Predicció de complicacions maternes i fetals en pacients amb Pre-eclàmpsia

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Presentada per

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per a aspirar al grau de "Doctor en Medicina"

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PRESENTATION

The present thesis has been structured following the regulation for PhD theses, as a collection of publications. It was approved by the Comisión de Doctorado de la Facultad de Medicina on the 2009. Projects included belong to the same research line, which resulted in three articles being published in international journals.

Article 1

Prognostic Role of Uterine Artery Doppler in Patients with Preeclampsia

Meler E., Figueras F., Mula R., Crispi F., Bennassar M., Gómez O., Gratacós E.

Fetal Diagn Ther 2010; 27: 8-13

Status: published. Journal Impact Factor 1.90

Article 2

The prognostic role of uterine artery Doppler investigation in patients with severe early-onset PE.

Meler E., Figueras F., Bennassar M., Gómez O., Crispi F., Gratacós E.

Am J Obstet Gynecol 2010; 202:559.e1-4

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Article 3

Role of maternal plasma levels of placental growth factor for the prediction of maternal complications in preeclampsia according to the gestational age at onset.

Meler E., Scazzocchio E., Peguero A., Triunfo S., Gratacós E., Figueras F.

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Table of contents

1-INTRODUCTION

1.1-Relevance of Preeclampsia (PE)

PE is a pregnancy-related condition, classically defined as high maternal blood pressure associated with protein in urine. The prevalence and the impact of PE vary according to the economic resources of the country: the lower the economic status of the country, the higher the prevalence and the impact in maternal and neonatal health. It affects about 2-3% of pregnancies in developed countries and up to 7 times higher in developing countries, and it is a major contributor to maternal and neonatal mortality. Preserving maternal health has become in the 21st century the main goal of maternal public health institutions in order to struggle against poverty and inequality. 99% of maternal deaths occur in low- and middle –income countries and PE and eclampsia is responsible for up to 10-15% of these. Consequently, many efforts have been made to improve the prediction and management of PE.

Globally, PE outcomes are often satisfactory, but it can be devastating and life threatening for both the mother and the foetus.

PE is classified as the third cause of global maternal deaths, with an estimated 100,000 deaths/year worldwide (1). In developed countries, PE is the primary cause of maternal admission to the intensive care unit (2) and the second cause of maternal death(3), after maternal haemorrhage. Maternal morbidity can be significant in impact, especially in cases of severe PE, in terms of renal insufficiency at one year follow-up, cerebral infarction, cardiac arrest or liver failure(4). PE is also associated with a higher rate of caesarean birth(5) and it has been postulated as a cause for postpartum depression associated with the unexpected experience of facing an admission to the intensive care unit or a very premature deliver(6). A contribution of PE to maternal cardiovascular disease in the long term has also been described(7) for a priori healthy patients, in terms of later ischemic heart failure, diabetes or impaired glucose tolerance.

PE is also associated with increased perinatal morbidity and mortality, being responsible for 10% of the six million perinatal deaths (8) and 15% of the eight

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million of premature deliveries worldwide (9). It also accounts for a substantial proportion of the 20 million low birth-weight infants in developing nations. These impacts are more prevalent when access to neonatal intensive care is limited: the infant mortality rate is 3 times higher in low-and middle-income countries than in high-income countries(10). Complications associated with premature delivery include respiratory distress, apnea, jaundice, kernicterus, feeding difficulties, hypoglicemia, seizures and periventricular leucomalacia, all of which usually prolong hospitalization (11).

For all these conditions, an accurate identification of those patients at highest risk of complications remains a goal for modern obstetrics in developed countries. This would lead to a more appropriate management of these high-risk patients with a more intensive strategy and the possibility of home monitoring for those patients with a low-risk of complications.

1.2-Physiopathology of PE

PE is a maternal endothelial disease, which exclusively appears during pregnancy and disappears with the extraction of the placenta, and currently, its physiopathology is not fully understood. It is known that a remodelling of spiral uterine arteries and formation of placental villi play a key role in the normal placental development, the vital structure that will guarantee maternal-foetal exchange functions and blood supply. This remodelling is believed to occur prior to the trophoblastic invasion and may be mediated by renin-angiotensin system and by maternal hormonal factors. There seems to be an associated secretion from the extravillous trophoblast of a wide variety of hormones and growth factors directly into the maternal blood. These factors, including angiogenic factors as Placental Growth Factor (PlGF) and Vascular Endothelial Growth Factor (VEGF), are suspected of being implied in the angiogenesis and vascular remodelling independently to the trophoblastic invasion of the vessel walls(12).

In normal pregnancy, arterial remodelling commences in the first weeks of gestation when the extravillous trophoblast cells from the placenta invade the uterus (Figure 1). The arterial vessel wall loses smooth muscle cells and elastic tissue, resulting in an expansion of the mouth of the arteries as they drain into the intervillous space within the placenta(13). Remodelled arteries will lose their capacity of constriction, ensuring the blood supply to the placenta. In addition, the velocity and pressure of the inflowing blood is significantly reduced, minimizing hemodynamic damage to the delicate placental villi.
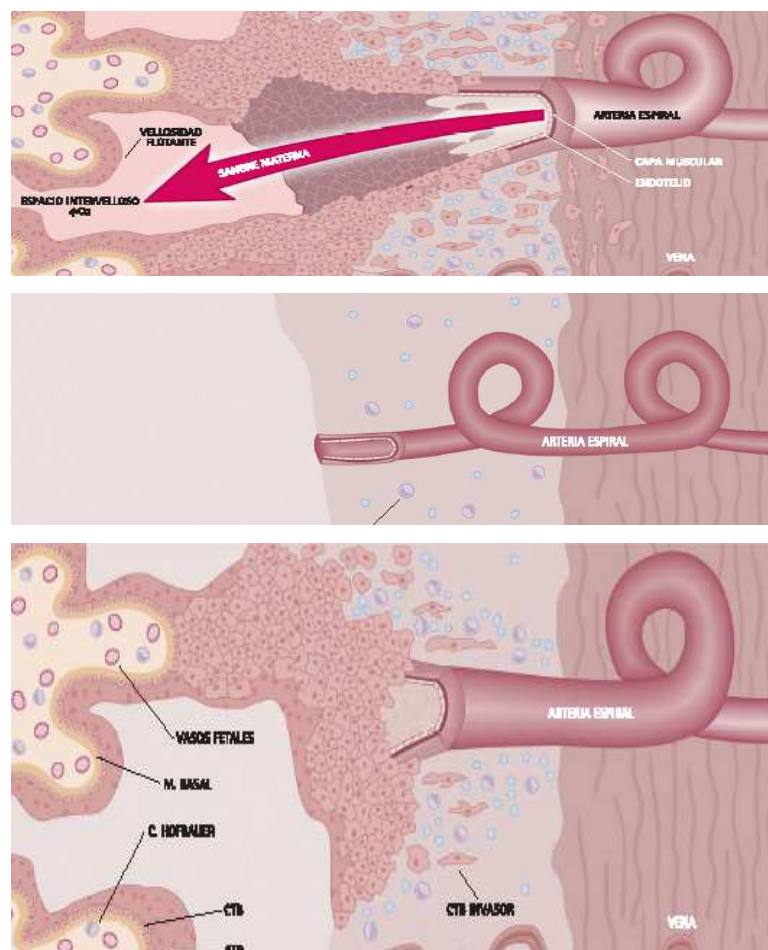
Figure 1. Physiological trophoblastic invasion (by Fàtima Crispi and Col.)
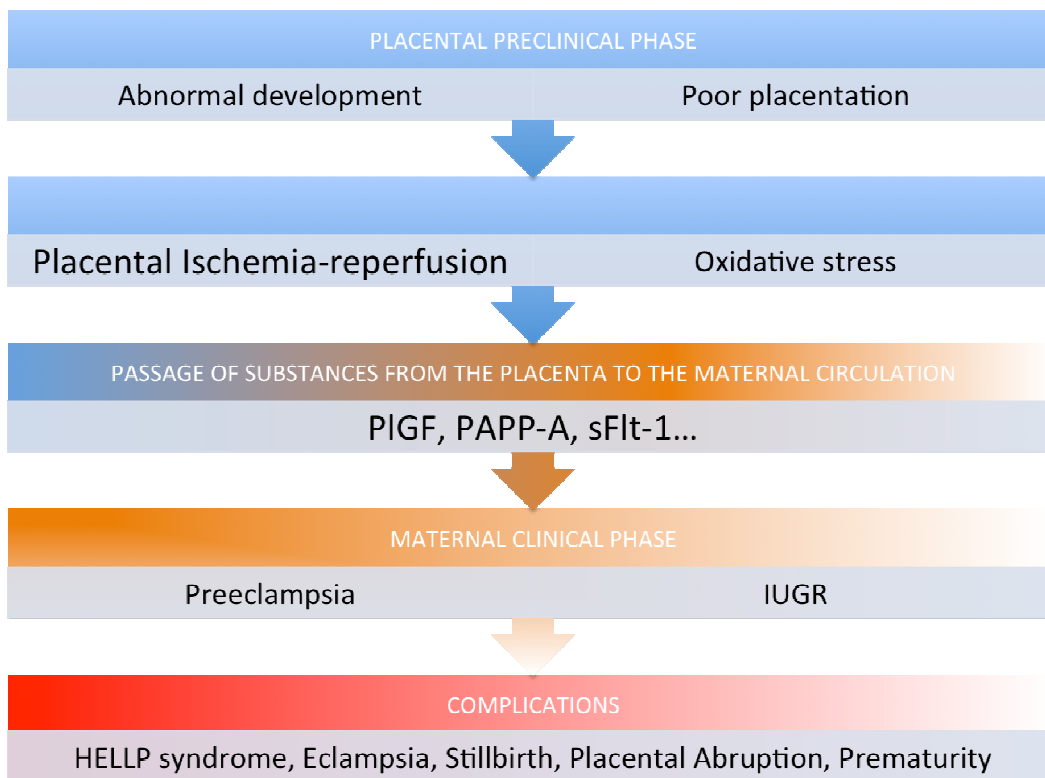
Figure 2. Impaired Trophoblastic invasion and Complications

Nevertheless, when these vascular transformations are incomplete and the transfer of substances between the foetal-maternal units is hindered, placental dysfunction emerges leading to endothelial dysfunction, the baseline of a maternal systemic syndrome called PE (Figure 2).

In the first phase, impaired invasion of trophoblast results in narrower spiral arteries with an increased capacity of constriction. In a second phase, the increase in the velocity of blood entry causes hemodynamic damage and oxidative stress in the intervillous space. Parallel, intermittent repetitive placental underperfusion due to the excessive constriction of the vessel walls, a condition easily studied through Uterine Artery Doppler (14), is said to be the main cause for placental oxidative stress. This condition leads to an ischemia/reperfusion type injury of the placenta (15) and an increased secretion of pro-inflammatory cytokines and angiogenic regulators by the abnormal placenta (16), triggering in consequence a maternal endothelial dysfunction(17). An imbalance between the anti-angiogenic

factors like soluble endoglin (sEng) and the soluble vascular endothelial growth receptor (sFlt-1) and pro-angiogenic factors like placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) may result in generalized endothelial dysfunction (18) leading to hypertensive syndrome and microangiopathy (19-21) in a third and last phase of the disease. Whether this imbalance of anti-angiogenic factors is due to excess of anti-angiogenic molecules or by a reduction in pro-angiogenesis molecules bio-synthesis, is today a matter of debate.

The systemic endothelial disease will sub-clinically impair, through a systemic maternal inflammatory response, certain physiological pathways: the vascular reactivity to modulation of vascular substances, the activation of the coagulation cascade and the increase of capillary permeability (22). Cytokines will inhibit vascular systemic relaxation and will increase the production of factors like Tromboxane, leading to an imbalance among the Tromboxane/Prostacicline ratio and producing the contraction of smooth muscle cells.

The end spectrum of this endothelial cascade will trigger the maternal clinical disease, with maternal hypertension and renal permeability impairment with proteinuria. Other maternal organ dysfunction as hepatic disease, neurological manifestations or haematological complications can also be present.

1.3-Definition and classification

Classical definition of PE according to the International Society Study of Hypertension in Pregnancy (ISSHP) was, up to this year, maternal hypertension defined as a resting blood pressure of ≥ 140/90 mmHg on two occasions at least 4 hours apart, and the presence of proteinuria defined as ≥0.3 g/24 hours or a 2+ urine dipstick, beyond 20 weeks of pregnancy in a previous normotensive woman. ISSHP has recently published an updated definition of PE based on the deeper knowledge about the physiopathology of the disease and the emerging concept that PE may indeed have several subtypes(23). This revised concept defined PE as maternal hypertension after the 20 weeks of gestation and the coexistence of one of the following new onset conditions:

- Proteinuria

- Maternal organ dysfunction: renal insufficiency, liver involvement, and neurological or haematological complications
- Utero-placental dysfunction: intrauterine growth restriction

How to define PE is of utmost importance. Nevertheless another essential concept is the gestational age at the clinical onset of the disease. Some studies have shown that gestational age at diagnosis of PE is associated with different biochemical and clinical features (17, 24) and it is one of the most important parameters in terms of prognosis. Early PE is defined as onset of PE before 34 weeks of gestation and Late PE defined as onset above 37 week. This gestational-age classification would seem more accurate than the classical one, based in the severity of clinical manifestations, and it would better reflect the scope of the disease. Nevertheless, both classifications are appropiated in approximately 80% of early PE cases, where the disease is manifested in a more severe way.

Although the pathophysiology of PE is not yet fully understood, evidence supports the concept that Early and Late PE would be two different entities (25, 26). Certainly, this distinction is not merely an academic issue; it has profound consequences for clinical practice, as disease onset before 32 weeks gestation is associated with a 20-fold increased risk of maternal mortality(22). Similarly, the likelihood of perinatal mortality or severe neonatal morbidity is 16 times higher in early-onset PE(27).

1.3.1-Early PE
An impairment of spiral arteries remodelling and an imbalance in angiogenic factors are believed to be the basis of Early PE. It is almost invariably associated with placental insufficiency because of defective trophoblastic invasion. Abnormal uterine artery Doppler is usually present (26). Early PE can have serious consequences for the mother and the baby and it is often associated with greater risk of foetal growth restriction (28) and higher maternal and perinatal morbidity and mortality (29, 30). Expectant management has demonstrated to improve neonatal outcome in selected cases and to decrease neonatal care intensive unit

admittance and neonatal respiratory distress (31, 32). In consequence, decision-making basically consists of a trade-off between reducing the risks of prematurity by prolonging gestation and minimizing the risks of maternal complications by delivery.

1.3.2-Late PE

Late PE form is fourfold more prevalent and in general, placental involvement is minimally present and in consequence low incidence of uterine artery Doppler impairment is observed. A pre-existing maternal systemic inflammation in a patient with maternal constitutional parameters – principally High Body Mass Index or maternal predisposing diseases for example cardiovascular disease or metabolic syndrome – would trigger a systemic cascade and oxidative stress in the context of a normal placenta. Although the maternal and foetal prognosis is much better, it is still associated with some maternal and foetal morbidity (27).

1.3.3-Severe PE

Severe PE is defined according to the ISSHP by the presence of the following criteria: blood pressure $\geq$ 160/110 mmHg in 2 or more determinations, proteinuria $\geq$5 g/24 hours, or the presence of maternal complications that included (1) eclampsia and other neurologic manifestations (visual disturbances or severe headache that persisted more than 24 hours), (2) HELLP syndrome (lactate dehydrogenase >600 IU/L, aspartate transaminase >62 IU/L, platelet count <100,000 /L), (3) acute renal failure defined as creatinine above 1,2g/dL, (4) subcapsular hepatic hematoma, (5) pulmonary oedema and (6) the presence of disseminated intravascular disease.

Nevertheless, evidence suggests that stratifying women, especially with early onset PE, as high and low risk for adverse events cannot confidently rely upon the classic severity criteria, which are based on non-specific clinical and laboratory markers(33-36).

1.4-Management of early and severe PE

Neonatal morbidity and mortality is inversely related with gestational age at delivery. Delivering below 28 weeks, especially in those IUGR foetuses usually associated with early PE, involves a mortality rate of at least 50% with a high risk of morbidity (37, 38). On the other hand, it is well known that the prognosis of neonates delivered above 34 weeks gestation in a tertiary care centre is excellent with a survival rate of almost 100%. Several studies have demonstrated that expectant management of early and severe cases could reduce neonatal morbidity with a low risk for the mother. Preterm delivery is associated with respiratory distress, necrotizing enterocolitis and intraventricular haemorrhage. Nevertheless, continuing with the pregnancy could worsen maternal clinical state and would expose the foetus to a hostile environment due to the unstable haemodynamics and the consequent risk of placental abruption and intrauterine death. Therefore, the election of when to deliver should be based on objectives and reliable parameters. Recent publications have defended an expectant management for early and severe cases, advocating a reduction of neonatal morbidity without increasing maternal morbidity and mortality(39).

1.5- Complications of preeclampsia and implications for management

PE can potentially result in a devastating disease for the mother and the foetus, forcing intensive care management in those cases at higher risk of complications. Although an appropriate treatment can avoid most of the severe complications, some patients will present them in any case and an appropriate identification of them is of utmost importance. The most prevalent ones are reviewed as follows

1- Eclampsia: It has the highest mortality rate at 1.8%. It is produced because of a vasospasm of cerebral arteries that leads to ischemia and endothelial disease. Its incidence has decreased because of the improvement in the management of these patients and the implementation of preventive treatment with magnesium sulphate. It occurs in 2-3% of severe PE and in 0.6% of mild PE and it presents more frequently before delivery. Persevering headache is present in 70% of the cases. It is important to highlight that almost 4 out of 10 patients with eclampsia neither have hypertension nor

proteinuria when convulsions happen. The presence of eclampsia will force to end up the gestation once the patient and the foetus will be hemodynamically stable.

2- HELLP syndrome: Its clinical presentation depends on the spread of endothelial disease and the deposits of fibrin. It affects 4-12% of those patients with severe PE and it is more frequent prior to the delivery. It is identified when haemolysis, elevation of liver enzymes and thrombocytopenia coexist, but in some cases, some of these criteria can be absent. Anaemia will be due to a mycroangiopathic Haemolysis and schistocytes can be observed. Liver disease will be secondary to the presence of fibrin in the hepatic sinusoids. The end spectrum of this affection will be subcapsular haematoma and liver rupture, resulting in a high-risk situation for the mother. Thrombocytopenia is produced because of platelets destruction and an increased adherence of those to the endothelial vessels. Its presence will be related with a higher incidence of disseminated intravascular coagulopathy (DIC) and placental abruption. There is still controversy whether we should terminate the pregnancy once HELLP syndrome is diagnosed.

3- Pulmonary acute oedema: its prevalence is 2-6% among those patients with severe PE and its origin is multifactorial: decrease in the oncotic pressure because of hemodilution, increase in the vessels permeability and cardiac ventricular dysfunction. This scenario could be impaired with the administration of glucocorticoids for the acceleration of foetal lung maturation. Its presence is frequently associated with other vital organs affections, so that termination of pregnancy will be of vital importance once the patient is stable.

4- Acute renal failure: It affects 1-8% of severe PE and it is frequently related with the presence of haemorrhage or DIC and its presence will lead to the pregnancy being terminated.

1.6- Prognostic challenges in PE

When a PE is suspected, some aspects must be taken into consideration. After initial assessment and stabilization of the patient based on blood pressure control and prevention of eclampsia if necessary, time of delivery is called into question.

For this reason, knowing how the mother and the baby is essential: a check must be made as to whether there is any evidence of a maternal end-organ complication or whether the foetus is growth-restricted and shows signs of foetal compromise. Even though delivery is the only definitive treatment, the goal of clinical management in developed countries, especially in early PE, is to manage the pregnancy expectantly (40), extending gestational age for enough time to decrease the risks of prematurity for the foetus or at least, to provide time for administration of corticosteroids for foetal maturation of lung, digestive system and brain. In developing countries with scarce resources, the identification of patients at highest risk of adverse outcomes could allow the transfer of these patients to a well-equipped hospital for their proper management.

Therefore it is of utmost importance to improve the stratification of women at risk of adverse maternal and foetal outcome. This would allow the establishment of subgroups of patients for surveillance either as outpatients or as inpatients with the possibility of immediate delivery(41, 42).

Current prognostic assessment is based on classical parameters defined by the ISSHP. Severity of maternal hypertension, presence of proteinuria, clinical evaluation and laboratory blood testing would detect maternal organ damage. Nevertheless, several authors have recently defended the concept that maternal symptoms or severe proteinuria should be used cautiously when taking clinical decision as these prognostic tools may have some limitations(35).

Low predictive values of blood pressure for severe adverse maternal and perinatal outcomes would limit its clinical utility(43, 44). Blood pressure can be affected by a variety of factors. Menzies (42) concluded that almost all the criteria defining a severe PE either in the Canadian or the American guideline do not predict adverse maternal nor perinatal outcome, and in consequence its utility, especially in those pregnancies far from term, should be taken with caution. In this studied cohort

among 737 pregnancies, dyspnoea, low platelets below 100×10^9/L, elevated liver enzymes or creatinine and HELLP syndrome were associated with maternal adverse outcome. Only diastolic blood pressure above 110 mmHg and suspicion of placenta abruption were associated with adverse perinatal outcome.

Proteinuria is a non-specific clinical sign, it is affected by the method of measurement and it is a poor predictor of adverse pregnancy outcome(45). The Pre-eclampsia Integrated Estimate of Risk (PIERS) study (35), a multicentre prospective cohort study carried on in 2011 among 2023 pregnant women with the diagnosis of PE, HELLP syndrome or superimposed PE whatever gestational age was, was designed to investigate the maternal risks associated with PE. Its main goal was to create a continuous quality improvement project for women hospitalized with preeclampsia. For that purpose, they collected all the candidate predictive variables, considered as those available, measurable and reliable ones within the first 48 hours after the hospitalization. In this study, proteinuria was not an independent valid predictor of maternal adverse outcome. Indeed, the range to consider severe proteinuria has been modified along the years and nowadays, some guidelines question whether it should be included in the severity criteria list.

Moreover, some of the criteria for severity as HELLP syndrome, although presenting an acceptable association with adverse maternal outcome, have limited capacity of prediction for foetal adverse outcome (46).

The PIERS study has proposed a multi parametric approach of maternal symptoms to assess more accurately maternal outcome. No symptom alone achieved an area under the ROC curve value above 0.7. The integrated model reached an area under the ROC curve of 0.88 to predict adverse maternal outcome in the first 48 hours after hospitalization and above 0.70 in the period between the second and the seventh day after the inclusion with 13% of false positive rate. In those early PE cases, the area under the curve reached 0.85. This algorithm incorporated gestational age at diagnosis, thoracic pain, creatinine levels, platelets concentration and aminotransferase aspartate and oxygen saturation (36).

When assessing for foetal prognosis, clinical maternal parameters perform even worse. The presence of IUGR has been proposed several times as a criteria for severity at onset of PE. Nevertheless, the ISSHP current classification of severity does not contemplate it. Although almost 75% of early PE present with IUGR, foetal growth restriction is much less present in late PE. In that sense, Doppler study has emerged as a useful tool in the evaluation of high-risk patients and there is strong evidence that abnormal individual vessel indices correlate with pregnancy outcomes (47, 48). Specifically, umbilical artery Doppler has demonstrated significant diagnostic efficacy in identifying foetal compromise in pregnancies complicated with PE (49-51), especially when integrated with other current foetal monitoring tests. Scarce data exists about the most frequently altered ultrasound parameters in the pathology (52) and even fewer, relating Doppler findings with foetal prognosis. Umbilical artery would have limited capacity in absence of IUGR.

The limitations in the prediction capacity of these prognostic tools could lead to an increased and often unnecessary intervention in many women. On the other hand, their non-specificity could lead to missed or delayed diagnosis of life-threating disease, resulting in a progression to severe PE or eclampsia.

Consequently, establishing new markers of severity has been identified as a major challenge for PE research in the upcoming years. In that sense, some of the parameters used in the prediction of the disease either at 1st or 2nd trimester have emerged as candidates to better classify those preeclamptic patients and to ensure an appropriate level of clinical surveillance. An evaluation about the placental dysfunction itself, throughout Uterine Artery Doppler or angiogenic biomarkers, has been proposed as a novel approach to the prognosis evaluation of PE.

1.6.1-Uterine Doppler

Third-trimester abnormal uterine artery Doppler has been related to worse perinatal outcomes among patients both with and without pregnancy complications(53). Furthermore, in PE clinical severity has been directly related to the extension of placental ischemia: the larger the ischemia, the more severe the clinical manifestations and the poorer the perinatal outcomes (54). Accordingly, the study of the uterus-placenta unit would seem an interesting parameter for the prognostic assessment of the disease. Few studies have evaluated uterine artery Doppler as a prognostic tool at the onset of PE.

Frusca et al. (55) evaluated uterine Doppler for the prediction of pregnancy outcome in the different Gestational Hypertension entities. Those preeclamptic patients with impaired Doppler presented poorer outcomes than those with normal Doppler evaluation. Furthermore, those patients with gestational hypertension, usually known as a milder affection, who also had abnormal uterine Doppler at diagnosis, presented pregnancy outcomes similar to those preeclamptic patients. Li H. and others (56, 57) concluded that abnormal Doppler is much more frequent at earlier the gestational age of onset of the disease. In that sense, the frequency of abnormal uterine artery blood flow was 87% in pregnancies delivered before 34 weeks, 71% in those delivered at 34-37 weeks and only 28% in term pregnancies.

Later, Ghi et al. (58) evaluated the utility of Uterine Doppler at hospital admission in those preeclamptic patients diagnosed above 34 weeks. Women with late-onset PE showed a higher risk of perinatal complications when abnormal uterine

Doppler was present. Nevertheless, this finding was not related to adverse maternal outcomes. These observations supported the idea that severe PE near term may coexist with low resistance placental circulation and may hence be promoted by factors others than those commonly advocated at earlier stage. Valensise et al (59) reported that patients who were diagnosed with Early PE had higher total vascular resistance and lower cardiac output, while late-onset patients had higher pre-pregnancy BMI, higher cardiac output and lower total vascular resistance.

However, the role of uterine artery Doppler evaluation in the identification of pregnancy at risk of maternal or foetal morbidity with early-onset PE has not been investigated. In that context, our first article evaluated the performance of Uterine Doppler in the prognostic assessment of adverse outcomes. Our second article further explored the performance of Uterine Doppler in early-PE.

1.6.2- Angiogenic factors

As we have described in the pathophysiology, the excess in anti-angiogenic factors produced by the placenta may cause damage to the vasculature and distal organs (60, 61). Karumanchi et al. (62) showed that excess sFlt-1 would mediate the multiple symptoms of PE. Parallel, circulating PlGF levels are much lower in those patients who would develop PE than in normal pregnancies(63). The concentration of circulating PlGF begins to decrease 9 to 11 weeks before the onset of pre-eclampsia, with substantial reductions during the 5 weeks before the onset of hypertension or proteinuria.

In that context, placental growth factor (PlGF) has emerged as a potential tool to be included in diagnostic and prognostic algorithms(64). Mainly produced in the placenta, PlGF induces vasodilatation of uterine arteries, and would therefore contribute to uterine vascular remodelling during pregnancy. This pro-angiogenic marker seems to be a more sensitive and precise predictor of PE than any other single biomarker, as it reflects placental function(65). Low concentrations of PlGF may reflect poor placentation and thus a response to oxidative stress in the placenta, which are mainly present in early PE(66, 67).

The Early-PE has been correlated with a more frequent presence of lesions consistent with utero-placental perfusion and with a lower PlGF (68). In a well-designed prospective study, Chappell *et al.* evaluated the diagnostic accuracy of Triage PlGF in those women presenting 20.0 to 40.6 weeks gestation with suspected PE for predicting PE requiring delivery within 14 days. The test performance had a sensitivity of 95% (86-98%), a specificity of 56% (49-63%) and a negative predictive value of 97% (92-99%). The author concluded that in women presenting suspected PE under 35 weeks' gestation, a low PlGF level would rule in women requiring preterm delivery and high PlGF would rule out preterm delivery within 14 days.(69)

Different assay platforms for the evaluation of PlGF have been commercialised, most of them only measureable in plasma sample. The Triage PlGF test (Alere, San Diego, USA) is a point-of-care fluorescence immunoassay that measures non-complexed biologically active form of PlGF. It has been designed to be compatible with EDTA plasma and whole blood samples. Samples must be frozen to -80ºC. Test results are typically obtained within 15 minutes and can be displayed and printed at the point-of-care. Valid reference ranges for PlGF in normal pregnancies have been published (70). The reportable range is 12-3000 pg/mL. Original cut-off levels based on the 5th centile of a PlGF level in normal healthy pregnant population would not differ significantly from an absolute threshold of 100 ng/ml in women presenting with signs and symptoms of PE before the 35th week of pregnancy (69). Therefore, the Pelican study supports that only two cut-offs (12 and 100 pg/mL) would be required for interpretation of a PlGF measurement in preterm PE:

PlGF concentration	Risk
PlGF<12 pg/mL	Highly abnormal and suggestive of severe placental dysfunction and likely to deliver within the next 2 weeks
PlGF ≥12 pg/mL and <100 mg/mL	Abnormal and suggestive of placental dysfunction with increased risk of preterm delivery
PlGF≥100pg/mL	Normal and suggestive of patients without placental dysfunction and unlikely to require delivery within the next two weeks

Table 1. Risk of PE according to PlGF concentration

Alere Triage® PlGF test, compared to the other platforms as Delfia® PlGF or Elecsys® PlGF presented significantly lower PlGF values among cases (71). In this study published by Benton, mean values for Triage® PlGF and Elecsys® PlGF in those cases with early PE were 12.0 and 62.5 pg/mL respectively (p<0.001) and 14.7 and 90.0 pg/mL in those late PE-onset cases (p<0.001)

Sibiude J. et al. (72) evaluated in a double-blind prospective study the predictive value of Triage for PE and adverse outcomes in patients with suspected PE or IUGR. Those preterm pregnancies below 34 weeks with PlGF level<12 pg/mL at admission experienced severe adverse outcomes in 96% of cases.

Several studies have also been published evaluating other platforms of PlGF that combine pro and anti-angiogenic biomarkers. An elevated sFlt1/PlGF ratio increases the risk of adverse outcomes 47 times (73). The addition of sFlt1/PlGF ratio to classical clinical parameters, for example hypertension and proteinuria, may increase the prediction capacity of complications.

However, this excellent profile of PlGF as a marker of early PE may limit its clinical applicability for prognostic assessment if a high number of women already have very low levels at the onset of PE. The median of PlGF was of 12 pg/mL in women with either early onset or preterm PE (71).

1.6.3-Combination of both parameters

Another approach in the current literature combines Doppler and angiogenic parameters in the prediction of adverse outcomes in preeclamptic women.

Molvarec et al. (74) in an observational retrospective study among 89 pregnant women with hypertensive disorders (12 HELLP syndrome, 19 PE and 17 with superimposed PE) compared the prediction capacity of foetal adverse outcomes for Triage PlGF and foetal Doppler evaluation at umbilical and foetal middle cerebral vessels. PlGF below 100pg/mL identified not only all women with hypertensive disorders who required urgent delivery following an abnormal foetal flow result but also those who had normal Doppler evaluation and needed to be delivered preterm due to pathological CTG or oligohydramnios.

Gómez-Arriaga PL et al.(75)evaluated in a cohort study of early-PE the performance of Uterine Doppler in combination with sFlt-1/PlGF ratio to predict adverse maternal and perinatal outcomes. The addition of these parameters to GA at diagnosis did not improve the predictive capacity of maternal complications. Regarding the perinatal prognosis, the addition of these parameters, especially sFlt-1/PlGF ratio, to GA at diagnosis could improve the prognostic accuracy and could help inform a decision regarding whether to pursue expectant management. Moreover, the antiangiogenic-angiogenic ratio demonstrated a good correlation with the expected time to delivery.

No information exists about what is the performance of these angiogenic parameters according to gestational age at diagnosis. In this context, the aim of the third article was to analyse the performance of PlGF for maternal adverse outcome according to the gestational age at onset.

2-HYPOTHESES

2.1- Main hypotheses

Preeclampsia has biochemical and biophysical features that could help to predict its complications and that could consequently modify its management in order to prevent them.

2.2-Specific hypotheses

* In early onset PE, uterine artery evaluation at the onset of clinical disease could help in the identification of those patients at higher risk of maternal and neonatal complications.

* In those cases with late PE, uterine artery evaluation at the onset of clinical disease could improve the identification of those patients at higher risk of adverse outcomes even better than classical Doppler fetal parameters as umbilical artery or middle cerebral artery.

* In preeclamptic patients, maternal plasma concentrations of placental growth factors could have a limited capacity in identifying those at higher risk of adverse outcomes, especially in early-onset PE.

3-OBJECTIVES

3.1-Main objective

To phenotypically characterize both early and late PE in terms of the uterine artery Doppler and angiogenic profile.

3.2-Specific objectives

* To evaluate the performance of uterine Doppler at onset of PE for the prediction of adverse pregnancy outcomes in early onset preeclampsia.

* To evaluate the performance of uterine Doppler at onset of PE for the prediction of adverse pregnancy outcomes in late onset preeclampsia.

* To evaluate the role of PlGF in the prognostic assessment of early PE according to the gestational age at onset.

4- STUDIES

STUDY 1

Prognostic Role of Uterine Artery Doppler in Patients with Preeclampsia

Meler E., Figueras F., Mula R., Crispi F., Bennassar M., Gómez O., Gratacós E.

Original Paper

Prognostic Role of Uterine Artery Doppler in Patients with Preeclampsia

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Abstract
Objectives: To evaluate the predictive capacity of umbilical, cerebral and uterine artery Doppler in women admitted for preeclampsia (PE). *Methods:* 190 consecutive singleton pregnancies admitted with PE were included. Umbilical, cerebral and uterine artery Dopplers were performed. The association with adverse perinatal outcome was evaluated from 2 ! 2 tables and multivariately by logistic regression. *Results:* A total of 82 (43%) women had an abnormal uterine artery Doppler on admission, being more prevalent in early-onset (! 32 weeks) than in the late-onset PE (62 vs. 27%, p ! 0.05). In both early- and late-onset forms, uterine artery Doppler showed a greater capacity than umbilical and middle cerebral artery Doppler for predicting adverse perinatal outcome. *Conclusion:* Uterine artery Doppler was the best predictive parameter for perinatal outcome in pregnancies with PE and may be included as a primary surveillance test.

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Introduction

Preeclampsia (PE) affects about 2–3% of pregnancies and is a major contributor to maternal mortality with an estimated 50,000 deaths/year worldwide [1]. PE is also associated with increased perinatal morbidity [2] and mortality [3].

In recent years, it has been established that early- and late-onset PE are associated with different biochemical and clinical features [4]: whereas the early-onset form is almost invariably associated with placental insufficiency and growth restriction, the late-onset form is more prevalent and, in general, placental involvement is minimally present. In patients with early-onset PE, expectant management improves neonatal outcome in selected cases, decreasing neonatal care intensive unit admittance and neonatal respiratory distress [5, 6]. Also in selected cases, mild PE could be managed in an outpatient regimen until term [7], providing a reassuring maternal and fetal assessment. While the criteria of maternal risk on which the selection of cases for expectant management could be based are well defined [7, 8], fetal criteria are not as well established.

Umbilical artery (UA) and middle cerebral artery (MCA) Dopplers are standard parameters in the manage-

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ment of pregnancies at risk of placental insufficiency [9]. Uterine artery (UtA) Doppler has been widely studied for the prediction of PE and intrauterine growth restriction, reflecting the involvement of a defective trophoblastic invasion. However, it has scarcely been evaluated as a prognostic tool at the onset of PE [10–12]. Only recently, it has been reported that women with late-onset PE show a higher risk of perinatal complications if uterine resistance is increased [13]. Also, preeclamptic women with abnormal uterine flow are at higher risk of recurrence during their next pregnancy [14]. The role of UtA Doppler in identifying pregnancies at risk of fetal morbidity in both early and late forms of PE has not been investigated. It is also unknown if its prediction capacity is superior to that of UA and MCA Doppler.

This study aimed to evaluate the prediction capacity of UA, MCA and UtA Doppler in women admitted for PE.

Material and Methods

Population

Between January 2002 and December 2008 a cohort was created of 190 women with singleton pregnancies and PE who were admitted to a referral hospital in Barcelona (Spain). PE was defined according to the International Society Study of Hypertension in Pregnancy as a resting blood pressure of $\geq 140/90$ mm Hg on 2 occasions at least 4 h apart, and the presence of proteinuria (≥ 0.3 g/dl) or a 2+ urine dipstick, beyond 20 weeks of pregnancy in previously normotensive women.

Measurements

On admission, all women underwent blood and urine workup according to current recommendations [7, 8]. Doppler examination was also performed on hospital admission using a Voluson 730 Pro or Voluson 730 Expert (GE Medical Systems, Milwaukee, Wisc., USA) equipment. All the scans were performed by 1 of 6 experienced observers. UtA Doppler was carried out by identifying the vessel in an oblique scan with the sample volume distal to the crossing with the external iliac artery. Pulsatility indexes (PIs) of the left and right arteries were measured and the mean PI was calculated. The UA Doppler flow spectrum was recorded from a free-floating portion of the umbilical cord. The MCA Doppler was recorded in a transverse view of the fetal brain, with the Doppler gate placed on the vessel about 1 cm distal to the circle of Willis. In all these vessels, the pulsed Doppler gate was placed over the whole width of the vessel once it had been ensured that the angle was <30°. Angle correction was then applied and the signal updated until 3 similar consecutive waveforms were obtained. Gestational age was calculated according to the crown-rump length at first trimester ultrasound [15]. The UtA Doppler investigation on admission and the last UA and MCA Doppler investigation within 1 week before delivery were considered for the analysis.

Definitions

Early PE was defined as that diagnosed before 32.0 weeks. Severe PE was defined as a blood pressure of $\geq 160/110$ mm Hg on 2 or more determinations, proteinuria of ≥ 5 g/24 h or the presence of maternal complications, including eclampsia and other neurological manifestations, HELLP syndrome (lactate dehydrogenase >600 IU/l, aspartate transaminase >62 IU/l, platelet count (10^9/l) <100,000), acute renal failure defined as creatinine >1.2 g/dl, subcapsular hepatic hematoma, pulmonary edema and the presence of disseminated intravascular disease. Small-for-gestational age (SGA) was defined as a birth weight <10th centile according to local customized curves [16]. Adverse perinatal outcome was defined as the presence of at least one of the following: fetal or neonatal demise, acidosis at birth (UA pH <7.10 and base excess >12 mEq/l), 5-min Apgar score <7, and admission to the neonatal intensive unit for more than 10 days.

Management

Magnesium sulfate seizure prophylaxis was administered to all women with severe PE, as well as first and second-line antihypertensive therapy with labetalol and hydralacine, respectively, when blood pressure was persistently $\geq 160/110$ mm Hg. Corticosteroid therapy for fetal lung maturity was administered to all pregnancies less than 34 weeks of gestational age. During admission, maternal blood pressure was recorded several times per day and laboratory testing at least twice a week. Fetal assessment was performed by daily fetal heart rate monitoring and Doppler at least every 3 days. Indications for delivery were severe PE beyond 32 weeks once pulmonary maturation was completed, uncontrollable blood pressure, maternal complications (defined above), abruptio placentae or decelerative fetal heart rate (>5 decelerations of more than 30 beats/min from basal line in 30 min). In addition, beyond 28 weeks indications for delivery also included UA Doppler with absent reversed end-diastolic velocities or persistent (>12 h apart) ductus venosus Doppler with absent or reversed atrial flow. Women without severity criteria [7] were discharged and outpatient management with weekly fetal and maternal assessment was performed. In cases with mild PE, delivery was induced after 37 weeks.

Statistical Analyses

Doppler parameters were transformed into z values for gestational age [17, 18]. Best cutoffs were chosen by means of receiver operator characteristics (ROC) curve analyses. Sensitivity, specificity and positive and negative likelihood ratios (LHRs) for the prediction of adverse outcome were calculated. Multivariate analysis for the occurrence of adverse perinatal outcome was performed by logistic regression. MedCalc 8.0 (MedCalc Software, Belgium) and SPSS 14.0 (SPSS Inc., USA) were used for the statistical analyses.

Results

Table 1 details the basal characteristics of the study population at admission. In 85% (162/190) of the patients, the criteria of severity were met; and in 44% (84/190) the clinical onset was before 32 weeks. Among the early-on-

set cases, 96% fulfilled the criteria of severity, whereas only 76% of the late-onset cases fulfilled them (p < 0.05).

Table 2 depicts the perinatal outcome. SGA was found in 120 neonates (63.2%), with a higher incidence in early-onset PE (85 vs. 44%, p < 0.05). A total of 51 (26.8%) infants had adverse perinatal outcomes, including non-exclusively 12 perinatal deaths, 9 cases with 5-min Apgar score of <7, 21 cases of UA pH <7.10, and 20 cases that required admission to the neonatal intensive care unit for more than 10 days. Early-onset cases showed a nonsignificant trend to a higher incidence of adverse outcome (33.3 vs. 21.4%, p = 0.06).

ROC curve analysis showed that whereas the area under the curve was 0.69 (95% CI 0.60–0.78; p < 0.001) for UtA PI, it was 0.59 (95% CI 0.50–0.69; p = 0.06) and 0.58 (95% CI 0.48–0.68; p = 0.09) for UA PI and MCA PI, respectively. The best cutoff for UtA PI was the 97.5th centile, and the 95th centile for UA PI and MCA PI. Overall, a total of 82 (43%) women had UtA PI >97.5th centile on admission. While this proportion was 62% (54/87) among early-onset PE, it was 27% (28/103) in cases with late-onset. Table 3 details the performance of Doppler parameters in predicting adverse perinatal outcome in both early- and late-onset clinical forms. Importantly, in both early- and late-onset forms, abnormal UtA Doppler showed a higher sensitivity and greater capacity for ruling in (+LHR) and out (–LHR) the occurrence of adverse outcome.

Multivariate regression analysis including standard severity criteria of PE showed (table 4) that abnormal UtA was the only parameter that significantly and independently predicted adverse perinatal outcome, with an OR of 4.17 (95% CI 1.97–8.81; p < 0.001). On stratification for early- and late-onset clinical forms, the ORs were 3.34 (95% CI 1.05–10.6; p = 0.04) and 5.18 (95% CI 1.63–16.47; p = 0.005), respectively.

Discussion

Our study demonstrates that UtA Doppler on admission for PE is superior to UA and MCA Doppler in identifying those cases at higher risk of adverse perinatal outcome. In addition, we found that none of the standard severity criteria for PE were significantly associated with this adverse outcome.

UtA Doppler is a validated noninvasive surrogate of trophoblastic invasion [19] and placental perfusion [20]. Thus, neonates of preeclamptic women with abnormal

Table 1. Characteristics of the study population

Basal characteristics	
Maternal age, years	31.5 (5.2)
Body mass index	26.9 (7)
Primiparity, %	62.6
Smoking, %	22
Risk factors for preeclampsia	
Body mass index >30, %	38
Diabetes mellitus, %	2.6
Autoimmune disease, %	3.2
Thrombophilia, %	3.7
Dyslipidemia, %	4.7
Previous preeclampsia, %	8.4
Characteristics on admission	
Gestational age at onset, weeks	32.4 (4)
Early-onset preeclampsia (<32 weeks), %	44
Diastolic blood pressure, mm Hg	105 (10)
Systolic blood pressure, mm Hg	172 (20)
24-hour proteinuria, g	2.3 (2.5)
Severity criteria, %	85

Data expressed as mean (standard deviation) or proportions.

Table 2. Perinatal outcome of the study population

Gestational age at delivery, weeks	33.5
Admission to delivery interval, days	9
Birth weight, g	1,833
Emergent CS for fetal distress, %	20
Admission to NICU for >7 days, %	20
SGA, %	63.1
Acidosis at birth, %	11.3
5-Min Apgar score <7, %	4.8
Perinatal mortality, %	6.3
Stillbirth, %	2.1
Neonatal death, %	4.3

Data expressed as mean (standard deviation) or proportions. CS = Cesarean section; NICU = neonatal intensive care unit; SGA = small-for-gestational age.

UtA Doppler are likely to have been exposed to more severe intrauterine hypoxia secondary to placental insufficiency, which explains the association with adverse outcome. The fact that UtA Doppler was more predictive than UA Doppler could speculatively be explained because the latter has been demonstrated to become abnormal only in advanced stages of placental dysfunction [21, 22]. Also, regional brain perfusion studies in IUGR fetuses showed that brain hypoxia is present long before

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Table 3. Prediction of Doppler parameters for adverse perinatal outcome

	Se	Sp	+LHR	–LHR	OR	95% CI	p
Overall							
Abnormal UtA	67	65	1.93	0.51	3.80	1.92–7.48	<0.001
Abnormal UA	45	63	1.23	0.87	1.42	0.74–2.72	0.29
Abnormal MCA	47	65	1.33	0.82	1.63	0.85–3.13	0.14
Early-onset							
Abnormal UtA	79	47	1.48	0.44	3.34	1.18–9.41	0.02
Abnormal UA	62	41	1.06	0.92	1.16	0.46–2.88	0.76
Abnormal MCA	62	52	1.29	0.73	1.75	0.71–4.36	0.23
Late-onset							
Abnormal UtA	50	79	2.38	0.63	3.77	1.40–10.15	0.06
Abnormal UA	23	79	1.08	0.98	1.1	0.35–3.43	0.86
Abnormal MCA	27	74	1.05	0.98	1.07	0.37–3.1	0.9

Se = Sensitivity; Sp = specificity; LHR = likelihood ratio; CI = confidence interval; OR = odds ratio; UtA = uterine artery; UA = umbilical artery; MCA = middle cerebral artery.

Table 4. Regression analysis of severity criteria for the prediction of adverse perinatal outcome

	OR	95% CI	p
Blood pressure ≥160/110 mm Hg	0.72	0.35–1.49	0.37
Prodromic neurological symptoms	1.53	0.72–3.23	0.27
AST >62 UI/l	0.6	0.19–1.86	0.37
LDH >600 mg/dl	0.94	0.38–2.29	0.88
Creatinine >1.2 mg/dl	0.84	0.07–10.55	0.89
24-Hour proteinuria >5 g/dl	1.67	0.57–4.94	0.35
Platelet count (10^9/l) <100,000/l	0.50	0.14–1.77	0.28
SGA	1.12	0.52–2.42	0.77
Abnormal mean UtA PI	4.17	1.97–8.81	<0.001
Abnormal UA PI	1.65	0.55–2.75	0.38
Abnormal MCA PI	1.73	0.87–2.59	0.25

UtA = Uterine artery; PI = pulsatility index; UA = umbilical artery; MCA = middle cerebral artery; AST = aspartate transaminase; LDH = lactate dehydrogenase; SGA = small-for-gestational age; CI = confidence interval.

significant changes in MCA are observed [23]. In keeping with this, Geerts and Odendaal [24] found that in severe PE, umbilical and cerebral Doppler parameters were not associated with adverse outcome, once adjusted for fetal size. On the contrary, our multivariate analysis that included SGA as a covariate confirmed the significant and independent value of UtA Doppler in predicting adverse outcome. In a group of 115 preeclamptic women, Simanaviciute and Gudmundsson [25] found that cerebral to uterine Doppler better predicted the necessity to deliver

prematurely than abnormal Doppler alone in either the UA or UtA. However, the authors did not report these results separately for early- and late-onset cases. Besides the interval between examination and delivery (mean 4.5 weeks) prevents strong conclusions from being drawn. In line with our results, Ghi et al. [13] found in late-onset PE that abnormal UtA was strongly associated with adverse perinatal outcome, but in this study the comparison of this predictive capacity with that of UA and MCA Doppler was not addressed.

The severity of PE is defined by biochemical and clinical maternal parameters [7], being essentially focused on maternal wellbeing. Interestingly, a survey including 18 experts [26] aimed at identifying tests (among 33 tests which included items of history, examination, and investigations) that could be clinically relevant in predicting maternal and fetal complications in women with PE, revealed that 'ultrasound including Doppler studies', unspecifically, was not rated among the potentially most useful predictors. Consistent with our findings, some previous studies [27, 28] have also suggested that presumed intrauterine stress reflected by the severity of maternal disease did not accurately predict neonatal outcome. It is therefore, of importance to define parameters that could better predict perinatal outcome. In our study, abnormal UtA Doppler at the onset of PE was the only parameter that significantly and independently predicted adverse perinatal outcome.

In keeping with the concept that early- and late-onset PE are associated with different biochemical and clinical features [4], we have found that while more than half

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of the early-onset cases of PE had an abnormal UtA Doppler, only one quarter of the cases of late-onset had this Doppler sign. These findings conferred to the UtA Doppler a different potential role for each clinical form. In early PE, where efforts are made to prolong the pregnancy to allow fetal maturation, the UtA performs better in ruling out the occurrence adverse outcome, allowing the safe prolongation of pregnancy. Our results do not support including abnormal UtA as an indication for early delivery. On the other hand, in late-onset PE, UtA Doppler is better at defining at-risk fetuses in whom the recommended outpatient management for mild PE [7] could pose an unnecessary risk. Ghi et al. [13] have recently reported how in late-onset PE UtA accounts for most cases of adverse outcome.

One of the limitations of our study is that most cases (85%) met the criteria of severity and it could be argued that our sample represents a population of preeclamptic women referred to a tertiary hospital rather than the overall population of preeclamptic women. This could have increased the sensitivity, specificity and LHRs of all the diagnostic tests, but could not explain the differences between them. Another limitation is that second-trimester Doppler was not available for analysis. This could have enabled knowing whether preeclamptic women with abnormal Doppler at onset correspond to those with abnormal flow in the second trimester. This could mean that an adverse outcome secondary to PE could be predicted earlier by second-trimester Doppler. However, Soregaroli et al. [29] reported that about half of the women with abnormal UtA Doppler at 24 weeks had normalization of Doppler indices by 34 weeks. As the proportion of abnormal Doppler in the second trimester [30] is the same as the proportion found in our study at clinical onset, one could speculatively conclude that some women convert from normal to abnormal and vice versa during the third trimester.

Our findings contribute to the premises for future studies evaluating management strategies based on UtA Doppler results at the clinical onset of PE.

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STUDY 2

The prognostic role of uterine artery Doppler investigation in patients with severe early-onset PE.

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OBSTETRICS

The prognostic role of uterine artery Doppler investigation in patients with severe early-onset preeclampsia

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OBJECTIVE: The purpose of this study was to evaluate the prediction capacity of uterine artery Doppler investigation for maternal and neonatal complications in women who are admitted with severe early-onset preeclampsia.

STUDY DESIGN: A uterine artery Doppler examination was performed at admission for patients with severe early-onset (<34 weeks of gestation) preeclampsia. The maternal and neonatal outcome of women with abnormal uterine Doppler results was compared with those with normal Doppler results.

RESULTS: One hundred twenty patients were included. In 53% of them, uterine Doppler results were abnormal. This group had a lower gesta-

tional age at delivery (30.2 vs 32.7 weeks; $P < .001$) and a higher proportion of small-for-gestational age infants (87.5% vs 67.9%; $P = .009$). Neonatal (40.6% vs 14.3%; $P = .01$) and maternal (28.1% vs 5.4%; $P = .001$) complications were more common in the abnormal uterine Doppler group.

CONCLUSION: Women with severe early-onset preeclampsia are at higher risk of maternal and neonatal complications if abnormal uterine blood flow is present.

Key words: early-onset preeclampsia, uterine artery Doppler investigation

Preeclampsia affects approximately 2-3% of pregnancies and is a major contributor to maternal death, with an estimate of 50,000 deaths a year worldwide.[1] In developing countries, preeclampsia is the second most common cause of maternal death[2] and the first cause of maternal admission to intensive care units.[3]

We and others have shown that early (developing at <34 weeks of gestation)

and late-onset preeclampsia are associated with different biochemical and clinical features.[4,5] Although the early-onset form is almost invariably associated with placental insufficiency and growth restriction because of defective trophoblastic invasion, the late-onset form is more prevalent; in general, placental involvement is minimally present. In patients with early-onset preeclampsia, expectant management improves neonatal outcome in selected cases and decreases neonatal care intensive unit admittance and neonatal respiratory distress.[6,7]

Uterine artery Doppler evaluation has been studied extensively for the prediction of preeclampsia and intrauterine growth restriction that reflects the involvement of a defective trophoblastic invasion. However, it has scarcely been evaluated as a prognostic tool at the onset of preeclampsia.[8,9] Only recently has it been reported that women with late-onset preeclampsia show a higher risk of perinatal complications when the uterine blood flow is abnormal.[10] The role of uterine artery Doppler evaluation in the identification of pregnancies that are at risk of maternal or fetal morbidity with early-onset preeclampsia has not been investigated.

This study was aimed to evaluate the prediction capacity of uterine artery Doppler investigation for maternal and neonatal complications in women who are admitted with severe early-onset preeclampsia.

MATERIALS AND METHODS
Population
Between January 2002 and December 2008, a cohort was created of singleton pregnancies with severe preeclampsia at <34 weeks of gestation that were admitted consecutively to a referral hospital (Barcelona, Spain). Exclusion criteria included threatened preterm labor, premature rupture of membranes, congenital malformations, and the presence of intrauterine fetal death at admission. The study protocol was approved by the Ethics Committee, and participants provided their written informed consent.

Measurements
At admission all women underwent a blood and urine work-up according to standard recommendations.[11] In addition, a Doppler examination was also performed on admission with a Voluson 730 Pro or Voluson 730 Expert (GE Medical Systems, Milwaukee, WI) equipment. All

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TABLE 1
Clinical characteristics of the study population according to uterine artery Doppler investigation at admission

Variable	Normal Doppler (n = 56)	Abnormal Doppler (n = 64)	P value
Maternal age, y[a]	31.8 ± 5	31.4 ± 5.1	.6[b]
Primiparity, n (%)	33 (58.9)	42 (65.6)	.45[c]
Body mass index >30 kg/m², n (%)	12 (21.4)	10 (15.6)	.41[c]
Previous preeclampsia, n (%)	4 (7.1)	7 (10.9)	.47[c]
Diabetes mellitus, n (%)	3 (5.4)	1 (1.6)	.34[d]
Autoimmune disease, n (%)	2 (3.6)	3 (4.7)	1.0[d]
Thrombophilia, n (%)	3 (5.4)	4 (6.3)	1.0[d]
Gestational age at onset, wk[a]	31 ± 2.4	29.6 ± 2.7	< .00[b]
Mean blood pressure, mm Hg[a]	128.5 ± 11	129.7 ± 12.5	.58[b]
24-hour proteinuria, g[a]	2.9 ± 2.5	3.1 ± 2.8	.78[b]

[a] Data expressed as mean ± SD; [b] Student *t* test; [c] χ² test; [d] Fisher's exact test.

Meler. Uterine artery Doppler investigation and severe early-onset preeclampsia. Am J Obstet Gynecol 2010.

scans were performed by 1 of 5 experienced observers. Uterine artery Doppler examination identified the vessel in an oblique plane, with the sample volume placed distal to the anatomic crossing with the external iliac artery. The pulsed Doppler gate was placed over the whole width of the vessel once it had been ensured that the angle was <30 degrees. Angle correction was then applied, and the signal was updated until 3 similar consecutive waveforms were obtained. Pulsatility indexes of the left and right arteries were measured, and the mean was calculated. Gestational age was calculated according to the crown-rump length at the first-trimester ultrasound examination.[12]

Definitions
Preeclampsia was defined according to the International Society Study of Hypertension in Pregnancy as resting blood pressure ≥140/90 mm Hg on 2 occasions at least 4 hours apart and the presence of proteinuria ≥0.3 g/dL after the 20th week of gestation in previously normotensive women. *Severe preeclampsia* was defined as blood pressure ≥160/110 mm Hg in ≥2 determinations, proteinuria ≥5 g/24 hours, or the presence of maternal complications that included (1) eclampsia and other neurologic manifestations (visual disturbances or severe headache that persisted >24 hours), (2) HELLP (hemolysis, elevated liver en-

zymes, and low platelet count) syndrome (lactate dehydrogenase, >600 IU/L; aspartate transaminase, >62 IU/L; platelet count [10⁹/L], <100,000), (3) *acute renal failure* defined as creatinine >1.2 g/dL, (4) subcapsular hepatic hematoma, (5) pulmonary edema, and (6) the presence of disseminated intravascular disease.

Small-for-gestational age was defined as birthweight <10th percentile according to local standards.[13] *Adverse neonatal outcome* was defined by the presence of any of the following criteria: (1) fetal or neonatal death, (2) acidosis at birth (umbilical artery pH at birth <7.10 and base excess >12 mEq/L), (3) 5-minute Apgar score <7, or (4) significant neonatal morbidity: seizures, intraventricular hemorrhage grade III or less, periventricular leukomalacia, hypoxic-ischemic encephalopathy, necrotizing enterocolitis, acute renal failure (serum creatinine >1.5 mg/dL), or cardiac failure that required ionotropic agents.

Management
Magnesium sulfate seizure prophylaxis was administered to all women; first- and second-line antihypertensive therapy was labetalol and hydralazine, respectively, when blood pressure was persistently ≥160/110 mm Hg. Corticosteroid therapy for fetal lung maturity was also administered at admission. Ma-

ternal blood pressure was recorded several times per day, and laboratory testing was performed at least twice a week. Fetal assessment was performed by daily cardiotocography and Doppler examination at least every 3 days. Indications for delivery were uncontrollable blood pressure, maternal complications (defined earlier), placental abruption, or cardiotocographic decelerations (>5 decelerations of >30 beats/minute from basal line in 30 minutes). Beyond 28 weeks, indications for delivery also included umbilical artery Doppler examination with reversed end-diastolic velocities or persistent (>12 hours apart) ductus venosus Doppler examination with reversed end-diastolic velocity. Elective delivery was performed at >32 weeks of gestation after completion of pulmonary maturation.

Analysis
Doppler parameters were transformed into z-values for gestational age[14,15] and were considered abnormal if they were >2 SDs. Variables were checked for normal distribution with the Kolmogorov-Smirnov test. Comparisons between groups were performed by Student *t*, χ², or Fisher's exact tests. From 2 × 2 tables, odds ratios (with 95% confidence intervals [CIs]) for both maternal and neonatal complications were calculated. The association between abnormal uterine Doppler results and neonatal complications was further adjusted for gestational age at delivery by logistic regression. Likewise, the association between abnormal uterine Doppler results and maternal complications was adjusted for gestational age at admission.

RESULTS
Overall, 129 women fulfilled the inclusion criteria. A total of 9 women (2 of whom met >1 criteria) were excluded because of congenital malformation (n = 1), premature rupture of membranes (n = 2), preterm labor (n = 5), or intrauterine fetal death at admission (n = 3), which left a study population of 120 pregnancies. Table 1 details the clinical characteristics of the study population. There were 11 cases of perinatal death,

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including 3 fetal deaths and 8 neonatal deaths.

In 53% of the cases (64/120), the uterine Doppler examination was abnormal, with a mean z-value of 0.32 and 2.35 in the normal and abnormal uterine Doppler groups, respectively. Among patients with abnormal uterine Doppler results, gestational ages at admission and delivery and birthweight were significantly lower. The proportion of small-for-gestational age infants and abnormal umbilical artery Doppler results was significantly higher in the abnormal uterine Doppler group. Overall, neonatal complications were also significantly more common in the abnormal uterine Doppler group (40.6% vs 14.3%), with an odds ratio of 4.1 (95% CI, 1.67–10.09). After adjustment by gestational age at delivery, the association between abnormal uterine artery Doppler results and neonatal complications remained significant (odds ratio [OR], 2.7; 95% CI, 1.04–7.2). Table 2 shows the perinatal outcome by study group.

No cases of eclampsia, subcapsular hepatic hematoma, or disseminated intravascular disease occurred. Maternal complications were more common in the abnormal uterine Doppler group (28.1% vs 5.4%), with an OR of 6.9 (95% CI, 1.9–25). After adjustment by gestational age at admission, the association between abnormal uterine artery Doppler results and maternal complications remained significant (OR, 6.8; 95% CI, 1.8–25.7). Table 2 shows the perinatal outcome by study group.

COMMENT

It has recently been reported that women with late-onset preeclampsia show a higher risk of neonatal complications if abnormal uterine blood flow is present.[10] Our study not only extends these results into severe early-onset preeclampsia but shows that abnormal uterine Doppler results at onset are also associated with maternal complications.

Uterine artery Doppler examination is a validated noninvasive surrogate of the trophoblastic invasion[16] and placental perfusion.[17] Thus, neonates from preeclamptic women with abnormal uterine artery Doppler results are likely to have been exposed to more severe intrauterine hypoxia that was caused by placental insufficiency. This speculatively explains the poorer neonatal outcome that was observed in our series in the abnormal uterine Doppler group, which is in keeping with previous reports.[8-10] This hypothesis is supported by our findings that most cases in abnormal uterine Doppler findings (78.1%) also had abnormal uterine blood flow.

The conception of preeclampsia as a 2-stage condition is well-established.[5,18] According to this concept, a first stage that is characterized by placental ischemia is followed by a systemic extension that leads to the clinical manifestations of the disease. Although the link between both stages has remained elusive, the syndrome may be initiated by placental factors that enter the maternal circulation and cause endothelial dysfunction that results in the clinical manifestations of this condition.[19,20] Circulating angiogenic factors that are produced by the placenta and that have been demonstrated in vivo to induce preeclampsia have received considerable attention in recent years.[21] We have provided evidence of the strong correlation between these factors and uterine artery Doppler abnormalities.[4] Thus, it could be speculated that abnormal uterine Doppler findings are markers of higher maternal endothelial dysfunction, which could explain the association between abnormal uterine Doppler findings and the maternal complications that were observed in our study.

The severity of preeclampsia is defined by biochemical and clinical maternal parameters.[11] A survey that included 18 experts aimed at identifying tests (among 33 tests that included items of history, examination, and investigations) that could be clinically relevant in the prediction of maternal and fetal complications in women with preeclampsia revealed that "ultrasound including Doppler studies" was not rated among the potentially most useful predictors.[22] However,

TABLE 2
Clinical outcome according to uterine artery Doppler findings at admission

Variable	Doppler finding		P value
	Normal (n = 56)	Abnormal (n = 64)	
Gestational age at delivery, wk[a]	32.7 ± 3	30.2 ± 2.6	< .001[b]
Cesarean delivery, n (%)	47 (83.9)	60 (93.8)	.08[c]
Birthweight, g[a]	1300 ± 677	1150 ± 485	< .001[b]
Small-for-gestational age, n (%)	38 (67.9)	56 (87.5)	.009[c]
Abnormal umbilical artery Doppler finding, n (%)	25 (44.6)	50 (78.1)	< .001[c]
Maternal complication, n (%)	3 (5.4)	18 (28)	.001[c]
HELLP syndrome	1	5	.21[d]
Neurologic manifestations	1	6	.12[d]
Acute renal failure	1	5	.21[d]
Pulmonary edema	0	3	.25[d]
Neonatal complication, n (%)	8 (14.3)	26 (40.6)	.001[c]
Acidosis at birth[e]	4	8	.32[c]
5-minute Apgar score <7[e]	2	9	.04[c]
Significant neonatal morbidity[e]	2	5	.27[d]
Perinatal death	1	10	.009[c]

[a] Data expressed as mean ± SD; [b] Student t test; [c] χ^2 test; [d] Fisher's exact test; [e] Cases of fetal death (n = 3) were excluded from the denominator.

Meler. Uterine artery Doppler investigation and severe early-onset preeclampsia. Am J Obstet Gynecol 2010.

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our findings and those of others[8-10] provide arguments to incorporate uterine Doppler investigation in the management of preeclampsia to identify patients who need more intensive maternal-fetal surveillance. Further studies should be required to define management strategies based on the uterine artery Doppler findings.

One of the strengths of this study is that it included a homogeneous population of early-onset cases with severe preeclampsia. Some previous studies that addressed the role of the uterine artery Doppler findings in women with preeclampsia that arose in the third trimester did not evaluate separately women with early-onset condition.[9] Another strong point of our study is that the prevalence of risk factors for preeclampsia was evaluated with no differences among the study groups, which allowed the establishment of the independent association of uterine Doppler investigation with maternal and neonatal complications. Despite the fact that our sample size compared favorably with previous studies,[10] it remains underpowered to analyze the association of uterine Doppler findings with some individual neonatal and maternal complications. This is, in part, due to the fact that both maternal and fetal complications were uncommon in our series. This could hamper the extrapolation of our results into populations with poorer clinical outcome. Also, a comparable gestational age at admission between those patients with normal and abnormal Doppler findings would have been desirable to evaluate the prognostic role of uterine Doppler findings without possible confounding factors. Adjustment by gestational age at delivery may have accounted for only a part of this confounding. Finally, another limitation is that a comparison of placental disease from women with normal and abnormal uterine Doppler findings was not performed. This would have allowed an insight into the relationship between Doppler abnormalities and clinical outcome.

Our findings build the premise for future studies that will evaluate the impact of uterine artery assessment in the management of early-onset preeclampsia. ∎

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STUDY 3

Role of maternal plasma levels of placental growth factor for the prediction of maternal complications in preeclampsia according to the gestational age at onset.

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ORIGINAL ARTICLE

Role of maternal plasma levels of placental growth factor for the prediction of maternal complications in preeclampsia according to the gestational age at onset[†]

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ABSTRACT

Objective This study aimed to describe the distribution of placental growth factor (PlGF) plasma levels in pregnancies complicated by preeclampsia (PE) according to the gestational age at clinical onset and to assess PlGF's predictive role for maternal complications.

Methods A total of 84 women whose pregnancies were complicated by PE before 37 weeks' gestation were enrolled. According to gestational age at onset, three groups were defined: group I, <28 weeks; group II, 28 to 31^{+6} weeks; and group III, 32 to 36^{+6} weeks. PlGF plasma levels were measured at diagnosis, and their association with maternal complications was investigated. Plasma PlGF levels below 12 pg/mL were designated as very low.

Results PlGF levels were very low in seven (87.5%) of eight women diagnosed before 28 weeks' gestation, 29 (78.4%) of 37 patients diagnosed between 28 and 32 weeks' gestation, and 16 (41%) of 39 cases diagnosed after 32 weeks' gestation. The sensitivity of very low PlGF values for predicting maternal complications was 76.9%, but the false positive rate was 65.5%. Positive and negative predictive values were 34.5% and 76.9%, respectively.

Conclusion The predictive role of a low PlGF level in predicting maternal complications in very early PE is limited because of both its low specificity and low positive predictive value. © 2014 John Wiley & Sons, Ltd.

INTRODUCTION

Preeclampsia (PE) is a leading cause of maternal and neonatal morbidity and mortality. It complicates approximately 2% to 8% of pregnancies, with a trend toward an increase in recent years.[1,2] Although the pathophysiology of PE is not yet fully understood, evidence supports the concept of two different entities.[3] On one end of the spectrum is early PE, characterized by significant placental dysfunction and thus a high risk of intrauterine growth restriction. On the other end is late PE, in which minimal placental involvement is present, and pre-existing maternal systemic inflammation is the most likely pathogenic pathway.[4] Certainly, this distinction is not merely an academic issue; it has profound consequences on clinical practice, as disease onset before 32 weeks' gestation is associated with a 20-fold increased risk of maternal mortality.[5] Similarly, the likelihood of perinatal mortality or severe neonatal morbidity is 16-fold higher in early-onset PE.[6]

In early-onset PE, decision making basically consists of a trade-off between reducing the risks of prematurity by prolonging gestation and minimizing the risks of maternal complications by delivery. Nevertheless, evidence suggests that stratifying women with early-onset PE as high and low risk for adverse events cannot confidently rely upon the classic severity criteria, which are based on nonspecific clinical and laboratory markers.[7–10] Consequently, establishing new markers has been identified as a major challenge for PE research in the upcoming years.[3]

Placental growth factor (PlGF) has emerged as a potential tool to be included in prognostic algorithms.[3] Mainly produced in the placenta, this pro-angiogenic marker seems to be a more sensitive and precise predictor of PE than any other single biomarker, as it reflects placental function.[11] Low concentrations of PlGF, detected even 5 weeks before clinical manifestations of the disease,[12] would reflect poor placentation and thus a response to oxidative stress in the placenta (which are mainly

present in early PE.[13,14] In a well-designed prospective study of early-onset PE, Chappell et al.[15] found that PlGF could aid decision making by reliably determining which women required delivery within 14 days of admission. However, this excellent profile of PlGF as a marker of early PE may limit its clinical applicability for prognostic assessment if a high number of women already have very low levels at the onset of PE.

The aims of the present study were to describe the distribution of PlGF plasma levels according to the gestational age at clinical onset of PE and to assess the role of PlGF as a predictor of maternal complications.

METHODS

Population

A prospective cohort was created of singleton pregnancies complicated by PE before 37 weeks, which were consecutively admitted to a referral hospital in Barcelona, Spain, between September 2011 and September 2012. Exclusion criteria included threatened preterm labor, premature rupture of membranes, congenital malformations, and intrauterine fetal death at admission. The study protocol was approved by our institution's ethics committee, and participants provided their written informed consent. Gestational age was calculated according to the crown–rump length on the first-trimester ultrasound examination.[16] Gestational age at onset was stratified into three groups: group I, <28 weeks; group II, 28 to 31^{+6} weeks; and group III, 32 to 36^{+6} weeks.

Definitions

Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy definition: new onset of hypertension after 20 weeks' gestation, defined as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher on at least two occasions 4 h apart, plus proteinuria of 300 mg or more in 24 h.

Severe PE was defined as a blood pressure ≥160/110 mmHg on two or more determinations, proteinuria ≥5 g/24 h, or the presence of maternal complications, including the following: (i) eclampsia; (ii) HELLP syndrome (lactate dehydrogenase >600 IU/L, aspartate transaminase >62 IU/L, platelet count <100 × 109/L); (iii) acute renal failure (creatinine >1.2 mg/dL); (iv) subcapsular hepatic hematoma; (v) pulmonary edema (dyspnea, low oxygen saturation, and compatible chest X-ray findings); (vi) placental abruption; or (vii) the presence of disseminated intravascular disease.

Small-for-gestational age was defined as a birth weight <10th customized percentile, according to local standards.[17]

Management

At admission, all women underwent blood and urine tests according to standard recommendations. Magnesium sulfate seizure prophylaxis was administered to all women on the basis of severity criteria. First-line and second-line antihypertensive therapy with labetalol and hydralazine, respectively, were administered when the blood pressure was persistently ≥160/110 mmHg. Corticosteroid therapy for fetal lung maturity was administered to all women admitted before

34.6 weeks. Maternal blood pressure was recorded several times per day, and laboratory tests were obtained at least twice per week. Fetal assessment was performed by cardiotocography every day and Doppler at least every 3 days. Indications for delivery irrespective of gestational age were uncontrollable blood pressure, maternal complications (as defined earlier), and cardiotocographic decelerations (more than five decelerations of more than 30 beats/min from baseline in 30 min). Beyond 28 weeks, indications for delivery also included umbilical artery Doppler with reversed end-diastolic velocities or persistent (>12 h apart) ductus venousus Doppler with reversed end-diastolic velocity. Elective delivery was performed beyond 32 weeks after completion of lung maturation in all women with severe PE and at 37 weeks (±2 days) in women with mild PE.

Placental growth factor measurements

At admission, a 5-mL blood sample was obtained and deposited into a tube containing ethylenediaminetetraacetic acid. The samples were immediately centrifuged for a minimum of 10 min at 4000 rpm. The supernatant (plasma) was aspirated and aliquoted into cryovials (500 μL each) for storage at −80 °C. Subsequently, samples were assayed for free PlGF using the Triage PlGF test. This test is a fluorescence immunoassay with a measurable range of 12–3000 pg/mL. Concentrations below 12 mg/mL are value-assigned on the basis of the calibration curve, but this value is displayed to the user as a qualitative result '<12 pg/mL' and qualified to very low concentrations, according to the manufacturer's manual. The results were also converted to a Z-score, according to the normal reference ranges of PlGF by gestational age interval published by Saffer.[18]

Statistical analysis

Descriptive statistics were used for the analysis of baseline characteristics. Linear trends across the gestational age at onset were analyzed by Jonckheere's trend test or linear-to-linear tests for continuous and categorical variables, respectively.

All p-values were two-sided, and values <0.05 were considered statistically significant. The software package SPSS 16.0 was used for the statistical analyses.

The study protocol was approved by our institution's ethics committee, 'Comitè d'Ètica en Investigació Clínica del Hospital Clínic de Barcelona', and participants provided their written informed consent.

RESULTS

A total of 84 patients were included in the analysis. There were 8 women (9.5%) with medical history: diabetes mellitus (n = 3), autoimmune diseases (n = 2), pre-existing hypertension (n = 2), and renal disorders (n = 1). Eight (9.5%) patients had PE onset before 28 weeks, 37 (44.1%) between 28 and 31^{+6} weeks, and 39 (46.4%) between 32 and 36^{+6} weeks. Clinical characteristics of the population are displayed in Table 1. Whereas 87.5% (7/8) of the patients with PE diagnosed below 28 weeks met severity criteria, this criteria was met by only 37.8% (14/37) and 23% (9/39) of the patients diagnosed at 28 to 32 and >32 weeks, respectively (linear p = 0.002).

Table 1 Clinical characteristics of the included patients

	Gestational age at preeclampsia onset			
	<28 weeks (n = 8)	28–32 weeks (n = 37)	>32 weeks (n = 39)	p[a]
Height (cm); mean (SD)	161.9 (6.4)	161.3 (5.2)	161.2 (6.2)	0.946
Maternal age (years); mean (SD)	30.9 (6.4)	32.2 (4.5)	32.0 (5.8)	0.825
Primiparity; n (%)	6 (75)	23 (62.2)	21 (53.8)	0.5
BMI (kg/m²); mean (SD)	28.3 (6.8)	24.5 (3.3)	25.3 (4.1)	0.077
GA at admission; mean (SD)	25.7 (1.3)	31.4 (1.6)	35.1 (1)	<0.001
Maximum dBP (mmHg); mean (SD)	128 (11.9)	131 (9.9)	125 (10.3)	0.078
Maximum sBP (mmHg); mean (SD)	220 (16.7)	220 (17.2)	220 (21.4)	0.076
Maximum mean BP (mmHg); mean (SD)	135.5 (12.6)	131.4 (11.0)	125.2 (12.6)	0.043
GA at birth (weeks); mean (SD)	27.7 (1.4)	31.3 (1.7)	34.8 (2.0)	<0.001
Birth weight (g); mean (SD)	687.5 (220.1)	1215.1 (290.4)	1643.6 (438.7)	<0.001
Small-for-gestational age; n (%)	7 (87.5)	25 (67.6)	17 (43.6)	0.006
Maternal complications[b]; n (%)	4 (50)	14 (37.8)	8 (20.5)	0.007
HELLP syndrome	3	7	7	
Pulmonary edema	0	5	0	
Renal failure	0	2	0	
Placental abruption	1	1	1	
Perinatal mortality; n (%)	2 (25)	0	0	0.03
Acidosis at birth; n (%)	0%	3 (8.1)	2 (5.1)	0.96
5-min Apgar score <7; n (%)	0%	5 (13.5)	1 (2.6)	0.39

BMI, body mass index; GA, gestational age; sBP, systolic blood pressure; dBP, diastolic blood pressure; BP, blood pressure; SD, standard deviation.
[a]Jonckheere's or linear-by-linear trend tests.
[b]Complications are listed nonexclusively (e.g. one woman in the 28–32 weeks PE onset group had two different complications).

Figure 1 depicts the PlGF values for each gestational age interval. Overall, PlGF was very low (below 12 pg/mL) in almost two-thirds (61.9%) of the patients (52/84). Stratified by gestational age interval at diagnosis, PlGF was very low in seven of eight patients (87.5%) below 28 weeks, 29 of 37 patients (78.4%) between 28 and 32 weeks, and 16 of 39 patients (41%) above 32 weeks (linear $p = 0.019$). The mean (range) PlGF raw values were 18.4 (12–63.4), 23.1 (12–182.4), and 32.8 (12–255) pg/mL (linear $p = 0.027$) for the <28 weeks, 28 to 32 weeks, and >32 weeks groups, respectively. The mean PlGF Z-scores were as follows (all values are negative numbers): 2.4 (19.9–10.9), 2.1 (6.7–3.2), and 1.8 (3.9–0.7) for these same groups, respectively (linear $p < 0.001$). Among those women with severity criteria ($n = 30$), only one (with a PE onset diagnosed at 32–34 weeks) had PlGF values above 12 pg/mL.

All eight cases diagnosed before 28 weeks were delivered because of the occurrence of a maternal ($n = 3$), fetal ($n = 3$), or both maternal and fetal ($n = 2$) complications. Among those cases diagnosed at 28 to 32 weeks, 27 (72.9%) were delivered for maternal and/or fetal complications, and ten (27%) were electively delivered at 32 to 36[1] weeks for the presence of severity criteria. Among those cases diagnosed at 32 to 37 weeks, 32 (82.5%) were delivered for maternal and/or fetal complications, and seven (17.9%) were electively delivered at 32 to 36[6] weeks for the presence of severity criteria.

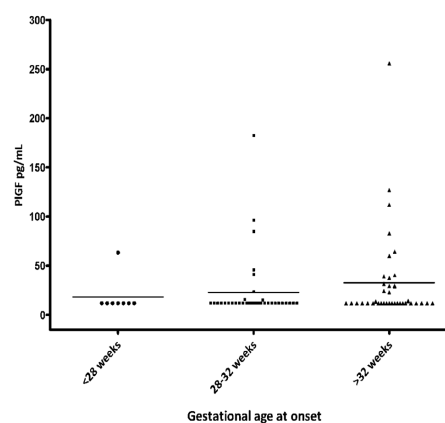Figure 1 Placental growth factor (PlGF) levels (pg/mL) according to gestational age at the onset of preeclampsia

There were 26 women with maternal complications (six of them postpartum); 20 of these women had very low PlGF values, yielding a sensitivity of 76.9 [95% confidence interval (CI), 56–91%]. However, among those women without complications, 65.5% (35/58) also had very low PlGF values,

constituting the false positive rate (95% CI, 51.9–77.5). The positive and negative predictive values were 34.5% (95% CI, 23.4–47.5%) and 76.9% (95% CI, 58.7–88.7%), respectively. The positive and negative likelihood ratios were 1.17 and 0.67, respectively.

DISCUSSION

Our study shows that 80% of patients with PE onset before 32 weeks have very low PlGF plasma levels and that the earlier the diagnosis, the higher the proportion of these very low values. These findings challenge the suggested role of PlGF as a prognostic marker of disease severity.

Despite advances in the understanding of PE, diagnosing this condition still relies on clinical findings (i.e. hypertension and proteinuria) that are poor markers of disease severity.[10] Indeed, there are cases of normotensive PE or PE without proteinuria in which the pathophysiology and risks are similar to those of classic PE.[19] In these instances of incomplete PE, in which either proteinuria or hypertension is not present, or in cases with signs of hemolysis or increased vascular permeability not fulfilling the criteria for PE, PlGF has been shown to identify the subgroup of patients with the highest risk for adverse outcome.[19,20] This suggests a role for PlGF in defining PE when the diagnosis is uncertain. In addition, PlGF has been proposed as a tool to aid in differentiating PE from chronic kidney disease,[21] lupus nephritis,[22] chronic hypertension,[23] and thrombocytopenia.[24] We agree with others that have urged for a redefinition of PE.[3] In this context, our results add to the body of evidence showing that reduced levels of PlGF are a consistent finding in early-onset PE,[25] profiling it as a strong candidate to be incorporated into a more comprehensive definition of the disease.

Furthermore, PlGF may also have a role as a predictor of both maternal[26] and fetal[27] adverse outcomes when the diagnosis of PE has been firmly established. According to our findings, PlGF values are very low before 32 weeks' gestation, regardless of the presence of severity criteria. This is consistent with the findings of Chaiworapongsa et al.,[28] who showed that among women with PE requiring preterm delivery, there were no differences in PlGF MoM values between mild and severe PE. Our findings suggest that decreased PlGF is inherent in early-onset PE and, therefore, can add little prognostic value over the gestational age at onset.

There is convincing evidence of a strong correlation between PlGF circulating levels and uterine artery Doppler abnormalities in PE.[29] It could be speculated that abnormal uterine Doppler is a marker of higher maternal endothelial dysfunction because of the adverse angiogenic profile. Indeed, we and others[30–32] have demonstrated that in patients with PE, uterine artery Doppler at admission identifies women with a higher risk of maternal complications. Espinoza et al.,[32] in a cohort of women in whom PlGF and uterine artery Doppler measurements were obtained at 22 to 26 weeks, found that a combination of both tests predicted the subsequent development of early and severe PE better than each individual parameter. It is unknown whether these findings can be extended to women with diagnosed PE. The combination of both parameters may reduce the high false positive rate we found for PlGF in women with early-onset PE.

It is surprising that we found very low PlGF values in such a large proportion of our patients. In a cohort of 96 women with suspected PE or intrauterine growth restriction, Sibiude et al.[20]

found that only 34 had very low PlGF values. This group used the same methodology for PlGF measurement as we did and also defined very low PlGF values as <12 pg/mL. Their lower proportion may reflect observations that the population of women with suspected PE differs from that of women with diagnosed PE. First, a number of women with suspected PE do not develop the disease. In addition, women with suspected PE are seen in a preclinical and earlier stage of the condition, which may account for a less severe antiangiogenic profile. Thus, our findings do not challenge the role of PlGF as a triage method in cases of suspected PE.

We acknowledge certain limitations of our study. First, there are several commercial PlGF assay platforms that can differentially detect the four different isoforms of the molecule. Indeed, in women with PE, lower PlGF values have been reported using the Alere Triage system than with other widely used platforms.[33–35] This may partly explain the high proportion of very low values found in our study. Second, we did not measure soluble fms-like tyrosine kinase-1 (sFlt-1) levels. The sFlt-1/PlGF ratio has been proposed as an index of antiangiogenic activity that, by reflecting alterations in both biomarkers, confers better prediction of PE[36] and PE requiring preterm delivery[37] than either measure alone. Moreover, sFlt-1 concentrations are directly correlated with the clinical and biochemical severity of PE.[38] It is possible that the sFlt-1/PlGF ratio would allow better resolution than PlGF alone for early-onset PE, but in a series of women with diagnosed PE, all cases with a normal ratio (n = 46) occurred after 32 weeks.[39] In addition, other series involving women with suspected PE demonstrated no benefit of the sFlt-1/PlGF ratio over PlGF alone to predict PE requiring delivery within 14 days (Chappell LC, Personal Communication). In addition, as the platform we used measures free PlGF (i.e. not bound to sFlt-1), it could be argued that it also reflects to a degree the sFlt-1 level. Third, a proportion of women diagnosed after 28 weeks were electively delivered after 32 weeks for the presence of severity criteria. Thus, it could be argued that in them the absence of maternal complications does not necessarily reflect a lack of severe disease, just that delivery often precedes the development of severe disease. Another limitation of our study was that the sample size might have rendered it underpowered to analyze individual adverse outcomes.

According to our study, very low PlGF is a highly prevalent finding in early onset PE, which thereby limits the usefulness of PlGF as a prognostic marker in these pregnancies.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Our manuscript could be the counterpoint to the emerging concept that placental biomarkers could be good predictors of maternal complications at the onset of preeclampsia. Studies had evaluated PlGF as a useful parameter to identify those patients at higher risk of adverse outcomes.

WHAT DOES THIS STUDY ADD?

- According to our results, the predictive role of a low PlGF level in very early PE would be limited as most patients had low concentrations at onset.

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5-RESULTS

5.1-Results from the first study

190 singleton pregnancies were included in this study and 85% (162/190) met the severity criteria. In 44% (84/190), the clinical onset of PE was before 32 weeks. Among the early-onset group, 96% of cases met severity criteria whereas 76% did in the late-onset group. Adverse perinatal outcomes were present in 1 out of 4 of all pregnancies (26.8%) and this prevalence tended to be higher in those early-onset cases.

When comparing the performance of uterine Doppler in predicting adverse perinatal outcome to umbilical artery or middle cerebral artery Doppler performance in patients with PE, the ROC curve analysis was 0.69 (95% CI 0.60-0.78), 0.59 (95% CI 0.50-0.69) and 0.58 (95% CI 0.48-0.68) respectively and uterine Doppler resulted in the only parameter significantly associated with adverse outcomes. The best cut-off for uterine artery pulsatility index (PI) was the 97.5[th] centile and the 95th centile for umbilical artery PI and middle cerebral artery PI. When evaluating the performance of those parameters according to the gestational age at the onset of the disease, those parameters performed much better in those early PE cases with a sensitivity of 79%, compared to 50% for late PE cases. Moreover, uterine artery PI remained the only parameter significantly related with adverse perinatal outcomes.

When comparing in a regression analysis those Doppler parameters to standard clinical and biochemical parameters for the prediction of severity of the disease, the only parameter that significantly and independently predicted adverse perinatal outcomes was uterine artery Doppler, with an OR 4.17 (95% CI 1.97-8.81; p<0.001). When stratifying for early- and late-onset clinical forms, the ORs were 3.34 (95% CI 1.05-10.6; p=0.04) and 5.18 (95% CI 1.63-16.47; p=0.005), respectively.

5.2-Results from the second study

In this study, 120 singleton pregnancies were analysed. Uterine artery Doppler was abnormal in 53% (64/120) of the cases.

Maternal and perinatal outcomes in the subgroup of severe early-onset PE were worse when the patients had an abnormal uterine Doppler at the onset of the disease. Gestational age at delivery was significantly lower in those patients with impaired Doppler (30.2 vs 32.7, p<0.001). The proportion of small-for-gestational age infants and abnormal umbilical artery Doppler was significantly higher in the abnormal uterine Doppler group. After adjustment by gestational age at delivery, those differences remained significant. Neonatal complications were significantly more common, especially in terms of 5-minute Apgar score <7 and perinatal death. Maternal complications in terms of HELLP syndrome, neurologic manifestations, acute renal failure and pulmonary oedema were also significantly more common (28% vs 5.4%, p=0.001) in those pregnancies with impaired Doppler. No cases of eclampsia, subcapsular hematoma or disseminated intravascular disease occurred.

5.3-Results from the third study

A total of 84 patients were included in the analysis. Eight (9.5%) patients had PE onset before 28 weeks, 37 (44.1%) between 28 and 31.6 weeks and 39 (46.4%) between 32 and 36.6 weeks. Earlier the onset of PE was, more frequent criteria of severity were present (linear p=0.002). In that sense, 87.5% of the patients with PE diagnosed below 28 weeks met severity criteria, 37.8% of those diagnosed at 28 to 32 weeks and 23% of those diagnosed above 32 weeks.

PlGF was undetectable in almost two-thirds of the patients, being much more frequent as earlier the onset of PE was. In consequence, PlGF was below 12 pg/mL in 87.5% of those PE diagnosed below 28 weeks, in 78.4% in those diagnosed between 28 and 32 weeks and in 41% of those above 32 weeks. (linear p=0.019).

Only one out of 30 patients with severity criteria did not present with undetectable PlGF values. On the other way, 65.5% (35/58) of patients without complications, also had undetectable PlGF values.

6-DISCUSSION

According to the results of the **first study**, uterine artery Doppler at clinical onset of early-severe PE could identify those patients at higher risk of adverse maternal and perinatal outcomes.

This hypothesis is similar to the one published by Ghi(58), who defended uterine Doppler at diagnosis to predict those patients at higher risk of neonatal complications in late-onset PE. In 2003 Frusca(55) also concluded that patients with gestational hypertension, the mildest affection of the gestational hypertension spectrum, with associated impaired uterine Doppler have similar outcomes to preeclamptic patients.

Uterine Doppler examination is a validated surrogate of the trophoblastic invasion and placental perfusion. Most of our cases (78.1%) with impaired uterine Doppler had also abnormal umbilical artery. The association of abnormal umbilical Doppler with adverse neonatal outcomes has widely been demonstrated in high-risk patients(50).

Although the association of impaired uterine Doppler with adverse maternal outcomes has not previously been described, abnormal uterine evaluation has strongly been related to findings in maternal circulating angiogenic factors compatible with endothelial dysfunction(24), the key parameter for the clinical maternal manifestations of PE. Evaluating angiogenic factors in our cases would have contributed interestingly to our results.

Our study is the first study focusing on a well-identified early onset PE group, homogeneous and with similar risk factors among those patients with normal and abnormal uterine Doppler.

The lower incidence of complications in our population and our acceptable but limited sample size resulted in some weak associations. This fact could constrain the extrapolation of our results into populations with poorer clinical outcomes.

In the **second study**, in which all Doppler parameters were compared for their prediction capacity of adverse outcomes, uterine Doppler at admission resulted to be the best predictive parameter for perinatal outcomes in pregnancies with PE.

Umbilical artery Doppler has been described as the most predictive parameter associated with adverse outcomes in high-risk patients(50). The presence of abnormal umbilical flow is the first step in the cascade of deterioration of IUGR pregnancies. Nevertheless, it becomes abnormal only in advanced stages of placental dysfunction, as it requires an ischemia affecting more than 50% of the placenta to become clinically evident. Middle cerebral artery, which reflects regional brain perfusion, shows in a second term brain hypoxia, but it is also clinically relevant in advanced stages(76). In this scenario, uterine Doppler emerges as a candidate to better predict adverse outcomes in high-risk pregnancies. In the line of our results, Li (57) found that UtA artery pulsatility index was the best indicator of adverse outcomes, followed by umbilical artery pulsatility, in preeclamptic patients. It is notable that a in a recently reported longitudinal series (77), approximately one-third of abnormal third-trimester uterine Doppler studies occurred in women with normal scans during the second trimester, suggesting that a segment of placental disease emerges late in pregnancy. Hence, the potential advantage of a third-trimester uterine Doppler is the ability to detect placental insufficiency of differing pathways.

Standard biochemical and clinical parameters have also been compared to uterine Doppler in the prediction of adverse perinatal outcomes in our second study. According to our results, abnormal uterine Doppler at the onset of PE was the only parameter that significantly and independently predicted adverse perinatal outcome. Doppler study may be even more predictive than blood pressure, maternal proteinuria or clinical prodromic neurological symptoms for the prediction of foetuses at higher risk. Zhang in the year 2001(44) already reported the limited capacity of blood pressure and proteinuria in the identification of perinatal risk but this study did not evaluate the results according to gestational age. More recently, the PIERS study(36) has concluded that maternal symptoms were poorly related to adverse perinatal outcomes. Alterations in biochemical parameters may reflect endothelial dysfunction in different organs of the maternal compartment may but may not be directly related the foetal compartment. Consequently, uterine Doppler could inform as to the affection of both compartments. In the case of impaired uterine Doppler, the risk of adverse

outcomes for the mother and the foetus may be higher. A new definition of PE published this year by the ISSHP(78) has even excluded proteinuria as a sign of severity. It is therefore important to define parameters that could foresee better perinatal outcomes. According to our results, the role of normal uterine Doppler in early-PE is to rule out the occurrence of adverse outcomes, allowing a safe prolongation of pregnancy and in consequence foetal maturation. In late-PE, uterine Doppler identifies those pregnancies at higher risk that would benefit from a more intensive management of the pregnancy.

The higher prevalence of severity criteria among our studied population could have increased the sensitivity and specificity but does not explain the differences between both groups. It would have also been interesting to know the prevalence of abnormal uterine Doppler at second trimester among our patients in order to evaluate whether those patients with persistent impaired Doppler would have worse outcomes than those with onset at third trimester.

Finally in our **third study**, we challenged the suggested role of PlGF as a prognostic marker of disease severity. According to our results, 80% of the patients with PE onset before 32 weeks had very low PlGF plasma levels. Our findings add evidence to the current approach in the diagnosis of the disease. The finding of low concentrations of PlGF maternal plasma levels may help in the diagnosis of PE and could be useful in distinguishing PE from other hypertensive disorders, such as gestational hypertension and chronic hypertension. Knudsen (79) in a case-control study among preterm pregnancies demonstrated high sensitivity and specificity of low PlGF concentrations for the diagnosis of PE.

According to our results, it is evident that the earlier the diagnosis is, the higher the proportion of these very low values is. Wikström (80) found that alterations in maternal plasma levels of angiogenic factors are more frequent and more pronounced in early-PE compared to late-onset PE: the median plasma concentration of PlGF was 21 and 5 times lower respectively. Nevertheless, our study is the first one to stratify the prevalence of low concentrations of PlGF in early-PE according to gestational age.

Furthermore, PlGF has also been proposed as a candidate to improve the prediction of both maternal and foetal adverse outcomes when the diagnosis has been firmly established. According to our results, PlGF values are very low before 32 weeks' gestation regardless of the presence of severity. These findings suggest that decreased PlGF is inherent in early-onset PE and therefore would add little prognostic value over the gestational age at onset. Chaiworapongsa (20) showed that in patients with early-PE, no significant differences in PlGF concentrations were found between mild and severe PE. Similarly, Sibiude (72) found that almost all patients (24 out of 25) with PE below 34 weeks and with severity criteria had PlGF concentrations below 12 pg/mL.

Gómez-Arriaga (75) concluded that even if gestational age at onset was the best predictor of maternal and perinatal adverse outcomes, uterine Doppler in combination with angiogenic factors could slightly improve its performance. The combination of both parameters may reduce the high false positive rate. According to our two previous studies and to some other studies corroborating our results, uterine Doppler at diagnosis would identify women at higher risk of maternal complications. Moreover, some evidence has been published about the relationship between impaired uterine Doppler and angiogenic factors. It could be speculated that abnormal uterine Doppler is a marker of higher maternal endothelial dysfunction because of the adverse angiogenic profile. A recent study by our group has found that both uterine Doppler and maternal circulating angiogenic factor contribute in the prediction of placental underperfusion(81), which, in turn, is a placental pattern contributing to adverse perinatal outcomes(82).

Some limitations of our study could be mentioned. PlGF can be measured by different assay platforms and the Alere Triage system has demonstrated lower values than other platforms. This could explain the high proportion of very low values found in our study. The sFlt-1/PlGF has been proposed as a candidate even better that PlGF alone for the prediction of adverse outcomes in preeclamptic patients. In our study, we did not measure sFlt-1 and this could allow better resolution than PlGF alone. Nevertheless, some series involving patients with

suspected PE demonstrated no benefit of the sFlt1/PlGF ratio over the PlGF alone to predict PE requiring delivery within 14 days.

It is evident that a better identification of preeclamptic patients at higher risk of adverse outcomes is of utmost importance in order to manage more accurately the disease. Uterine Doppler could perform effectively as a predictor of adverse outcomes, in early and late PE. The role of angiogenic factor in the prediction of adverse outcomes needs to be more clearly defined, but it shows a potential in late preeclampsia.

7-CONCLUSIONS

1- Uterine Doppler was the best predictive parameter for perinatal outcomes in pregnancies with PE and it was even more effective than classical clinical parameters

2- Uterine Doppler should be incorporated in the management strategy of PE at the clinical onset of the disease

3- Early-onset preeclamptic patients with impaired uterine Doppler are at higher risk of maternal and neonatal complications.

4- Uterine Doppler may help in the prognostic evaluation of early-PE and should be incorporated in the management strategy at its clinical onset

5- Very low PlGF is a highly prevalent finding in early onset PE leading to its low specificity and low positive predictive value

6- The predictive role of a low PlGF level in predicting maternal complications in very early PE is limited

To conclude, the study of the uterus-placenta unit would seem an interesting parameter for the prognostic assessment of the disease.

Future studies based in the combination of angiogenic factors and Doppler measurements could improve the prediction of complications in patients with PE.

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