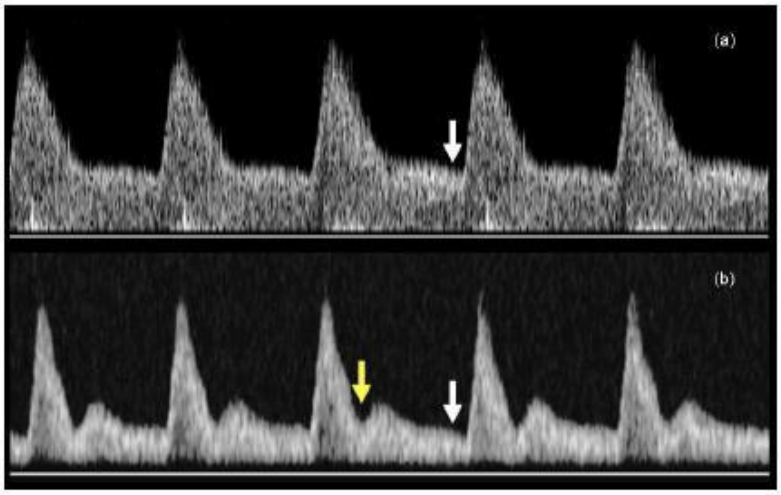

Figure 4 – (a) Normal uterine artery blood flow Doppler waveform, with an abundance of end-diastolic flow (arrow); (b) waveform characteristic of high-resistance blood flow, with a steeper systolic upslope when compared to the normal waveform, with an early diastolic notch (yellow arrow) and reduced end-diastolic flow velocity (courtesy of Prof Kypros Nicolaides, Fetal Medicine Foundation, UK).

The PI is defined by the difference between the peak systolic velocity and the minimum diastolic velocity, divided by the peak systolic velocity. The RI is determined by the same difference divided by the mean velocity.

On transabdominal or transvaginal ultrasound, the uterine artery is identified with the use of colour Doppler and pulsed wave is applied to measure blood flow velocity. In the first trimester or at a transvaginal ultrasound at any stage, the uterine artery is identified at the level of the internal cervical os. After measuring the right and left uterine arteries PI, the mean UtPI is expressed in MoM values after adjustment for maternal characteristics and gestational age.

During transabdominal ultrasound in the second trimester, due to difficult visualisation of the cervix, this vessel is identified in its apparent cross-over with the external iliac artery. When technically possible, transvaginal measurements should be avoided, as they result in higher PI values (Plasencia *et al.*, 2008; Plasencia *et al.*, 2011).

1.5.3.2. Biochemical markers

A large number of biochemical markers have been evaluated for clinical use in the prediction of PE. At present, no marker used in isolation can accurately predict the occurrence of PE. In general, the altered levels of biochemical markers in patients who will later experience PE are a preclinical sign of impaired placentation, release of inflammatory factors, platelet activation, endothelial dysfunction, abnormal oxidative stress or maternal renal impairment. Two of the most extensively investigated biomarkers are PAPP-A and PLGF, placental products that are significantly lower, even in the first trimester, in patients who will later experience early and severe forms of PE (O'Gorman *et al.*, 2016b). They can be measured in automated machines with reproducible results provided within 30 to 40 minutes after blood sampling. These markers are also influenced by gestational age and different maternal characteristics. Thus, their results should also be expressed in MoM values, after adjustment for these confounders (Spencer *et al.*, 2006; Poon *et al.*, 2009b; Lai *et al.*, 2013; Lai *et al.*, 2014; Tsiakkas *et al.*, 2015; Wright *et al.*, 2015a; Khalil *et al.*, 2016; Tsiakkas *et al.*, 2016a; Andrietti *et al.*, 2017). Some other markers, such as sFLT-1, are only predictive when used in the second or third trimester, being no different in the first trimester in patients who will later develop PE from normotensive controls (Akolekar *et al.*, 2010).

Pregnancy-associated plasma protein A (PAPP-A)

PAPP-A is secreted by the syncytiotrophoblasts and plays an essential role in placental growth and development. Its measurement is widely used in first-trimester screening for fetal chromosomal abnormalities, given the fact that pregnancies affected by trisomy 21 (Down's syndrome), trisomy 18 (Edward's syndrome) and trisomy 13 (Patau's syndrome), are associated with significantly reduced PAPP-A levels (Bersinger *et al.*, 1994; Spencer *et al.*, 1999; Kagan *et al.*, 2008). Pregnancies affected by PE have also been shown to have significantly lower PAPP-A at all stages of pregnancy before the onset of the disease (Poon *et al.*, 2009b). However, the isolated use of PAPP-A below the 5th percentile (0.4 MoM) predicts only 8-23%

of the cases of PE, with reported odds ratio (OR) between 1.5 and 4.6 (Smith *et al.*, 2002; Yaron *et al.*, 2002; Dugoff *et al.*, 2004; Spencer *et al.*, 2005).

Placental growth factor (PLGF)

PLGF is an angiogenic factor, part of the vascular endothelial growth factor family, secreted by the cytotrophoblasts. Its levels are significantly decreased in the first trimester in women who will experience PE (Ahmad and Ahmed, 2004; Levine *et al.*, 2004; Stepan *et al.*, 2007a; Stepan *et al.*, 2007b; Akolekar *et al.*, 2008; O'Gorman *et al.*, 2016b).

1.5.5. Inversion of the pyramid of prenatal care

The current approach to antenatal care is based on a booking visit in which the health professional obtains a detailed maternal medical history to identify risk factors for pregnancy complications. The intervals between visits are large up until the third trimester, being then shortened to fortnightly or weekly visits, aiming to identify as early as possible complications that typically appear in the second half of the pregnancy, such as PE, gestational diabetes mellitus and obstetric cholestasis. Hence, the BP should be measured in all antenatal visits. In 1929, the Ministry of Health in the UK issued a Memorandum on Antenatal Clinics recommending that women should first be seen at 16 weeks, then at 24 and 28 weeks, fortnightly thereafter until 36 weeks and then weekly until delivery (Ministry of Health Report, 1929). However, there is a clear trend in the literature in recent years toward the attempt to predict pregnancy complications in the first trimester, allowing for early initiation of preventive measures and close surveillance of high-risk pregnancies. This concept has been referred to as “inversion of the pyramid of antenatal care” (Nicolaides, 2011) (Figure 5).

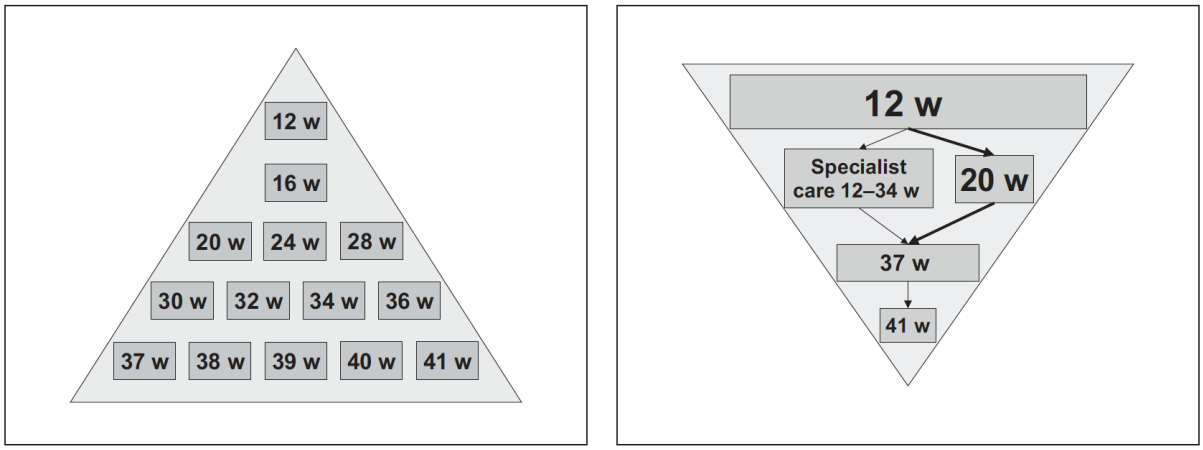

Figure 5 – Left: the traditional model of prenatal care established in 1929; Right: the inverted pyramid of antenatal care with screening for pregnancy complications at 12 weeks and stratification of care after the first visit; taken from Nicolaides (2011).

1.5.6. Combined screening

An alternative method of screening for PE is based on the combination of biophysical and biochemical markers with maternal characteristics and medical history to obtain an individual probability of developing PE, in a similar approach to that of the combined screening test for Down's syndrome. In these predictive models, the *a priori* risk based on maternal characteristics and history is then adjusted (Bayes' theorem) after addition of biomarkers measurement through a mathematical equation derived from logistic regression or competing risk models (O'Gorman *et al.*, 2016b).

Logistic regression models produce risk estimates based on the multiplication of regression coefficients by the individual value of each variable used in the model when predicting a categorical outcome variable. This combined method of screening is performed using equations obtained by logistic regression analysis to derive the *a priori* risks of PE from maternal characteristics. The maternal factors-related *a priori* risks are then multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the *a posteriori* risks (Akolekar *et al.*, 2011). It is hypothesised that for each predictor in the model development, ten events should have occurred to avoid overfitting (Vittinghoff and McCulloch, 2007).

In a competing risk model (Kalbfleisch and Prentice, 2002), it is assumed that if the pregnancy were to continue indefinitely, all women would develop this

condition. Therefore, there is a competition between the delivery with PE and delivery for other reasons. The distribution of gestational age at birth with PE is then obtained by applying Bayes' theorem (Wright *et al.*, 2012; Akolekar *et al.*, 2013; Wright *et al.*, 2015b; Gallo *et al.*, 2016; O'Gorman *et al.*, 2016b) to combine the prior distribution based on maternal characteristics with likelihoods of the time to delivery with PE given by biomarkers measurements (adjusted for confounding characteristics and expressed in MoM values). A recent study by O'Gorman *et al.* (2016b), including 35,948 pregnancies between 11 and 13 weeks of gestation, reported that while maternal characteristics and history alone would detect only 49% of the cases of preterm PE, screening by combining maternal characteristics and history with MAP, mean UtPI and PLGF predicted 89% of the cases of PE requiring delivery before 32 weeks, 75% of the cases of preterm PE and 47% of the cases of term PE, for a fixed false-positive rate (FPR) of 10%. In this study, the addition of PAPP-A to the predictive model did not significantly increase the detection rates when PLGF was included, suggesting some degree of overlap and interaction between the two biochemical markers (O'Gorman *et al.*, 2016b).

Different combinations of markers yield various detection rates for the same FPR and would be useful, for example, in low-income settings where biochemical markers measurement might not be available. Another study, performed in a low-resource area of Brazil using only maternal factors and MAP in the first trimester predicted 67% of the cases of preterm PE at a 10% FPR (Rocha *et al.*, 2017).

Predictive models have been criticised because of the possibility of a better performance of the algorithm in the cohort used to develop the model than in prospective validation studies, a phenomenon known as overfitting. Other authors criticise the use of these predictive models because they often lack cross-validation and external validation studies (Oliveira *et al.*, 2014; Brunelli and Prefumo, 2015; Kleinrouweler *et al.*, 2016).

1.6. Prevention of preeclampsia with aspirin

Aspirin is one of the oldest medications still in widespread use. The utilisation of aspirin-related compounds derived from the willow tree bark, "*salix*", and reports

can be found in Egyptian papyrus scrolls dating back to 1534 BCE (Ebers, 2018). Hippocrates and his disciples also extracted powder from the same tree to treat headaches and fevers (Miner and Hoffhines, 2007). In 1828, Johann Buchner extracted the active ingredient of the willow plant and called it "*salicin*", word that represents willow in Latin; a few years later the sodium salicylate was treated with acetyl chloride to produce acetylsalicylic acid, and the first tablets were industrially produced in 1915 (Miner and Hoffhines, 2007). In 1971, Vale, Samuelson and Bergstrom were awarded a Nobel prize after elucidating the mechanism of action of the drug; nowadays, aspirin is one of the most commonly prescribed medications, taken by more than 50 million people for prevention of cardiovascular disease and with about 40,000 tons consumed every year (Navaratnam *et al.*, 2016).

Several interventions, including non-pharmacological (such as bed rest and reduction of salt intake) and pharmacological measures, have been studied for the prevention of PE, and aspirin has been the most widely considered pharmacological intervention with this purpose.

1.6.1. First reports

In 1978, Goodlin *et al.* (1978) reported in a prestigious medical journal a case of a woman who was pregnant for the third time, having had two previous pregnancies affected by severe hypertension, thrombocytopaenia and fetal growth restriction, requiring premature deliveries at around 30 weeks of gestation. In the third pregnancy, platelets were markedly reduced already at 15 weeks of gestation, but after initiation of treatment with aspirin (600 mg three times a day), the platelet count slowly increased to twice as high values, and no signs of hypertension were present at 32 weeks of gestation. At that stage, aspirin was ceased due to the fear of side effects to the fetus such as premature closure of the arterial duct, and there was an acute development of hypertension and thrombocytopaenia again, suggesting a potential beneficial effect of aspirin in high-risk cases.

One year later, Crandon and Isherwood (1979) published, in the same periodic, the results of a questionnaire survey administered to 964 primigravidae women, and those who had frequently taken aspirin or non-steroidal anti-

inflammatory drugs during pregnancy had a significantly lower incidence of PE compared to those who did not take these drugs regularly.

1.6.2. Mechanism of action of aspirin

The mechanism by which aspirin could potentially prevent PE is unclear. Theories include the inhibition of platelet activation that occurs in PE through changes in the prostacyclin and thromboxane A2 cascade, improvement in placentation, and anti-inflammatory effects on endothelial cells (Redman *et al.*, 1978; Crandon and Isherwood, 1979).

An *in vitro* study with a model of placental cells and the addition of serum from women with and without PE has shown that in PE the production of PLGF and its gene expression are reduced compared to controls, cytokines levels are altered, the differentiation of placental cells is abnormal, and markers of cellular apoptosis are increased. All these alterations were reverted to normal levels with the addition aspirin to the experiment (Panagodage *et al.*, 2016).

1.6.3. Randomised trials

In the first randomised trial on the effect of aspirin on pregnancy complications, Beaufils *et al.* (1985) randomised 102 women at high risk of PE or fetal growth restriction (FGR) to receive 150 mg of aspirin daily or no treatment from 12 weeks of gestation. PE occurred in six patients and severe FGR or fetal death in nine patients in the non-treatment group, but none of the patients in the aspirin group was affected by these complications.

Similarly, a small placebo-controlled trial randomised 46 primigravidae women judged to be at high risk of hypertensive disorders at 28 weeks of gestation to receive aspirin at a daily dose of 60 mg or placebo. The incidence of PE and PIH was markedly reduced in the aspirin group (Wallenburg *et al.*, 1986) without a significant increase in side-effects.

Many other intervention studies followed, and part of the excitement about the potential beneficial effect of aspirin vanished when a large landmark study known as

CLASP trial (Collaborative Low-dose Aspirin Study in Pregnancy) showed that, despite being safe for the mother and the child, aspirin at a daily dose of 60 mg did not reduce the incidence of PE when compared to placebo (CLASP Collaborative Group, 1994). The study included 9,364 women at risk of PE or FGR due to medical history and also pregnancies already diagnosed with PE and FGR. There was, however, a significant trend towards progressively greater reductions in PE the more preterm the delivery occurred. It is now believed that the negative results of this trial could have been due to the low dose used (60 mg), inadequate selection of high-risk patients and late initiation of aspirin (treatment was started at different stages between 12 and 32 weeks).

1.6.4. Meta-analyses

In 2001, a meta-analysis including more than 30,000 pregnancies from 39 trials reported a 15% reduction in the risk of PE among women who used anti-platelet agents during pregnancy (relative risk 0.85, 95% CI 0.78-0.92) (Duley *et al.*, 2001).

Similarly, the PARIS (Perinatal Antiplatelet Review of International Studies) study was a meta-analysis of individual patient data including 31 randomised trials evaluating the effect of anti-platelet agents on the prevention of PE, 23 of which used aspirin (Askie *et al.*, 2007). This study found a significant but very modest 10% reduction of PE in the treatment group compared to placebo or no treatment (relative risk 0.90, 95% CI 0.84-0.97), but no differences in stillbirth or delivery of small for gestational age infants. Aspirin again appeared to be safe, as adverse events were similar between the groups. In the trials included in the PARIS study, 14 different definitions of PE were used. A large proportion of the studies used aspirin at a daily dose that was lower than 100 mg (21/23), and 20 of 23 studies started the treatment after 15 weeks of gestation.

Evaluating the platelet function in pregnant women making use of aspirin, Caron *et al.* (2009) demonstrated that, at 81 mg, the standard dose in North America, about 30% of the women were non-responders, with no alterations in platelet function. When the dose was doubled (162 mg), non-responsiveness was

less than 5%. This finding was supported by a later meta-analysis including 20,909 pregnant women, which reported a dose-dependent and a significant reduction and in PE cases when aspirin is initiated before 16 weeks of gestation, but not when treatment is started after this gestational age or with lower doses (Roberge *et al.*, 2017b). Previous meta-analyses published by the same group have also shown a significant risk reduction for PE, FGR and perinatal death with aspirin started before 16 weeks of gestation (Roberge *et al.*, 2012a; Roberge *et al.*, 2013b). Equally, there was a very pronounced reduction in the occurrence of preterm PE, but no statistically significant reduction of PE at term (Roberge *et al.*, 2012a). The studies by Roberge *et al.* were not meta-analyses of individual patient data and, for this reason, could have overestimated the beneficial effect of aspirin. However, their results show a benefit only when aspirin is started before 16 weeks of gestation, at a dose higher than 100 mg and when the outcome measure is preterm PE rather than term PE or all PE cases.

In a recent secondary analysis of the PARIS study (Meher *et al.*, 2017), the authors did not find differences when the results were stratified by gestational age at which treatment was initiated (before or after 16 weeks of gestation) and by dose of aspirin given (higher or lower than 75 mg), but the authors did not analyse a combination of both (dose > 75 mg initiated before 16 weeks of gestation) (Roberge *et al.*, 2017a).

In view of the conflicting results reported in the literature, the aims of the publications included in this thesis are, first, to prospectively test and validate the FMF algorithm for prediction of PE in a large European population; second, to compare this method of screening to the performance of currently used guidelines; third, to evaluate a possible beneficial effect of aspirin initiated at 11 to 14 weeks of gestation and at a dose of 150 mg in the prevention of PE in a multicentre double-blind placebo-controlled randomised trial; and fourth, to analyse a potential role of cell-free DNA testing in the prediction of PE. My contribution for each one of the studies included in this thesis is summarised in the Appendix.

2. Validation of combined screening for preeclampsia

The second chapter of this thesis consists of three publications. The first two report the findings of a validation study performed before the initiation of the Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE) trial, denominated Screening Quality Study. The third one reports the validation of a similar approach to screening for PE in the Australian population, in the second trimester of pregnancy, using maternal factors, MAP and UtPI.

In the first publication (O'Gorman *et al.*, 2017b), we report the accuracy of the screening test in a prospective application of the FMF algorithm for prediction of preterm PE in the first trimester in hospitals participating of the subsequent ASPRE trial. This study was proposed to test the screening prospectively in a large European population. Patients attending first-trimester combined screening for chromosomal abnormalities at 11 to 13 weeks of gestation were also offered combined screening for PE at the same time using collection of maternal characteristics and medical history, measurement of MAP and UtPI according to previously published techniques (Plasencia *et al.*, 2007; Poon *et al.*, 2012) and maternal serum biochemistry. Inclusion criteria were defined as maternal age > 18 years; singleton pregnancy; live fetus at 11 to 13 weeks of gestation; no serious mental illness or learning difficulty; and informed and written consent. The individual risk for preterm PE was calculated and was not disclosed to the patients. 8,775 patients were screened, and the incidence of PE was 2.7% (239), with PE requiring delivery before 32 weeks of gestation affecting 0.2% (17) and preterm PE 0.7% (59) of the pregnancies. The detection rates were 89% (95% CI 79-96%), 75% (95% CI 70-80%) and 47% (95% CI 44-51%) for PE < 32 weeks, preterm PE and term disease, respectively. These figures were similar to those reported in the development of the algorithm (O'Gorman *et al.*, 2016b).

In the second publication (O'Gorman *et al.*, 2017a), we compared the performance of the screening test to current policies of screening based on maternal factors alone (NICE guidelines and ACOG recommendations) in the same cohort of 8,775 patients. Whereas combined screening detected 75% (95% CI 70-80%) of the cases of preterm PE at a 10% FPR, screening with use of NICE guidelines identified 39% (95% CI 27-53%) of PE < 37 weeks at 10.2% FPR and screening with use of ACOG risk factors detected 90% (95% CI 79-96%) of PE <37 weeks, at a much

higher FPR of 64.2%. Screening based on the ACOG recommendations for the use of aspirin detected only 5% (95% CI 2-14%) of the preterm PE cases. This illustrates that methods based on maternal history and characteristics alone have either low detection rates or high FPR.

Other studies also validated the first trimester combined screening by applying the FMF algorithm in different populations, with similar results (Park *et al.*, 2013; Park *et al.*, 2015; Lobo *et al.*, 2017).

A similar way of screening through the combination of multiple markers with maternal factors can also be used in the second trimester (Gallo *et al.*, 2016) or the third trimester (Tayyar *et al.*, 2014; Tsiakkas *et al.*, 2016b). This is particularly important to reassess risks in models in which care is stratified according to previous screening tests results (Litwinska *et al.*, 2017) or in settings where a significant proportion of patients attend antenatal care too late for first-trimester screening, after 14 weeks of gestation (Gross *et al.*, 2012; Muhwava *et al.*, 2016). Although no intervention appears to reduce the incidence of PE when started after 20 weeks of gestation, cases identified late as high risk may benefit from increased surveillance. Hence, in the third publication presented in this chapter (Al-Amin *et al.*, 2018), we prospectively tested the combined screening for PE in the Australian population in the second trimester, in a cohort of 543 women attending fetal morphology ultrasound at 19 to 22 weeks of gestation. Combined screening detected all cases of preterm PE, performing better and with a lower screen-positive rate than NICE guidelines and ACOG recommendations criteria.

Strengths and limitations

The main limitation of the three studies presented in this chapter is the relatively small incidence of early-onset and preterm PE, leading to inevitably wide confidence intervals. Specifically, the number of women screened in the third study is limited. Nevertheless, the detection rates with the predictive model in all three studies are similar to those reported in previously published studies (Wright *et al.*, 2012; Akolekar *et al.*, 2013; Gallo *et al.*, 2016; O'Gorman *et al.*, 2016b).

Publications

1. https://www.ncbi.nlm.nih.gov/pubmed/28067011

O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, Akolekar R, Cicero S, Janga D, Jani J *et al.* Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2017;49: 751-755.

2. https://www.ncbi.nlm.nih.gov/pubmed/28295782

O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, Carbone IF, Dutemeyer V, Fiolna M, Frick A *et al.* Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017;49: 756-760.

3. https://www.ncbi.nlm.nih.gov/pubmed/28850663

Al-Amin A, Rolnik DL, Black C, White A, Stolarek C, Brennecke S, da Silva Costa F. Accuracy of second trimester prediction of preterm preeclampsia by three different screening algorithms. Aust N Z J Obstet Gynaecol 2018;58: 192-196.

3. Development and design of the ASPRE trial

This chapter is based on the publication of the Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE) study protocol in an open access journal (O'Gorman *et al.*, 2016a). The ASPRE trial was designed to address the controversy as to whether aspirin reduces or not the risk of PE, brought to light by previous studies that were very heterogeneous regarding the selection of high-risk patients, dose and time of initiation of treatment with aspirin. Given that results of recent meta-analyses showed an apparent effect of aspirin at doses of 100 mg or higher and start of treatment before 16 weeks of gestation on the incidence of preterm (but not term) PE (Bujold *et al.*, 2010; Roberge *et al.*, 2012b), we designed a large multicentre, double-blind, placebo-controlled trial starting aspirin at 11 to 14 weeks of gestation at a dose of 150 mg daily, at night, to women deemed to be at high risk by the FMF combined predictive model at the same time of the 11 to 13 weeks ultrasound for chromosomal abnormalities screening, until 36 weeks of gestation or birth if it happened before 36 weeks.

All patients attending first-trimester combined screening for chromosomal abnormalities at 11 to 13 weeks of gestation would also be offered combined screening for PE at the same time using collection of maternal characteristics and medical history, measurement of MAP and UtPI on ultrasound by previously published techniques (Plasencia *et al.*, 2007; Poon *et al.*, 2012) and measurement of serum PAPP-A and PLGF. The individual risk of preterm PE would be calculated through equations embedded in the ultrasound reporting software once the results were received and the patient would be informed about the risk. Patients considered to be at high risk, with the probability of developing preterm PE above a certain threshold chosen to result in a 10% screen-positive rate, would then be invited to participate in the randomised trial of aspirin versus placebo.

There were 13 academic hospitals participating in the trial, including six centres in the UK; three in Spain, and one in each of Italy, Belgium, Greece and Israel. Adherence to the study protocol would be monitored by the Comprehensive Clinical Trials Unit, University College London (CCTU-UCL).

Inclusion criteria for screening were defined as maternal age > 18 years; singleton pregnancy; live fetus at 11 to 13 weeks of gestation; English, Italian,

Spanish, French, Dutch or Greek-speaking (otherwise interpreters would be used); and informed and written consent. Patients younger than 18 years, multiple pregnancies, cases of major fetal abnormality identified at the 11 to 13 weeks assessment and women who were unconscious, severely ill, those with learning difficulties or serious mental illness would be excluded from screening.

High-risk patients would be excluded from randomisation if they were taking low-dose aspirin regularly, previously diagnosed with bleeding disorders (such as Von Willebrand' s disease), peptic ulceration, hypersensitivity to aspirin or they were already on long-term non-steroidal anti-inflammatory medication. Cases of concurrent participation in another drug trial at any time within the previous 28 days, or with any other reason the clinical investigators thought would prevent the potential participant from complying with the trial protocol would also be excluded from the randomised study.

The sample size was calculated assuming that the screen-positive rate would be 10%, that 76% of the cases of preterm PE would be identified and that 60% of the high-risk women would agree to participate in the study. We would need to screen 29,330 pregnancies to identify 2,933 high-risk cases and randomise 1,760 to detect a possible significant effect of aspirin in reducing the incidence of preterm PE by 50% when compared to placebo at a 5% significance level and with 90% statistical power. This number was also calculated to account for attrition, expecting a 10% loss to follow-up or drop-out rate.

Strengths and limitations

The ASPRE trial was designed to be the largest multicentre, double-blind, placebo-controlled trial to date to examine the effect of aspirin in women at high-risk for preterm PE identified in the first trimester. To maximise the potential benefit of aspirin in the prevention of preterm PE and given the findings of recent literature, a higher dose of aspirin (150 mg) would be used to avoid resistance. The main limitation of the study would be the lack of long-term follow-up of the offspring, as the outcome collection was limited to the early neonatal period.

Publication

1. https://www.ncbi.nlm.nih.gov/pubmed/27354081

O'Gorman N, Wright D, Rolnik DL, Nicolaides KH, Poon LC. Study protocol for the randomised controlled trial: combined multimarker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE). *BMJ Open* 2016;6: e011801.

4. Multicentre double-blind placebo-controlled trial with aspirin – the ASPRE trial

This chapter consists of the main publication of this work. It reflects the results of the Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention trial (ASPRE), a large multicentre double-blind, placebo-controlled intervention study (Rolnik *et al.*, 2017b).

My contribution to the ASPRE study included overall coordination of the trial, training of staff in all sites prior to commencement, recruitment and follow-up of patients at King's College Hospital (London, UK), reporting of adverse events to CCTU-UCL, oversight of data collection in all sites, collection of pregnancy outcomes, quality assurance of biomarker measurements in all sites, and writing of the manuscript.

In total, 26,941 women underwent first-trimester screening for PE by a combination of maternal characteristics and history, MAP, UtPI, PAPP-A and PLGF. Of those, 2,971 (11%) were found to be at high risk for developing PE, using a risk cut-off of 1 in 100. After counselling, 1,776 (60%) high-risk patients were eligible and agreed to randomisation, 878 allocated to blindly receive aspirin at a dose of 150 mg and 898 to receive placebo daily from 11 to 14 until 36 weeks of gestation. The final number of patients in each group were 798 for aspirin and 822 for placebo, as 152 (8.6%) patients withdrew consent and four patients (0.2%) were lost to follow-up. For the primary outcome (PE with delivery before 37 weeks of gestation), we reported adjusted odds ratio (OR) with 95% CI, and for secondary outcomes OR with 99% CI (PE with delivery before 34 weeks, term PE, stillbirth, neonatal death, poor fetal growth).

All patients participating in the randomised trial received follow-up phone calls at 16 and 28 weeks of gestation in order to assess the occurrence of side-effects and compliance to treatment and were advised to attend follow-up visits including blood pressure measurement, fetal growth assessment by ultrasound, evaluation of side-effects (which were strictly reported to the CCTU-UCL), as well as assessment of compliance by counting the remaining tablets at 22 to 24^{+6}, 32 to 34^{+6} and 36 to 36^{+6} weeks of gestation.

Baseline characteristics were similar between the groups. Adherence to treatment was good, with 80% of the patients taking 85% or more of the tablets. Aspirin reduced the incidence of preterm PE by 62% (OR 0.38, 95% CI 0.20-0.74,

p=0.004). This reduction was also significant in the survival analysis. There was also a non-significant reduction in early-onset PE (OR 0.18, 99% CI 0.03-1.03), early-onset fetal growth restriction (OR 0.53, 99% CI 0.16-1.77), stillbirth before 37 weeks (OR 0.78, 99% CI 0.31-1.95) and preterm placental abruption (OR 0.52, 99% CI 0.06-4.91). The study was not powered for such secondary outcomes.

There was no increase in side effects, fetal abnormalities or other adverse events in the aspirin group when compared to the placebo group.

The results of the ASPRE trial confirm that daily aspirin initiated before 16 weeks of gestation, at a dose of 150 mg per day, to high-risk patients identified by first trimester combined screening, reduces the incidence of preterm PE. This study has been cited several times since publication, and its results were practice-changing, with increasing implementation of first trimester screening for PE worldwide and prescription of a higher dose of aspirin (150 mg) to high-risk patients compared to the commonly prescribed doses before publication (100 mg or less).

New meta-analyses performed after the publication of the ASPRE trial including its results also confirm the beneficial dose-dependent effect of aspirin in the prevention of preterm PE (Roberge *et al.*, 2018a; Seidler *et al.*, 2018).

Strengths and limitations

Unlike previous trials, this was the first large multicentre, double-blind, placebo-controlled trial to include high-risk women by means of first trimester combined screening. Aspirin was initiated before 16 weeks and with a dose higher than usual based on the apparent dose-dependent effect in the prevention of preterm PE. Measurements of biomarkers were obtained in a standardised manner by trained operators. Adherence to treatment was carefully assessed during follow-up visits, and compliance with the study protocol was rigorously monitored by an independent entity, the Comprehensive Clinical Trials Unit, University College London. An independent statistician performed the statistical analysis. Funding organisations had no role in study design, collection, analysis or interpretation of the data, or writing of the manuscripts.

The main limitation of the study is the lack of long-term follow-up of the offspring, as the outcome collection was limited to the early neonatal period. The incidence of preterm PE was lower than what was anticipated, possibly a consequence of differences between the demographic characteristics of the trial population and those of the cohort used in the development of the algorithm.

Publication

1. https://www.ncbi.nlm.nih.gov/pubmed/28657417

Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M *et al.* Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med* 2017;377: 613-622.

5. Secondary analyses of the ASPRE trial

This chapter is comprised by five sections, each containing one publication with a secondary analysis of the ASPRE trial.

5.1. Performance of combined screening during the ASPRE trial

This section includes a publication describing the performance of screening in the actual randomised study (Rolnik *et al.*, 2017c). Of 26,941 women screened, 1,144 (4.2%) were excluded due to loss of follow-up or study withdrawal (n = 716), miscarriage (n = 243) or termination of the pregnancy (n = 185), leaving 25,797 patients in the study. During the randomised trial, the performance of screening was very similar to that of published studies using similar first trimester algorithms for prediction of PE (Poon *et al.*, 2009a; Akolekar *et al.*, 2013; O'Gorman *et al.*, 2016b). The analysis was adjusted for the beneficial effect of aspirin by estimating the number of events (preterm PE) in those receiving treatment if aspirin had no effect. No significant effect of aspirin was seen on PE at term in the trial.

Using a risk cut-off of 1 in 100 to define the high-risk population, screening for PE had a detection rate of 76.7% for preterm PE and 43.1% for term PE, at a screen-positive rate of 10.5% and false-positive rate of 9.2%.

Strengths and limitations

The randomised controlled trial showed a significant effect of aspirin in reducing the incidence of preterm PE. The analysis in the evaluation of the screening had to be adjusted for this effect and, therefore, this was not a non-intervention validation study.

Publication

1. https://www.ncbi.nlm.nih.gov/pubmed/28741785

Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M *et al.* ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol* 2017;50: 492-495.

5.2. Incidence of preterm preeclampsia in patients fulfilling ACOG and NICE criteria according to risk by the FMF algorithm

This section is based on a publication that reports the rate of preterm PE in women that fulfil the screening criteria of the NICE guidelines and ACOG risk factors and compares the incidence of preterm PE in those that are screen-positive (risk > 1 in 100) and screen-negative (risk ≤ 1 in 100) by the FMF algorithm in the first trimester.

In this publication (Poon *et al.*, 2018), a secondary analysis of the ASPRE study was performed including all women screened during the validation phase (O'Gorman *et al.*, 2017b) and the randomised trial period (Rolnik *et al.*, 2017b). In total, 34,573 singleton pregnancies delivering at ≥ 24 weeks of gestation were included, and 239 (0.7%) were affected by preterm PE.

At least one of the ACOG risk factors was fulfilled in 64.5% of the population, and the incidence of preterm PE in this group was 0.97% (95% CI 0.85-1.11%); in the subgroup that was screen-positive by FMF (risk > 1 in 100), the incidence of preterm PE was 4.8% (95% CI 4.14-5.55%), whereas in the subgroup that screened negative by the FMF algorithm, the incidence of preterm PE was 0.25% (95% CI 0.18-0.33%). At least one of the NICE guidelines high-risk factors was present in 4.0% of the screened population, and the incidence of preterm PE in this group was 5.17% (95% CI 4.13-6.46%); in the subgroups of screen-positive and screen-negative by the FMF algorithm the incidence was 8.71% (95% CI 6.93-10.89%) and 0.65% (95% CI 0.25-1.67%), respectively. At least two of the NICE guidelines moderate-risk factors were present in 6.8% of the study population and the incidence of preterm PE in this group was 1.74% (95% CI 1.28-2.35%); in the subgroups of screen-positive and screen-negative by the FMF algorithm the incidence was 4.91% (95% CI 3.54-6.79%) and 0.42% (95% CI 0.20-0.86%), respectively.

These findings are consistent with our previous studies (O'Gorman *et al.*, 2017a; Al-Amin *et al.*, 2018) that show that risk-based methods of screening are superior to current guidelines based solely on maternal characteristics and medical history. Additionally, it is important to highlight that screening negative by the FMF

algorithm in the first trimester is reassuring even in the presence of risk factors, such as the history of a previous pregnancy affected by PE.

The results of this study provide further evidence to support combined screening for PE. In women fulfilling criteria by history-based screening methods and with a low risk according to first-trimester risk calculation by the FMF algorithm, the risk of preterm PE is reduced to within or below background levels.

Strengths and limitations

This study included a large population and collected data prospectively on maternal characteristics and history, biophysical and biochemical markers in women attending routine care between 11 and 13 weeks, a range widely used for screening of chromosomal abnormalities and other major fetal malformations. Consistency on biomarkers measurement was maintained throughout the study following training of all investigators according to standardised protocol and adherence to the study protocol was monitored by an independent entity (CCTU-UCL).

The main limitation of the study relates to the low number of cases of women with certain risk factors, such as systemic lupus erythematosus or anti-phospholipid syndrome, leading to wide confidence intervals for relative incidences. Nonetheless, there was a consistent reduction in the incidence of preterm PE in the FMF screen-negative group compared to that in the screen-positive group.

Publication

2. https://www.ncbi.nlm.nih.gov/pubmed/29380918

Poon LC, Rolnik DL, Tan MY, Delgado JL, Tsokaki T, Akolekar R, Singh M, Andrade W, Efeturk T, Jani JC et al. ASPRE trial: incidence of preterm pre-eclampsia in patients fulfilling ACOG and NICE criteria according to risk by FMF algorithm. Ultrasound Obstet Gynecol 2018;51: 738-742.

5.3. Influence of adherence to treatment on the effect of aspirin in the prevention of preterm preeclampsia

This section is based on a publication evaluating the impact of adherence to treatment on the beneficial effect of aspirin (Wright *et al.*, 2017). Several studies have shown that patients who are adherent to drug therapies have better outcomes than those with poor compliance (Coronary Drug Project Research, 1980; Cramer, 2002; Avorn, 2006; Simpson *et al.*, 2006; Abbott-Mitchell, 2007; Cramer *et al.*, 2008). Although randomised trials are often conducted on an intention-to-treat basis, their results could be influenced by the level of adherence to treatment of the participants.

In this secondary analysis of the ASPRE trial (Rolnik *et al.*, 2017b), we examined the factors affecting compliance to treatment and its influence on the beneficial effect of aspirin in preventing preterm PE. Compliance was assessed by counting the tablets that were returned by the patients at each visit and by the participants' reporting of tablet counts during each telephone interview. The total number of tablets taken was calculated by subtracting the number of tablets returned from the number of tablets prescribed. Compliance was calculated as a percentage of the reported intake to the total number of tablets that participants were expected to have taken between the date of randomisation and the date of the visit at 36 weeks of gestation or the date of delivery if it occurred before 36 weeks. Compliance to the treatment of 90% or more was associated with a significantly greater reduction in the incidence of preterm PE (OR 0.24, 95% CI 0.09-0.65), when compared to compliance below 90% (OR 0.59, 95% CI 0.23-1.53). Adherence to treatment was positively associated with family history of PE and negatively associated with smoking, maternal age below 25 years, Afro-Caribbean and South Asian ethnic origins and history of PE in a previous pregnancy.

A possible explanation for the surprising finding of lower compliance among women with previous pregnancies affected by PE is that poor compliance may also be associated with an unknown attribute or with other maternal characteristics that are also associated with PE, such as ethnic origin.

Strengths and limitations

The main limitation of this study is that it consists of a secondary analysis of the ASPRE trial, which was not adequately powered for such secondary analysis. Furthermore, it relied on the counting of tablets as a method of ascertaining compliance rather than a more objective measurement of a biomarker. There is a wide range of platelet activation and function assays that have been used to assess responsiveness to aspirin, but these assays suffer from limited reproducibility and poor agreement between them, and suboptimal suppression of platelet activation does not necessarily reflect poor compliance (Navaratnam *et al.*, 2016). Nevertheless, the findings of this study support previous literature reports (Coronary Drug Project Research, 1980; Cramer, 2002; Avorn, 2006; Simpson *et al.*, 2006; Abbott-Mitchell, 2007; Cramer *et al.*, 2008) and are in agreement with the intuitive perception that good compliance results in a better effect of any given treatment. Future studies evaluating methods to ensure good compliance to treatment are necessary.

Publication

3. https://www.ncbi.nlm.nih.gov/pubmed/28888591

Wright D, Poon LC, Rolnik DL, Syngelaki A, Delgado JL, Vojtassakova D, de Alvarado M, Kapeti E, Rehal A, Pazos A *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: influence of compliance on beneficial effect of aspirin in prevention of preterm preeclampsia. *Am J Obstet Gynecol* 2017;217: 685 e681-685 e685.

5.4. Subgroup analyses according to maternal characteristics and medical history

This section includes a publication in which we performed a secondary analysis of the ASPRE trial (Rolnik *et al.*, 2017b) to examine whether there are differences in the effect of aspirin on the incidence of preterm PE according to maternal characteristics and medical history (Poon *et al.*, 2017). Subgroup analyses were performed to assess evidence of differences in the effect of aspirin in subgroups of maternal age (< 30 and ≥ 30 years), BMI (< 25 and ≥ 25 kg/m^2), ethnic background, method of conception, cigarette smoking, family history of PE, and history of chronic hypertension.

Interaction tests were performed on the full dataset of patients in the intention-to-treat population, as well as on the subgroup of patients with good adherence to treatment (≥ 90% of the tablets consumed), with a Bonferroni correction for multiple comparisons. There was no evidence of heterogeneity in the aspirin effect in subgroups according to maternal characteristics and obstetric history. However, in patients with chronic hypertension, there was no evidence of reduction in the incidence of superimposed PE requiring delivery before 37 weeks of gestation, neither in the full dataset (10.2% in the aspirin group, 8.2% in the placebo group, adjusted OR 1.29, 95% CI 0.33-5.12), nor in the good adherence to treatment subgroup (adjusted OR 2.06, 95% CI 0.40-10.71). The test of interaction was highly significant in the subgroup with chronic hypertension and good compliance. Patients without chronic hypertension and with good adherence to treatment had a 95% reduction in the incidence of preterm PE (adjusted OR 0.05, 95% CI 0.01-0.41).

Regarding the biological plausibility, there is some evidence of differences between chronic hypertension and other factors in the pathogenesis of PE. Chronic hypertension, found in 1-2% of pregnancies, is the strongest risk factor for PE compared to other factors in maternal demographic characteristics and medical history (Wright *et al.*, 2015b). The results of this study suggest that the beneficial effect of aspirin may not apply to women with chronic hypertension, favouring the hypothesis that in this group of patients an impaired cardiovascular system may be implicated in the origin of the disease (Kalafat and Thilaganathan, 2017). In chronic

hypertension, there is endothelial dysfunction and inflammation even before pregnancy and it is possible that in this condition PE can develop in the absence or less severe degree of impaired placentation because the pre-existing endothelial dysfunction is exacerbated by the physiological changes of pregnancy (Roberts and Hubel, 2009; Brandes, 2014; Panaitescu *et al.*, 2017).

Strengths and limitations

The main limitation of this study is that it consists of a secondary analysis of the ASPRE trial, which was powered for a global test of the aspirin effect in a high-risk population. Subgroup analyses will inherently have lower statistical power, and only more substantial interaction effects are likely to be identified. Furthermore, this problem is exacerbated by the need to account for multiple comparisons using a conservative technique such as Bonferroni correction. It is reassuring that the effect of aspirin was consistent across maternal characteristics and history factors other than chronic hypertension, but the broad confidence intervals do not allow the exclusion of other clinically important interaction effects.

More studies are needed to investigate novel treatments in women with chronic hypertension and for the prevention of term PE, and to identify high-risk women who do not benefit from aspirin intake. Ongoing trials are now evaluating the possible beneficial effect of pravastatin, metformin and esomeprazole in the prevention of PE.

Publication

4. https://www.ncbi.nlm.nih.gov/pubmed/28784417

Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, Leipold G, Akolekar R, Shearing S, De Stefani L *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. *Am J Obstet Gynecol* 2017;217: 585 e581-585 e585.

5.5. Effect of aspirin on the length of stay in the neonatal intensive care unit

In the ASPRE trial, the use of aspirin significantly reduced the incidence of preterm PE by more than 60% when compared to placebo (Rolnik *et al.*, 2017b). Surprisingly, however, despite the reduction in the incidence of PE, the rate of admission to the neonatal intensive care unit (NICU) was not significantly affected (OR 0.93, 95% CI 0.62-1.40).

In the publication included in this section (Wright *et al.*, 2018), we report a secondary analysis of the impact of treatment with aspirin on the length of stay in NICU. In the trial, there were 1,620 participants included in the final analysis, and these pregnancies resulted in 1,571 live births. The total length of stay in NICU was compared between the two intervention groups, as were the average lengths of stay in NICU among infants that were admitted and in the whole trial population (defining length of stay in NICU as zero days for infants who were not admitted to NICU). Bootstrapping was used for the comparison of the mean length of stay.

The total length of stay was substantially longer in the placebo group than in the aspirin group (1,696 and 531 days, respectively). The mean duration of stay among infants admitted to NICU was 31.4 days in the placebo group and 11.1 days in the aspirin group, a mean difference of 20.3 days (95% CI 7.0-38.6, p=0.008). This difference was mainly due to the reduction of PE requiring delivery before 32 weeks of gestation, which contributed to 83.3% of the total length of stay in NICU. Overall, in the whole population, including zero lengths of stay for those that were not admitted to NICU, the mean length of stay was longer in the placebo than aspirin group (2.06 vs 0.66 days; reduction of 1.4 days [95% CI 0.45-2.81; p=0.014]). This corresponds to a reduction in length of stay of 68% (95% CI 20-86%). Since babies may be admitted to NICU for observation only and soon be discharged, length of stay is probably a better indicator of morbidity and, perhaps, predictor of long-term adverse outcomes.

In a population of 10,000 pregnancies undergoing first-trimester screening for PE, 1,000 women would be classified as high risk with the expected screen-positive rate of 10%. Had these high-risk women not received aspirin, the expected total

length of stay in NICU would be 2,060 days, whereas if they had, the expected total length of stay in NICU would be 660 days. If the daily cost of the stay in NICU were £2,000, the cost saving from such care by a policy of screening 10,000 pregnancies and treating the high-risk group with aspirin would be £2,000 x (2,060 – 660) = £2,800,000. This is equivalent to £280 per patient screened, which is well in excess of the cost of screening and treating with aspirin. This calculation is based only on the reduction of length of admission in NICU, without considering a possible reduction in other prematurity-associated adverse neonatal outcomes that carry a significant burden, such as chronic lung disease and cerebral palsy.

Although it is difficult to estimate the cost-effectiveness of a policy of screening while the effect of such policy on neonatal outcomes is not well-established in the literature, a cost-effectiveness study published in 2012 demonstrated that screening would be cost-effective under various scenarios (Shmueli *et al.*, 2012). Another recent study found screening and subsequent treatment of high-risk patients to be cost-saving when compared to absence of screening (Ortved *et al.*, 2018).

Effective first-trimester screening is provided by a combination of maternal demographic characteristics and medical history, and measurement of MAP, UtPI and PLGF. Recording of medical history and measurement of BP are an integral part of current prenatal care. The marginal cost of PLGF in a screening scenario, when the equipment is already in place, is estimated at £10 per patient. Measurement of UtPI can be carried out by the same sonographers and ultrasound machines used for the routine scan at 11 to 13 weeks of gestation; however, the sonographers will require training, and the measurement would add two to three minutes to the current 20-30 minutes used for the scan.

Strengths and limitations

This was a secondary analysis of the ASPRE study. The trial was powered to detect differences between the treatment arms on the incidence of the primary outcome (preterm PE). Therefore, the statistical power for the detection of less

common outcomes, such as admission to NICU and length of stay in this unit is inevitably poor.

The findings that infants born before 32 weeks of gestation contributed to more than 80% of the total length of stay in NICU, that the incidence of delivery before 32 weeks was lower in the aspirin group and that, as a consequence, the total length of stay in NICU was substantially reduced in the aspirin group are not surprising.

Future trials, powered for specific neonatal outcomes, as well as long-term follow-up studies and meta-analyses, will be important to demonstrate the effect of aspirin in improving other neonatal outcomes due to the reduction of iatrogenic extreme prematurity.

Publication

5. https://www.ncbi.nlm.nih.gov/pubmed/29505771

Wright D, Rolnik DL, Syngelaki A, de Paco Matallana C, Machuca M, de Alvarado M, Mastrodima S, Tan MY, Shearing S, Persico N et al. Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin on length of stay in the neonatal intensive care unit. Am J Obstet Gynecol 2018;218: 612 e611-612 e616.

6. Arguments against universal prophylaxis with aspirin

With increasing evidence that aspirin reduces the incidence of preterm PE and the publication of the results of the ASPRE trial (Rolnik *et al.*, 2017b), there is now little doubt as to its beneficial effect in pregnancies at high risk for PE. For this reason, some authors have been suggesting that, given the clear benefit, the low cost and the favourable safety profile of aspirin, this medication should be given to all pregnant women (Werner *et al.*, 2015; Mone *et al.*, 2017), arguing that the screening test is complex and expensive.

In a recent editorial (Rolnik *et al.*, 2017a), we have summarised the main findings of the literature on this matter along with the results of the ASPRE study, highlighting some of the issues that would be faced in a policy of universal prophylaxis:

- *Efficacy:* Aspirin is highly effective in reducing preterm PE when given to women at increased risk from before 16 weeks of gestation and at doses higher than 100 mg. Consistent evidence concerning its efficacy in the general obstetric population including low-risk women is currently lacking. In fact, the results of the ASPRE trial indicate that the number needed to treat (NNT) when aspirin is given to women identified as being at high risk is 38. If we gave aspirin to all pregnant women regardless of their risk and assumed that efficacy and compliance would be maintained within the general obstetric population, which is unlikely, the NNT would be 202. Preterm PE affects 0.7% to 0.8% of the pregnancies (O'Gorman *et al.*, 2017a; Rolnik *et al.*, 2017c), and more than 99% of the women would not benefit from aspirin use.
- *Adherence to treatment:* Very few studies evaluated adherence to treatment among women without a perceived high risk for developing PE. Nevertheless, pregnant women are naturally reluctant to take medication unnecessarily or in the absence of a significant risk factor for adverse pregnancy outcome, due to concerns regarding safety for the fetus (Nordeng *et al.*, 2010; Lynch *et al.*, 2018). A randomised trial in which aspirin or placebo were given to nulliparous women with no other risk factors listed as inclusion criteria reported poorer compliance rates (Subtil *et al.*, 2003). Another trial that recruited 3,647 of all women attending antenatal clinics between 12 and 32 weeks of gestation reported that only 55% of the participants had compliance above 80% (Rotchell *et al.*, 1998).

- *Long-term safety:* The vast majority of the studies performed to date, including the ASPRE trial, have not shown an increase in serious side-effects or adverse events for both the mother and the child, and aspirin is considered a safe medication in pregnancy. However, information on long-term outcomes for women exposed to aspirin in pregnancy and their children is scarce. Although a previous study has shown no increase in mortality at two years of age with the use of aspirin at a daily dose of 60 mg throughout pregnancy (CLASP Collaborative Group, 1995), other authors have raised concerns regarding long-term outcomes for the infant in observational studies, but these findings may have been subjected to biases due to the influence of confounders (Petersen *et al.*, 2018). In non-pregnant adults, long-term use of aspirin has been shown to increase the incidence of haemorrhagic events (De Berardis *et al.*, 2012), and a recent meta-analysis has shown a possible hazardous effect of aspirin in increasing the risk of placental abruption when the medication is initiated after 16 weeks of gestation (Roberge *et al.*, 2018b).

For the reasons mentioned above, caution is recommended, and the widespread prescription of aspirin to millions of pregnant women without targeting a specific high-risk population should be avoided.

7. Cell-free DNA in screening for preeclampsia

This chapter is based on two publications. Numerous studies have been investigating new markers that may enhance predictive algorithms in the near future. Given that predictive algorithms detect about 75% of the cases of preterm PE, there is room for improvement. Although new markers might be different in patients with PE compared to normotensive controls, their effects on predictive models need to be tested in combination with established markers with which they may interact and overlap.

Several studies have reported that in patients with established PE both total and fetal cell-free DNA are higher than in normotensive controls (Lo *et al.*, 1999; Zhong *et al.*, 2001; Alberry *et al.*, 2009; Miranda *et al.*, 2013; Zeybek *et al.*, 2013), possibly due to accelerated apoptosis of placental cells in cases of PE. Following the discovery of fetal DNA on maternal blood (Lo *et al.*, 1997), cell-free fetal DNA testing has been rapidly incorporated into clinical practice due to its high accuracy in detecting fetal chromosomal abnormalities. Higher incidence of PE was also found among patients with failed cell-free DNA test due to a low fetal fraction (Chan *et al.*, 2017). There is conflicting data as to whether these altered levels precede the onset of the disease (Martin *et al.*, 2014).

In the first study, published in 2015, we performed a case-control study of 20 cases of early PE, 20 cases of late PE and 200 normotensive controls, in which neither the quantities of cell-free DNA nor the fetal fraction were different in patients who developed PE compared to normotensive controls after adjustment for confounders (Rolnik *et al.*, 2015).

In a larger cohort study, however, our group demonstrated a clear association of fetal fraction with first trimester markers for PE and predicted risks (Rolnik *et al.*, 2018), even after adjustment for maternal characteristics and gestational age. This suggests that fetal fraction on cell-free DNA testing also reflects placentation. However, the degree of overlap with other markers and a possible role of incorporating cell-free DNA or fetal fraction into predictive models are yet to be determined. The possibility of using this test in patients undergoing cell-free DNA testing for aneuploidies screening instead of first trimester combined screening should be addressed in future research.

Strengths and limitations

In the case-control study, the number of pregnancies affected by PE may be inadequate for concluding that cell-free DNA levels are not altered in all types of PE.

The second study, despite the large number and the associations found, is retrospective in nature and did not include pregnancy outcomes. Therefore, no conclusions regarding differences in cell-free DNA between patients who developed PE and normotensive controls can be drawn.

The search for new markers remains the focus of several studies, and with the increasing use of cell-free DNA testing for aneuploidy screening, the potential role of this test in improving current predictive algorithms should be explored further.

Publications

1. https://www.ncbi.nlm.nih.gov/pubmed/25252010

Rolnik DL, O'Gorman N, Fiolna M, van den Boom D, Nicolaides KH, Poon LC. Maternal plasma cell-free DNA in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2015;45: 106-111.

2. https://www.ncbi.nlm.nih.gov/pubmed/29318732

Rolnik DL, da Silva Costa F, Lee TJ, Schmid M, McLennan AC. Association between fetal fraction on cell-free DNA testing and first trimester markers for pre-eclampsia. *Ultrasound Obstet Gynecol* 2018, DOI 10.1002/uog.18993.

8. Conclusions and future perspectives

8.1. Clinical implementation of screening and recommendations for clinical practice

The clinical implementation of first-trimester combined screening for PE requires specific training for MAP and UtPI measurements and testing PLGF on maternal blood, but the addition of these biomarkers would lead to a minimal increase in costs in settings where first-trimester combined screening for chromosomal abnormalities is routinely performed.

Different combinations of markers with partial models of screening can be used with reasonable detection rates in low-resource settings where applying the full algorithm may be challenging. In settings where analysing maternal serum biochemistry is challenging, for instance, a combination of maternal history with biophysical markers such as MAP can lead to reasonable detection rates. Similar to screening for chromosomal abnormalities, a quality assurance process for biomarkers measurement should be in place, and the cut-off selected by different health systems may vary depending on the resources and the baseline risk of PE in a given population.

Aspirin at doses of less than 100 mg does not seem to be effective in the prevention of preterm PE and therefore the daily dose used should be higher than 100 mg (ideally 150 mg daily). Because the standard dose varies from country to country, adjustments may be necessary. For example, in the UK, where the standard dose is 75 mg, and in the USA, where the standard dose is 81 mg, two tablets can be taken daily. In countries where the standard dose is 100 mg, the daily recommended intake should be one tablet or one tablet and a half.

For successful clinical implementation, it is crucial to achieve a global consensus regarding screening, dose and timing of aspirin prescription in light of the emerging body of evidence.

8.2. Future studies

Identifying which maternal characteristics predict poor response to aspirin and research on new preventive measures will be essential to find alternative treatments for this specific group and for women with chronic hypertension. Additionally, term PE is responsible for the majority of the burden of the disease, as this form of PE is more common than preterm PE (Duley, 2009; Bahado-Singh *et al.*, 2017). Its prediction is poor (Akolekar *et al.*, 2013; O'Gorman *et al.*, 2016b) and its incidence is not reduced by aspirin intake (Roberge *et al.*, 2012b; Rolnik *et al.*, 2017b). Therefore, new predictive markers for term PE and novel preventive strategies, including the use of pravastatin, metformin and proton-pump inhibitors such as esomeprazole are subject of ongoing research (Cluver *et al.*, 2015; Brownfoot *et al.*, 2016a; Brownfoot *et al.*, 2016b; Syngelaki *et al.*, 2016; Girardi, 2017; Kaitu'u-Lino *et al.*, 2018).

In summary, further research needs to be undertaken to identify women with poor response to aspirin, to improve the prediction of term PE and to investigate new preventive strategies for term PE and for PE in women with chronic hypertension.

8.3. Final considerations

Preeclampsia is a serious complication of pregnancy, and a significant proportion of the cases that will require premature delivery can be identified in the first trimester through a screening test that combines maternal characteristics, medical history and biophysical and biochemical markers. This approach is superior to current guidelines based on maternal characteristics and history alone.

The ASPRE trial is a landmark study in the investigation of screening and prevention of preeclampsia. It was well designed and the first multicentre trial to apply combined screening in a large population. The intervention was carefully chosen considering the latest evidence. Its impact is reflected in the publication of the results in a high impact medical journal, the large number of citations in a short period time and, above all, in the changes in clinical practice worldwide.

Aspirin, at a daily dose of 150 mg and given to high-risk patients from 11 to 14 weeks until 36 weeks of gestation, reduces the incidence of the severe forms of PE that require premature delivery and, as a consequence, the length of stay in NICU, saving costs that clearly exceed the cost of screening. The effect of the medication, unsurprisingly, depends on the adherence to treatment and is questionable in patients with chronic hypertension.

Universal prophylaxis with aspirin that has been suggested in the literature is problematic, as it would probably result in low compliance and long-term safety data is currently lacking.

Further research is warranted to improve current predictive algorithms and to investigate new preventive measures for term PE, and for preterm PE in specific groups of women.

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241. Wright D, Rolnik DL, Syngelaki A, de Paco Matallana C, Machuca M, de Alvarado M, Mastrodima S, Tan MY, Shearing S, Persico N *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin on length of stay in the neonatal intensive care unit. *Am J Obstet Gynecol* 2018;218: 612 e611-612 e616.

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APPENDIX

A. Aims, studies and articles characteristics

Aim	Study	Articles	Characteristics
1. Prospective validation of predictive algorithms	**1. ASPRE study (part 1: validation)**	1.O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, Akolekar R, Cicero S, Janga D, Jani J *et al.* Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2017;49: 751-755.	**Multicenter European study** **Funding:** Fetal Medicine Foundation, European Union 7th Framework Programme **Blind allocation process:** online (www.sealedenvelope.com) **Oversight:** Comprehensive Clinical Trials Unit (CCTU) – University College London
2. Prospective validation of predictive algorithms	**1. ASPRE study (part 1: validation)**	2. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, Carbone IF, Dutemeyer V, Fiolna M, Frick A *et al.* Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017;49: 756-760.	**Multicenter European study** **Funding:** Fetal Medicine Foundation, European Union 7th Framework Programme **Oversight:** Comprehensive Clinical Trials Unit (CCTU) – University College London
	2. Australian screening cohort	3. Al-Amin A, Rolnik DL, Black C, White A, Stolarek C, Brennecke S, da Silva Costa F. Accuracy of second trimester prediction of preterm preeclampsia by three different screening algorithms. Aust N Z J Obstet Gynaecol 2018;58: 192-196.	**Prospective external validation of second trimester screening and comparison with current guidelines** **Funding:** none

Aim	Study	Articles	Characteristics
3. Evaluation of the effect of aspirin initiated at 11 to 14 weeks of gestation and at a dose of 150 mg in the prevention of PE (and secondary analyses)	**1. ASPRE study (part 2: RCT)**	4. O'Gorman N, Wright D, Rolnik DL, Nicolaides KH, Poon LC. Study protocol for the randomised controlled trial: combined multimarker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE). *BMJ Open* 2016;6: e011801. 5. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med 2017;377: 613-622. 6. Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M *et al.* ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol* 2017;50: 492-495. 7. Poon LC, Rolnik DL, Tan MY, Delgado JL, Tsokaki T, Akolekar R, Singh M, Andrade W, Efeturk T, Jani JC *et al.* ASPRE trial: incidence of preterm pre-eclampsia in patients fulfilling ACOG and NICE criteria according to risk by FMF algorithm. *Ultrasound Obstet Gynecol* 2018;51: 738-742. 8. Wright D, Poon LC, Rolnik DL, Syngelaki A, Delgado JL, Vojtassakova D, de Alvarado M, Kapeti E, Rehal A, Pazos A *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: influence of compliance on beneficial effect of aspirin in prevention of preterm preeclampsia. *Am J Obstet Gynecol* 2017;217: 685 e681-685 e685. 9. Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, Leipold G, Akolekar R, Shearing S, De Stefani L *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. *Am J Obstet Gynecol* 2017;217: 585 e581-585 e585. 10. Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, Leipold G, Akolekar R, Shearing S, De Stefani L *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. *Am J Obstet Gynecol* 2017;217: 585 e581-585 e585.	**Multicenter European study** **Funding:** Fetal Medicine Foundation, European Union 7th Framework Programme **Blind allocation process:** online by the responsible investigator in each site (www.sealedenvelope.com) **Oversight:** Comprehensive Clinical Trials Unit (CCTU) – University College London; Trial Steering Committee; Independent Data and Safety Monitoring
4. Investigation of the potential role of cell-free DNA testing in the prediction of PE.	**3. British cell-free DNA study**	11. Rolnik DL, O'Gorman N, Fiolna M, van den Boom D, Nicolaides KH, Poon LC. Maternal plasma cell-free DNA in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2015;45: 106-111.	**Case control study** **Funding:** Fetal Medicine Foundation, and Sequenom, Inc, San Jose, CA, USA.
	4. Australian cell-free DNA cohort	12. Rolnik DL, da Silva Costa F, Lee TJ, Schmid M, McLennan AC. Association between fetal fraction on cell-free DNA testing and first trimester markers for pre-eclampsia. *Ultrasound Obstet Gynecol* 2018, DOI 10.1002/uog.18993.	**Retrospective cohort study in Australia** **Funding:** none

B. My contribution for each publication and RDPUB forms

My contribution to the ASPRE study, including the validation of the algorithm, involved overall coordination of data collection, training of staff in all sites prior to commencement, recruitment, randomisation and follow-up of patients at King's College Hospital (London, UK), reporting of adverse events to CCTU-UCL, performing quality assurance of biomarker measurements in all sites, and writing of the manuscript. Although the statistical analysis of the main publication was performed by an independent statistician, I contributed to all secondary analysis of the trial.

In the studies performed in Australia, my contribution included the study design and a significant proportion of data collection, statistical analysis and writing of the manuscripts.

My contribution for each publication is summarised in the following Table.

Reference	Collection of data	Writing of manuscript	Statistical analysis	Study design
O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, Akolekar R, Cicero S, Janga D, Jani J *et al.* Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2017;49: 751-755. †	**50%**	**50%**	**10%**	**50%**
O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, Carbone IF, Dutemeyer V, Fiolna M, Frick A *et al.* Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017;49: 756-760. †	**50%**	**50%**	**10%**	**50%**
Al-Amin A, Rolnik DL, Black C, White A, Stolarek C, Brennecke S, da Silva Costa F. Accuracy of second trimester prediction of preterm preeclampsia by three different screening algorithms. Aust N Z J Obstet Gynaecol 2018;58: 192-196. §	**50%**	**50%**	**80%**	**60%**
O'Gorman N, Wright D, Rolnik DL, Nicolaides KH, Poon LC. Study protocol for the randomised controlled trial: combined multimarker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE). *BMJ Open* 2016;6: e011801. ¶	**50%**	**50%**	**-**	**60%**
Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med 2017;377: 613-622. *	**60%**	**70%**	**0%**	**50%**
Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M *et al.* ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol* 2017;50: 492-495. †	**60%**	**70%**	**30%**	**60%**
Poon LC, Rolnik DL, Tan MY, Delgado JL, Tsokaki T, Akolekar R, Singh M, Andrade W, Efeturk T, Jani JC *et al.* ASPRE trial: incidence of preterm pre-eclampsia in patients fulfilling ACOG and NICE criteria according to risk by FMF algorithm. *Ultrasound Obstet Gynecol* 2018;51: 738-742.†	**60%**	**50%**	**10%**	**40%**
Wright D, Poon LC, Rolnik DL, Syngelaki A, Delgado JL, Vojtassakova D, de Alvarado M, Kapeti E, Rehal A, Pazos A *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: influence of compliance on beneficial effect of aspirin in prevention of preterm preeclampsia. *Am J Obstet Gynecol* 2017;217: 685 e681-685 e685. ¶¶	**60%**	**50%**	**10%**	**40%**
Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, Leipold G, Akolekar R, Shearing S, De Stefani L *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. *Am J Obstet Gynecol* 2017;217: 585 e581-585 e585. ¶¶	**60%**	**50%**	**10%**	**40%**

Reference	Collection of data	Writing of manuscript	Statistical analysis	Study design
Wright D, Rolnik DL, Syngelaki A, de Paco Matallana C, Machuca M, de Alvarado M, Mastrodima S, Tan MY, Shearing S, Persico N *et al.* Aspirin for Evidence-Based Preeclampsia Prevention trial: effect of aspirin on length of stay in the neonatal intensive care unit. *Am J Obstet Gynecol* 2018;218: 612 e611-612 e616. ¶¶	**60%**	**50%**	**10%**	**40%**
Rolnik DL, O'Gorman N, Fiolna M, van den Boom D, Nicolaides KH, Poon LC. Maternal plasma cell-free DNA in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2015;45: 106-111. †	**70%**	**80%**	**20%**	**50%**
Rolnik DL, da Silva Costa F, Lee TJ, Schmid M, McLennan AC. Association between fetal fraction on cell-free DNA testing and first trimester markers for pre-eclampsia. *Ultrasound Obstet Gynecol* 2018, DOI 10.1002/uog.18993. †	**60%**	**90%**	**80%**	**70%**

Scientific Journal	Impact factor for 2017
† Ultrasound in Obstetrics and Gynecology	5.654
§ Australian & New Zealand Journal of Obstetrics and Gynaecology	1.766
¶ BMJ Open	2.413
* New England Journal of Medicine	79.258
¶¶ American Journal of Obstetrics and Gynecology	5.732

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Manchester Metropolitan University

PhD BY PUBLISHED WORK (ROUTE 1/2): CONTRIBUTION TO PUBLICATIONS

This form is to accompany an application for registration for PhD where the PhD is by Published Work. A separate form should be completed for each publication that is submitted with the proposal and should accompany the RD1 form.

1. The Candidate

First Name(s):	Daniel	Preferred Title:	Mr.
Surname:	Lorber Rolnik		
MMU e-mail address:	DANIEL.LORBER-ROLNIK@stu.mmu.ac.uk	Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Accuracy of competing risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction, methods, results and discussion, together with Prof. Kypros Nicolaides and Neil O'Gorman.

Approximate percentage of contribution:

Neil O'Gorman – 40%

Daniel Rolnik – 40%

Alan Wright, Ranjit Akolekar, Simona Cicero, Deepa Janga, Jacques Jani, Francisca S. Molina, Catalina de Paco Matallana, Nikos Papantoniou, Nicola Persico, Walter Plasencia, Mandeep Singh – 5%

David Wright, Liona Poon, Argyro Syngelaki, Kypros Nicolaides – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Neil O'Gorman		neilmogorman@gmail.com
Kypros Nicolaides		kypros@fetalmedicine.com

5. Statement by Director of Studies/Advisor

I confirm that I have read the above publication and am satisfied that the extent and nature of the candidate's contribution is as indicated in section 4 above.

Signature:	Carol Haigh	Date:	01/06/1
	(Director of Studies/Advisor)		

6. Signature of Faculty Research Degrees Administrator			
Signature:	Chris Wills	Date:	01/06/2018
	(Faculty Research Degrees Administrator)		

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MMU e-mail address:	DANIEL.LORBER-ROLNIK@stu.mmu.ac.uk	Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Multicenter screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison to NICE guidelines and ACOG recommendations

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction, methods, results and discussion, together with Prof. Kypros Nicolaides and Neil O'Gorman.

Approximate percentage of contribution:

Neil O'Gorman – 40%

Daniel Rolnik – 40%

Mercedes de Alvarado, Ilma F. Carbone, Vivien Dutemeyer, Magdalena Fiolna, Alex Frick, Natalia Karagiotis, Sofia Mastrodima, Catalina de Paco Matallana, George Papaioannou, Andrea Pazos, Walter Plasencia – 5%

David Wright, Liona Poon, Argyro Syngelaki, Kypros Nicolaides – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Neil O'Gorman		neilmogorman@gmail.com
Kypros Nicolaides		kypros@fetalmedicine.com

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Signature:	Carol Haigh	Date:	01/06/18
	(Director of Studies/Advisor)		

6. Signature of Faculty Research Degrees Administrator			
Signature:	Chris Wills	Date:	01/06/2018
	(Faculty Research Degrees Administrator)		

Signe

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MMU e-mail address:		Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Accuracy of second trimester prediction of preterm preeclampsia by three different screening algorithms

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate did the statistical analysis and wrote the part of the methods, results and discussion.
Approximate percentage of contribution:
Ahmed Al-Amin – 40%
Daniel Rolnik – 40%
Carin Black, Adrienne White, Caroline Stolarek, Shaun Brennecke – 10%
Fabricio Costa – 10%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Ahmed Al-Amin		ahmed.alamin@hotmail.com
Fabricio Costa		fcosta@monashultrasound.com.au

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Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

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Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Study protocol for the randomised controlled trial: combined multimarker screening and randomised patient treatment with ASpirin for evidencebased PREeclampsia prevention (ASPRE)

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate participated in the trial design. Contributed to the paper writing introduction, methods, and discussion, together with Prof. Kypros Nicolaides and N. O'Gorman.

Approximate percentage of contribution:

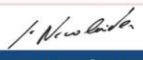
Neil O'Gorman – 40%

Daniel Rolnik – 40%

David Wright, Kypros H. Nicolaides, Liona Poon – 20%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Neil O'Gorman		neilmogorman@gmail.com
Liona Poon		liona.poon@cuhk.edu.hk
Kypros Nicolaides		kypros@fetalmedicine.com

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2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction, methods, results and discussion, together with Prof. Kypros Nicolaides and Liona Poon.

Approximate percentage of contribution:

Daniel Rolnik – 60%

David Wright, Neil O'Gorman, Argyro Syngelaki, Catalina de Paco Matallana, Ranjit Akolekar, Simona Cicero, Deepa Janga, Mandeep Singh, Francisca S Molina, Nicola Persico, Jacques Jani, Walter Plasencia, George Papaioannou, Kinneret Tenenbaum-Gavish, Hamutal Meiri, Sveinbjorn Gizurarson, Kate Maclagan - 10%

Liona Poon, Kypros Nicolaides – 30%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Liona Poon		chiu_yee_liona.poon@kcl.ac.uk
Kypros Nicolaides		kypros@fetalmedicine.com

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Surname:	Lorber Rolnik		
MMU e-mail address:	DANIEL.LORBER-ROLNIK@stu.mmu.ac.uk	Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

ASPRE trial: performance of screening for preterm pre-eclampsia

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction and discussion, together with Prof. Kypros Nicolaides.

Approximate percentage of contribution:

Daniel Rolnik – 70%

David Wright, Liona Poon, Argyro Syngelaki, Neil O'Gorman, Catalina de Paco Matallana, Ranjit Akolekar, Simona Cicero, Deepa Janga, Mandeep Singh, Francisca S Molina, Nicola Persico, Jacques Jani, Walter Plasencia, George Papaioannou, Kinneret Tenenbaum-Gavish- 10%

Kypros Nicolaides – 20%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Kypros Nicolaides		kypros@fetalmedicine.com

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	(Director of Studies/Advisor)		

6. Signature of Faculty Research Degrees Administrator

Signature:	Chris Wills	Date:	01/06/2018
	(Faculty Research Degrees Administrator)		

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PhD BY PUBLISHED WORK (ROUTE 1/2): CONTRIBUTION TO PUBLICATIONS

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1. The Candidate

First Name(s):	Daniel	Preferred Title:	Mr.
Surname:	Lorber Rolnik		
MMU e-mail address:		Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

ASPRE trial: incidence of preterm preeclampsia in patients fulfilling ACOG and NICE criteria according to risk by the FMF algorithm

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction and discussion, together with Prof. Kypros Nicolaides and Liona Poon.
Approximate percentage of contribution:
Liona Poon – 40%
Daniel Rolnik – 40%
Other Co-Authors – 5%
Kypros Nicolaides – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Liona Poon		liona.poon@cuhk.edu.hk
Kypros Nicolaides		kypros@fetalmedicine.com

5. Statement by Director of Studies/Advisor

I confirm that I have read the above publication and am satisfied that the extent and nature of the candidate's contribution is as indicated in section 4 above.

Signature:	Carol Haigh	Date:	01/06/18
	(Director of Studies/Advisor)		

RDPUB, version 1.0, 22/08/2014

6. Signature of Faculty Research Degrees Administrator			
Signature:	Chris Wills	Date:	01/06/2018
	(Faculty Research Degrees Administrator)		

Research and Knowledge Exchange

Graduate School

Form **RDPUB (ROUTE 1 AND 2)**

Manchester Metropolitan University

PhD BY PUBLISHED WORK (ROUTE 1/2): CONTRIBUTION TO PUBLICATIONS

This form is to accompany an application for registration for PhD where the PhD is by Published Work. A separate form should be completed for each publication that is submitted with the proposal and should accompany the RD1 form.

1. The Candidate

First Name(s):	Daniel	Preferred Title:	Mr.
Surname:	Lorber Rolnik		
MMU e-mail address:	DANIEL.LORBER-ROLNIK@stu.mmu.ac.uk	Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

ASPRE trial: influence of adherence on beneficial effect of aspirin in prevention of preterm preeclampsia

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction and discussion, together with Prof. Kypros Nicolaides and D. Wright.

Approximate percentage of contribution:

David Wright – 40%

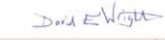
Daniel Rolnik – 40%

Liona Poon, Argyro Syngelaki, Juan Luis Delgado, Denisa Vojtassakova, Mercedes de Alvarado, Evgenia Kapeti, Anoop Rehal, Andrea Pazos, Ilma Floriana Carbone, Vivien Dutemeyer, Walter Plasencia, Nikos Papantoniou– 5%

Kypros Nicolaides – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
David Wright		D.Wright@exeter.ac.uk
Kypros Nicolaides		kypros@fetalmedicine.com

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	(Director of Studies/Advisor)		

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Signature:	Chris Wills	Date:	01/06/2018
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Surname:	Lorber Rolnik		
MMU e-mail address:		Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

ASPRE trial: effect of aspirin in prevention of preterm preeclampsia in women with chronic hypertension

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction and discussion, together with Prof. Kypros Nicolaides and Liona Poon.

Approximate percentage of contribution:

Liona Poon – 40%

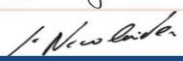
Daniel Rolnik – 40%

David Wright, Argyro Syngelaki, Juan Luis Delgado, Theodora Tsokaki, Gergo Leipold, Ranjit Akolekar, Siobhan Shearing, Luciana De Stefani, Jacques Jani, Walter Plasencia, Nikolaos Evangelinakis, Otilia Gonzalez-Vanegas, Nicola Persico – 5%

Kypros Nicolaides – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Liona Poon		liona.poon@cuhk.edu.hk
Kypros Nicolaides		kypros@fetalmedicine.com

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First Name(s):	Daniel	Preferred Title:	Mr.
Surname:	Lorber Rolnik		
MMU e-mail address:	DANIEL.LORBER-ROLNIK@stu.mmu.ac.uk	Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	18000337

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

ASPRE trial: effect of aspirin on length of stay in the neonatal intensive care unit

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate recruited patients in the UK sites, participated in the data collection, received and managed data from all sites on a weekly basis and participated in the statistical analysis. Contributed to the paper writing introduction and discussion, together with Prof. David Wright and Prof. Kypros Nicolaides. Approximate percentage of contribution:

Daniel Rolnik – 40%

David Wright, Kypros H. Nicolaides – 50%

Other co-authors - 10%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
David Wright	David E Wright	david.wright@plymouth.ac.uk
Kypros Nicolaides	K. Nicolaides	kypros@fetalmedicine.com

5. Statement by Director of Studies/Advisor

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	(Director of Studies/Advisor)		

6. Signature of Faculty Research Degrees Administrator

Signature:	Chris Wills	Date:	01/06/2018

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First Name(s):	Daniel	Preferred Title:	Mr.
Surname:	Lorber Rolnik		
MMU e-mail address:		Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Maternal plasma cell-free DNA in the prediction of preeclampsia

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate collected and analysed the samples, participated in the data collection and in the statistical analysis. Contributed to the paper writing introduction, results and discussion, together with Prof. Kypros Nicolaides and Liona Poon

Approximate percentage of contribution:

Daniel Rolnik – 60%

Neil O'Gorman, Magdalena Fiolna, Dirk van den Boom – 10%

Kypros Nicolaides – 15%

Liona Poon – 15%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Kypros Nicolaides		kypros@fetalmedicine.com
Liona Poon		liona.poon@cuhk.edu.hk

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MMU e-mail address:		Contact Number:	07472803783
Personal e-mail address:	daniel.rolnik2@gmail.com	Student ID Number:	

2. Title of PhD Proposal

Prediction of Preeclampsia and prevention with low-dose aspirin

Title of Research Output

Association between fetal fraction on cell-free DNA testing and first trimester markers for pre-eclampsia

3. Candidate's contribution to the research output

(State nature and approximate percentage contribution of each author)

The candidate has analysed the data and written the manuscript

Approximate percentage of contribution:

Daniel Rolnik – 70%

Other Co-Authors – 5%

Andrew McLennan – 25%

4. Co author(s):

I confirm that the contribution indicated above is an accurate assessment of the contribution by the candidate to the research output named in section 3.

Name	Signature	Current e-mail address
Fabricio Costa		FCosta@monashultrasound.com.au
Andrew McLennan		AMcLennan@sufw.com.au

5. Statement by Director of Studies/Advisor

I confirm that I have read the above publication and am satisfied that the extent and nature of the candidate's contribution is as indicated in section 4 above.

Signature:	Carol Haigh	Date:	01/06/18
	(Director of Studies/Advisor)		

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Signature:	Chris Wills	Date:	01/06/2018
	(Faculty Research Degrees Administrator)		