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Identification of Patients at Risk for Early Onset and/or Severe Preeclampsia With the Use of Uterine Artery Doppler Velocimetry and Placental Growth Factor

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Abstract

OBJECTIVE: Preeclampsia has been proposed to be an anti-angiogenic state that may be detected by the determination of the concentrations of the soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and placental growth factor (PIGF) in maternal blood even before the clinical development of the disease. The purpose of this study was to determine the role of the combined use of uterine artery Doppler velocimetry (UADV) and maternal plasma PIGF and sVEGFR-1 concentrations in the second trimester for the identification of patients at risk for severe and/or early onset preeclampsia.

STUDY DESIGN: A prospective cohort study was designed to examine the relationship between abnormal UADV and plasma concentrations of PIGF and sVEGFR-1 in 3348 pregnant women. Plasma samples were obtained between 22 and 26 weeks of gestation at the time of ultrasound examination. *Abnormal UADV* was defined as the presence of bilateral uterine artery notches and/or a mean pulsatility index above the 95th percentile for the gestational age. Maternal plasma PIGF and sVEGFR-1 concentrations were determined with the use of sensitive and specific immunoassays. The primary outcome was the development of early onset preeclampsia (≤ 34 weeks of gestation) and/or severe preeclampsia. Secondary outcomes included preeclampsia, the delivery of a small for gestational age (SGA) neonate without preeclampsia, spontaneous preterm birth at ≤ 32 and ≤ 35 weeks of gestation, and a composite of severe neonatal morbidity. Contingency tables, chi-square test, receiver operating characteristic curve, and multivariate logistic regression were used for statistical analyses. A probability value of $< .05$ was considered significant.

RESULTS: (1) The prevalence of preeclampsia, severe preeclampsia, and early onset preeclampsia were 3.4% (113/3296), 1.0% (33/3296), and 0.8% (25/3208), respectively. UADV was performed in 95.4% (3146/3296) and maternal plasma PIGF concentrations were determined in 93.5% (3081/3296) of the study population. (2) Abnormal UADV and a maternal plasma PIGF < 280 pg/mL were independent risk factors for the occurrence of preeclampsia, severe preeclampsia, early onset preeclampsia, and SGA without preeclampsia. (3) Among patients with abnormal UADV, maternal plasma PIGF concentration contributed significantly in the identification of patients

destined to develop early onset preeclampsia (area under the curve, 0.80; $P < .001$) and severe preeclampsia (area under the curve, 0.77; $P < .001$). (4) In contrast, maternal plasma sVEGFR-1 concentration was of limited use in the prediction of early onset and/or severe preeclampsia. (5) The combination of abnormal UADV and maternal plasma PIGF of <280 pg/mL was associated with an odds ratio (OR) of 43.8 (95% CI, 18.48-103.89) for the development of early onset preeclampsia, an OR of 37.4 (95% CI, 17.64-79.07) for the development of severe preeclampsia, an OR of 8.6 (95% CI, 5.35-13.74) for the development of preeclampsia, and an OR of 2.7 (95% CI, 1.73-4.26) for the delivery of a SGA neonate in the absence of preeclampsia.

CONCLUSION: The combination of abnormal UADV and maternal plasma PIGF concentration of <280 pg/mL in the second trimester is associated with a high risk for preeclampsia and early onset and/or severe preeclampsia in a low-risk population. Among those with abnormal UADV, a maternal plasma concentration of PIGF of <280 pg/mL identifies most patients who will experience early onset and/or severe preeclampsia.

Keywords

gestational hypertension; placental growth factor (PIGF); preeclampsia small for gestational age; soluble vascular endothelial growth factor receptor-1 (sVEGFR-1); uterine artery Doppler velocimetry; vascular endothelial growth factor (VEGF)

INTRODUCTION

Preeclampsia (PE) is a leading cause of pregnancy-related maternal death.¹⁻³ The earlier the gestational age at diagnosis, the higher the risk of maternal death exists.¹ For example, the risk of maternal death is 4 times higher if preeclampsia develops between 32 weeks of gestation than after this gestational age. Thus, the identification of patients at risk for severe and/or early onset preeclampsia followed by prophylactic interventions may prevent or delay the clinical presentation of the disease and/or reduce its severity.

Abnormal uterine artery Doppler velocimetry (UADV)⁴⁻⁸ as well as abnormal maternal plasma concentration of proangiogenic and antiangiogenic factors are risk factors for the subsequent development of preeclampsia.⁹⁻¹⁴ Recently, it has been reported that UADV between 22 and 25 weeks of gestation is the “best test” for the identification of patients destined to develop preeclampsia, compared with biochemical indicators in the maternal plasma, such as markers for (1) lipid peroxidation (F2-isoprostane), (2) total antioxidant capacity of plasma (ferric reducing ability of plasma and uric acid concentrations), (3) antioxidant enzymes in erythrocytes (catalase, superoxide dismutase, and glutathione peroxidase), (4) putative markers for endothelial cell dysfunction (von Willebrand factor, plasminogen activator inhibitor types 1 and 2, and thrombomodulin), and (5) pro- and antiangiogenic factors (placental growth factor [PIGF], vascular endothelial growth factor [VEGF], and soluble vascular endothelial growth factor receptor-1 [sVEGFR-1]).¹⁵ The purpose of this study was to determine whether the maternal plasma concentration of the angiogenic factor PIGF and the antiangiogenic factor sVEGFR-1 in the mid trimester of pregnancy can improve the risk assessment determined by UADV for severe and/or early onset preeclampsia.

MATERIAL AND METHODS

Study design

A prospective cohort study was conducted between January 1998 and April 2004 to examine the relationship between UADV and plasma concentrations of PIGF and sVEGFR-1 in pregnant women. Plasma samples were obtained at the time of ultrasound examination between 22 and 26 weeks of gestation. Preeclampsia was diagnosed in the presence of gestational

hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, 6 hours to 1 week apart) and proteinuria (≥ 300 mg in a 24-hour urine collection or 1 dipstick measurement $\geq 2+$). Patients with preeclampsia were sub-classified as either early-onset (≤ 34 weeks of gestation) or late-onset (> 34 weeks of gestation) disease according to the gestational age at which preeclampsia was diagnosed. *Severe preeclampsia* was defined as severe gestational hypertension (diastolic blood pressure ≥ 110 mmHg) and mild proteinuria, or mild gestational hypertension and severe proteinuria (a 24-hour urine sample that contained ≥ 3.5 g protein or a urine specimen $\geq 3+$ protein by dipstick measurement). Patients with an abnormal liver function test (aspartate aminotransferase > 70 IU/L) and thrombocytopenia (platelet count $< 100,000/\text{cm}^3$) were also classified as having severe preeclampsia. *Small for gestational age* (SGA) neonate was defined as a birthweight of $< 10^{\text{th}}$ percentile for the gestational age at birth, according to the national birthweight distribution of a Hispanic population.¹⁶ Patients with chronic hypertension, multiple pregnancies, fetal anomalies, or chronic renal disease were excluded from the study. All women provided written informed consent before the collection of plasma samples. The collection and use of samples was approved by the Human Investigation Committee of the Sotero del Rio Hospital, Santiago, Chile (an affiliate of the Pontificia Catholic University of Santiago), and the Institutional Review Board of the National Institute of Child Health and Human Development of the National Institutes of Health.

Uterine artery Doppler velocimetry

Five experienced sonographers performed Doppler ultrasound of the uterine arteries at the time of blood sampling using real-time ultrasound equipment (ACUSON 128-XP Acuson Corporation, Mountain View, CA) with a 3.5-MHz or a 5-MHz curvilinear probe. The right and left uterine arteries were identified in an oblique plane of the pelvis at the crossover with the external iliac arteries, and the Doppler signals were sampled. When 3 similar consecutive waveforms were obtained, the pulsatility index of the right and left uterine arteries were measured, and the mean pulsatility index of the 2 vessels was calculated. The presence of an early diastolic notch in the uterine arteries was determined according to the criteria proposed by Bower et al.¹⁷ An *abnormal UADV* was defined as the presence of bilateral uterine artery notches and/or a mean pulsatility index of $> 95^{\text{th}}$ percentile for the gestational age.

Sample collection and human sVEGFR-1 immunoassay

Venipuncture was performed, and the blood was collected into tubes that contained EDTA. The samples were centrifuged for 10 minutes at 4°C and stored at -70°C until assayed. The concentrations of sVEGFR-1 were measured with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The details of the method have been described previously.¹⁸ The inter- and intraassay coefficients of variation for human sVEGFR-1 immunoassay in our laboratory were 4.8% and 6.9%, respectively. The sensitivity of the assay was 17.8 pg/mL. The maternal plasma concentrations of sVEGFR-1 were determined only among patients with abnormal UADV.

Human PIGF assays

A specific and sensitive enzyme-linked immunosorbent assay was used to determine concentrations of PIGF in maternal plasma (R&D Systems). Briefly, human plasma samples were incubated in duplicate wells of the microtiter plates, which had been coated with monoclonal antibodies to human PIGF. During this incubation, plasma PIGF (antigen) binds to monoclonal antibodies of PIGF to form antigen-antibody complexes. After unbound substances were washed away, horseradish peroxidase-conjugated polyclonal antibodies specific for PIGF were added to each well of the microtiter plate. After the second incubation, the unbound antibody-enzyme reagent was removed by repeated washing, and a substrate

solution was added. Color developed in proportion to the amount of PIGF in each well. The intensity of color was measured by a programmable spectrophotometer (Ceres 900 Micro plate Workstation, Bio-Tek Instruments, Winooski, VT). Concentrations of the samples were derived by interpolation of the absorbance readings from a standard curve that was generated with known concentrations of PIGF. Calculated inter- and intraassay coefficients of variation for PIGF immunoassays in our laboratory were 4.60% and 2.27%, respectively. The detection limit (sensitivity) of the assay was 10.7 pg/mL.

Study outcomes

The primary outcome was the diagnosis of early onset preeclampsia and/or severe preeclampsia. Secondary outcomes included preeclampsia, SGA without preeclampsia, spontaneous preterm delivery at ≤ 35 and ≤ 32 weeks of gestation, examination-to-diagnosis interval among patients who developed preeclampsia, and a composite of severe neonatal morbidity that included intraventricular hemorrhage, necrotizing enterocolitis and hyaline membrane disease with sonographic/radiological confirmation. Additional secondary outcomes included abruptio placentae and eclampsia. We have also included non-objective definitions of early onset preeclampsia (such as “preeclampsia requiring delivery at ≤ 34 weeks,”^{19,20}) to provide the basis for comparison with previous reports.

Statistical analysis

Comparisons between proportions were performed with chi-square or Fisher's exact test. Receiver operating characteristic curves were constructed to describe the relationship between sensitivity and the false-positive rate (1-specificity) of plasma PIGF and sVEGFR-1 in the identification of patients destined to develop early onset and/or severe preeclampsia.

Survival analysis was used to compare the examination-to-diagnosis interval in patients who developed preeclampsia, according to the results of the UADV and maternal plasma PIGF concentration. Logistic regression analysis was used to explore the relationship between the occurrence of the outcomes and the following explanatory variables: maternal plasma PIGF concentration, maternal age of >35 years, previous preeclampsia, nulliparity, first trimester body mass index of $>30\text{kg/m}^2$, smoking status, and sample storage time. A power analysis indicated that this study had adequate power ($>90\%$) in determining the role of the combination of abnormal UADV and maternal plasma PIGF concentration in the prediction of the outcomes except for spontaneous preterm delivery at <32 weeks of gestation. The statistical packages used were SPSS software (version 12.0; SPSS Inc, Chicago, IL), MedCalc software (version 7.4.4.1; MedCalc Software, Mariakerke, Belgium), and PASS software (NCSS, Kaysville, UT). A probability value of <0.05 was considered significant.

RESULTS

Prevalence of the outcomes

This study included 3348 patients (52 patients were lost to follow-up evaluations). The prevalence of preeclampsia, severe preeclampsia, and early onset preeclampsia was 3.4% (113/3296), 1.0% (33/3296), and 0.8% (25/3208), respectively. UADV was performed in 95.4% (3146/3296) and plasma PIGF concentrations were determined in 93.5% (3081/3296) of the study population. Early onset preeclampsia developed in 5 patients, severe preeclampsia developed in 13 patients, and both early onset and severe preeclampsia developed in 20 patients (this group does not include the former 18 patients).

Diagnostic indices, predictive values, and likelihood ratios of UADV

An abnormal UADV was present in 11.3% (354/3146) of the study population. The diagnostic indices, predictive values and likelihood ratios for the primary outcomes are displayed in Table I. Logistic regression analysis indicated that an abnormal UADV, between 22 and 26 weeks of gestation, was an independent explanatory variable for the occurrence of preeclampsia, early onset preeclampsia, severe preeclampsia, and SGA without preeclampsia, after an adjustment was made for maternal age of >35 years, previous preeclampsia, nulliparity, smoking, first trimester body mass index of >30 kg/m², maternal plasma PIGF, and sample storage time (Table 2).

Diagnostic indices, predictive values, and likelihood ratios of maternal plasma PIGF concentration

Receiver operating characteristic curve analysis was performed to examine the diagnostic performance of maternal plasma PIGF concentrations in the identification of the patient destined to develop early onset and/or severe preeclampsia; a cut-off of 280 pg/mL was selected. Logistic regression analysis indicated that a maternal plasma concentration of PIGF <280 pg/mL was an independent explanatory variable for the occurrence of preeclampsia, early onset preeclampsia, severe preeclampsia, and SGA without preeclampsia after an adjustment was made for the aforementioned covariates and abnormal UADV. The odds ratio and 95% CI of a low maternal plasma PIGF concentration (<280 pg/mL) in the identification of the outcomes are displayed in Table 2, and the diagnostic indices of a low maternal plasma concentration for the primary outcomes are given in Table 3.

The combination of abnormal UADV and maternal plasma PIGF <280 pg/mL in the identification of the outcomes

Table 4 gives the diagnostic indices, predictive values and likelihood ratios of the combination of abnormal UADV and maternal plasma PIGF <280 pg/mL for the identification of the primary outcomes. This combined approach improved the positive predictive value of an abnormal UADV in the prediction of the primary study outcomes without a significant reduction in the sensitivity. Multivariate logistic regression analysis indicated that the combination of abnormal UADV and maternal plasma PIGF <280 pg/mL was an independent explanatory variable for the outcomes after being controlled for the aforementioned covariates. This parameter combination was associated with an odds ratio of 43.8 (95% CI; 18.48-103.9) and 37.4 (95% CI, 17.6-79.1) to develop early onset preeclampsia and severe preeclampsia, respectively. Thus, abnormal UADV and low maternal plasma concentration of PIGF conferred a much higher risk for the development of early onset and/or severe preeclampsia than abnormal UADV alone (Tables 2 and 5).

Maternal plasma PIGF and sVEGFR-1 in the identification of patients destined to have early onset and/or severe preeclampsia according to the results of the UADV

A sub-analysis indicated that, among patients with an abnormal UADV result, the maternal plasma PIGF concentration contributed significantly to the identification of patients destined to have early onset preeclampsia (area under the curve, 0.80; $P < .001$) and severe preeclampsia (area under the curve, 0.77; $P < .001$; see Figure 1A and 1B). Indeed, 89% of the women (16/18) with abnormal UADV results who had early onset preeclampsia and 84% of the women (21/25) who had severe preeclampsia had a plasma PIGF concentration <280 pg/mL. In contrast, maternal plasma sVEGFR-1 concentration was of limited value in the prediction of early onset (area under the curve, 0.49; $P = .9$) and severe preeclampsia (area under the curve, 0.54; $P = .5$; Figure 1A and 1B).

Among patients with normal UADV, the maternal plasma PIGF concentration did not contribute to the identification of patients destined to develop early onset preeclampsia (area under the curve, 0.56; $P = .6$) or severe preeclampsia (area under the curve, 0.62; $P = .2$).

Demographic and clinical characteristics of the study population, according to UADV and maternal plasma PIGF concentration

Table 6 and Table 7 display the demographic and clinical characteristics of the population as well as the outcomes according to the results of the UADV and maternal plasma PIGF concentration of <280 pg/mL, respectively. There were no differences in gestational ages at ultrasound examination among the study groups. Patients with abnormal UADV and maternal plasma PIGF concentrations <280 pg/mL had a higher frequency of preeclampsia, early onset preeclampsia, severe preeclampsia, SGA without preeclampsia, placental abruption, eclampsia, and a composite of severe neonatal morbidity than both patients with normal UADV results and those with abnormal UADV results and a maternal plasma concentration of PIGF ≥ 280 pg/mL (chi square for trend; $P < .001$).

Sequential screening with UADV and maternal plasma PIGF concentration in the identification of patients destined to develop early onset and/or severe PE

Figures 2 and 3 display the distribution of the study population according to the results of sequential assessment with the use of UADV followed by maternal plasma PIGF determinations in the second trimester. The prevalence of early onset and/or severe preeclampsia among patients with abnormal UADV and PIGF concentrations <280 pg/mL was 11 times higher than among those with abnormal UADV and PIGF concentrations ≥ 280 pg/mL (15.7% [22/140] vs. 1.4% [3/207]; $P < .001$) and 30 times higher than among patients with normal UADV results, regardless of the maternal plasma PIGF concentration (15.7% [22/140] vs. 0.5% [13/2792]; $P < .001$; Figure 3).

Figure 4 displays the distribution of the study population as a result of the sequential determination of maternal plasma concentration of PIGF followed by UADV. This flow diagram was generated because many centers in the United States do not use UADV.

The survival analysis indicated that patients with abnormal UADV and low PIGF have a shorter examination-to-diagnosis interval than those in the other 2 groups (log rank test, 37.9; $P < .001$; Figure 5).

COMMENT

The results of this study indicate that a combination of an abnormal UADV and a maternal plasma PIGF concentration of <280 pg/mL, between 22 and 26 weeks of gestation, identifies patients at a very high risk for preeclampsia, early onset preeclampsia and severe preeclampsia.

These novel observations are consistent with previous reports indicating that a low maternal plasma concentration of PIGF in the first^{9,12} or second trimester of pregnancy^{9-11,21} and abnormal UADV results between 23 and 25 weeks of gestation⁵⁻⁸ are risk factors for the development of preeclampsia. Similarly, a low urine concentration of PIGF between 25 and 28 weeks has been associated recently with a high risk for preeclampsia.¹³

The results presented herein differ from those reported recently,²² indicating a lack of association between abnormal UADV and low PIGF. Differences in sample size, gestational age at ultrasound, and study outcomes may account for these discrepancies. A recent longitudinal study that included 81 patients at risk for preeclampsia reported that the maternal plasma PIGF concentration at 24 weeks of gestation contributed significantly to the prediction of the disease.²³ However, this study did not include enough patients to determine the value

of maternal plasma PIGF for the prediction of early onset preeclampsia, and the authors cautioned that “large prospective cohort studies in unselected women are required to ascertain any clinical usefulness.”²³

The regulation of vascular growth and remodeling, also known as angiogenesis, is considered to be central to normal placental and fetal growth and development.²⁴⁻²⁶ In the human placenta, angiogenesis is biphasic, with peaks at mid gestation and at term as the result of endothelial proliferation early in pregnancy and vascular remodeling in the second half of pregnancy.²⁷ This is consistent with the model of placental angiogenesis proposed by Kingdom et al,²⁸ whereby branching angiogenesis is predominant in the first trimester and is associated with high placental production of VEGF. In contrast, nonbranching angiogenesis is predominant in the third trimester and is associated with a high placental production of PIGF.²⁸

Angiogenesis is regulated by at least three growth factor families, which include VEGFs, angiopoietins, and ephrins.²⁹ Other nonspecific factors that have been proposed to regulate angiogenesis include fibroblast growth factors, transforming growth factors α and β , tumor necrosis factor α , Interleukin-8, hepatocyte growth factor, angiogenin, and members of the Notch family.^{26,30,31} Recent evidence indicates that angiogenesis requires the sequential activation of several receptors (which include Tie1, Tie2, and platelet-derived growth factor receptor β) by ligands in endothelial and mural cells.³² However, VEGF signaling represents a critical rate-limiting step in physiological angiogenesis.³²

VEGFs are a family of dimeric proteins whose members include VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF.³¹ The function of VEGF is to promote survival, migration, and differentiation of endothelial cells as well as to mediate vascular permeability.^{30,31} VEGF exerts its biologic effect through VEGFR-2, whereas the precise function of VEGFR-1 is still a subject of debate. Most investigators believe that VEGFR-1 might not be a receptor that transmits a mitogenic signal, but rather a “decoy” receptor that prevents the binding of VEGF to VEGFR-2.³¹ The decoy function can be performed not only by the transmembrane, but also by the soluble isoform (sVEGFR-1).³¹ An additional mechanism by which sVEGFR-1 may regulate the bioavailability of VEGF is the formation of heterodimers with the VEGF receptors in the cell surface, which abolishes their signal transduction.³³ Thus, sVEGFR-1 is considered an antiangiogenic factor.

PIGF is another ligand for VEGFR-1 that enhances the angiogenic response of VEGF.^{34,35} This has been proposed to be accomplished by (1) intermolecular cross-talk between VEGFR-1 and VEGFR-2 (transphosphorylation and activation of VEGFR-2 following activation of VEGFR-1 by PIGF); (2) PIGF displacement of VEGF from sVEGFR-1, which makes more VEGF available to bind VEGFR-2, and (3) PIGF homodimers that can destabilize inactive heterodimers of VEGFR-2 and sVEGFR-1, which makes more VEGFR-2 available for the formation of functional homodimers.^{34,35}

The results of the current study indicate that both an abnormal UADV and a maternal plasma PIGF concentration of <280 pg/mL are independent factors for the prediction of the outcomes. The predictive value of an abnormal UADV between 22 and 26 weeks of gestation for the occurrence of preeclampsia and early onset preeclampsia in the study population is consistent with previous reports.⁵⁻⁸ However, this study further demonstrates that the combination of abnormal UADV and a maternal plasma PIGF of <280pg/mL in the second trimester confers a much higher risk for preeclampsia and early onset or severe preeclampsia than abnormal UADV alone. Moreover, patients with abnormal UADV results and a plasma PIGF concentration of <280 pg/mL, which represented only 4.5% of the population (140/3146

women), contained 60% of the patients (22/38 women) who developed early onset and/or severe preeclampsia.

The sequential determination of UADV and maternal plasma concentration of PIGF, or vice versa, identifies patients at a very high risk for early onset and/or severe preeclampsia. The flow diagrams in Figures 2 and 3 indicate that centers favoring the use of UADV first can obtain the same results if the determination of maternal plasma PIGF concentrations is offered only to patients with abnormal UADV (approximately 10% of the population), rather than the whole study population. This is because a maternal plasma concentration of PIGF did not contribute to the identification of patients at risk for early onset and/or severe preeclampsia among patients with normal UADV results. Among centers that may favor the determination of maternal plasma concentration of PIGF followed by UADV, the identification of patients at risk for early onset and/or severe preeclampsia can be accomplished if UADV is offered only to those with a plasma concentration of PIGF of <280 pg/mL. However, this will require offering UADV to about one-half of the population.

It is noteworthy that patients with abnormal UADV results and a plasma PIGF of <280 pg/mL also had a higher proportion of spontaneous preterm delivery than patients with normal UADV results and those with abnormal UADV results and a PIGF concentration of ≥ 280 pg/mL. However, the difference did not reach statistical significance. This observation is consistent with a growing body of evidence showing that chronic uteroplacental ischemia may represent the mechanism of disease in a subset of patients with preterm delivery.³⁶⁻³⁸ Additional studies, with larger sample sizes, may be required to determine the risk of spontaneous preterm birth among patients with abnormal UADV results and a low PIGF in the second trimester.

sVEGFR-1 has recently been implicated recently in the pathophysiologic condition of preeclampsia.^{11,18,39} Indeed, clinical and experimental evidence indicates that a high maternal plasma concentration of sVEGFR-1 in patients with preeclampsia is associated with a reduction in the bioavailability of the free form of VEGF and PIGF,³⁹ with the subsequent endothelial cell dysfunction. The observation that maternal plasma concentration of sVEGFR-1 among patients with abnormal UADV results were of limited value in the prediction of early onset and/or severe preeclampsia is consistent with recent reports that the elevation of this antiangiogenic factor occurs rather late in the course of the disease (approximately 5 weeks before the clinical presentation of preeclampsia).^{13,14} We did not determine the maternal plasma concentration of sVEGFR-1 among patients with normal UADV results, given that sVEGFR-1 did not improve the diagnostic indices of an abnormal UADV (Figure 1).

An Abnormal UADV result between 22 and 26 weeks of gestation is considered to be a surrogate marker of chronic uteroplacental ischemia. Evidence in favor of this view includes the following observations: (1) embolization of the uterine arterioles and spiral arteries with Gelfoam particles in pregnant animals reduced the uterine blood flow and increased the uterine artery pulsatility index in a dose dependant manner^{40,41}; (2) an abnormal UADV result is associated with failure of physiologic transformation of the myometrial segment of the spiral arteries in placental bed biopsy specimens obtained from patients with preeclampsia and intrauterine growth restriction⁴²⁻⁴⁷; and (3) an abnormal UADV result is associated with placental ischemic/hypoxic changes (excessive syncytial knots, proliferation of villous cytotrophoblast cells, intervillous fibrin deposition, thickening of the basement membranes, and mature small villi) in patients with preeclampsia⁴⁸ and intrauterine growth restriction.⁴⁹

In vitro studies had demonstrated that hypoxia may have opposite effects in the expression of angiogenic factors in the trophoblast.^{50,51} Indeed, the incubation of isolated human term syncytiotrophoblast under hypoxic conditions increased VEGF messenger RNA expression 8-fold but reduced the PIGF messenger RNA expression by 75%.⁵¹ Similarly, in primary

cytotrophoblast cultures, hypoxia increases the VEGF messenger RNA expression (but not the release of free VEGF in the supernatant) and reduces the PlGF concentrations in the supernatant.⁵⁰ Thus, it is possible that chronic uteroplacental ischemia may account for the low maternal plasma PlGF concentration observed among patients destined to develop early onset and/or severe preeclampsia and that the combination of an abnormal UADV result and a maternal plasma PlGF concentration of <280 pg/mL identifies patients at risk for preeclampsia whose primary insult is a chronic reduction in the uteroplacental blood flow.

Abnormal UADV results^{4,5,50,52-56} and biochemical markers^{10,15,57-59} in the second trimester had been reported to have low positive predictive values in the prediction of preeclampsia and early onset preeclampsia. Therefore, most patients with a positive test will not experience the disease; any prophylactic intervention that targets the screen-positive group will expose a large proportion of patients that will not benefit from the intervention. Preeclampsia is 1 of the great obstetrical syndromes,⁶⁰ and several mechanisms of disease had been implicated in the pathophysiologic makeup of this complex disease, including (1) uteroplacental ischemia,⁶¹ (2) immune maladaptation,⁶¹ (3) very low-density lipoprotein toxicity,⁶¹ (4) genetic imprinting,⁶¹ (5) increased trophoblast apoptosis/necrosis,⁶²⁻⁶⁴ and (6) an exaggerated maternal inflammatory response to deported trophoblast.⁶⁵⁻⁶⁸ The heterogeneity of the disease is exemplified by the observation that chronic uteroplacental ischemia may be more relevant in the pathogenesis of early onset preeclampsia than in term or post-term preeclampsia.^{69,70} Indeed, an abnormal UADV result in the second trimester is associated with a higher risk for preeclampsia at ≤ 34 weeks of gestation than at >34 weeks of gestation⁵⁻⁸. Moreover, early onset preeclampsia is more severe⁷¹ and has a higher proportion of growth-restricted fetuses,⁷¹ a higher risk of maternal death,¹ and a higher frequency of placental pathologic condition^{72,73} than late onset preeclampsia.

Because of the syndromic nature of preeclampsia,⁷⁴ it is unlikely that a single marker or combination of markers will identify all patients destined to develop this pregnancy complication, regardless of their mechanism of disease. We have provided the diagnostic indices of a combination of abnormal UADV result and low PlGF concentration for the prediction of the outcomes to provide the basis for comparison with previous reports. However, we propose that risk assessment of preeclampsia should not focus on the prediction of this heterogeneous disorder, but on the identification of patients at high risk for the early and/or a more severe form of preeclampsia, in whom prophylactic interventions are more likely to reduce the morbidity and mortality rates associated with this obstetrical syndrome.

Collectively, the results presented herein indicate that a combination of abnormal UADV result and a maternal plasma concentration of PlGF <280 pg/mL between 22 and 26 weeks of gestation is associated with a high risk for preeclampsia, early onset preeclampsia, and severe preeclampsia. Moreover, a maternal plasma PlGF concentration of <280 pg/mL can identify most patients at risk for early onset and/or severe preeclampsia among patients with abnormal UADV results. The identification of this subset of patients may allow for prophylactic interventions, such as the modulation of the antiangiogenic state before the clinical presentation of early onset and/or severe preeclampsia, which potentially could reduce the perinatal and maternal mortality rate that is associated with this syndrome.

The results of 2 recent randomized clinical trials of vitamin C and E supplementation for the prevention of preeclampsia in low-⁷⁵ and high-risk⁷⁶ patients yielded negative results. Thus, there is a need for alternative prophylactic interventions. VEGF and PlGF have been proposed as potential candidates to modulate the antiangiogenic state in severe preeclampsia.⁷⁷ Recently, it has been reported that the administration of recombinant VEGF121 to a rat model of preeclampsia, which was induced by administration of adenovirus expressing the sVEGFR-1 gene, attenuated the preeclamptic phenotype. Indeed, the authors reported that the

subcutaneous administration of 100, 200 or 400 μ /kg of VEGF121 reduced the systolic blood pressure, limited the kidney damage (as indicated by a lower glomerular lesion index), and reduced proteinuria among the treated animals.⁷⁸ The authors proposed that VEGF121 could be a potential therapeutic agent for preeclampsia in humans.⁷⁸ Similarly, VEGF and PlGF had been reported to stimulate angiogenesis in an animal model of myocardial and limb ischemia.⁷⁹ However, PlGF appears to be a better candidate for clinical trials, because it did not induce the side effects associated with VEGF (such as edema, hypotension, bleeding and the formation of fragile capillaries that were prone to regression).⁷⁹ If clinical trials prove that proangiogenic factors are effective in delaying the clinical presentation of preeclampsia and/or reducing its severity, we have estimated that 15 patients with abnormal UADV results (95% CI, 11-26) in the second trimester would need to be treated in order to prevent 1 case of early onset and/or severe preeclampsia. In contrast, only 7 patients with abnormal UADV results and low PlGF concentrations (95% CI, 5-11) would need to be exposed to these interventions to prevent 1 case of severe and/or early onset preeclampsia.

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Figure 1.

Maternal plasma PIGF concentration (solid line) contributed significantly to the prediction of patients destined to develop **A**, early onset preeclampsia (*PE*; $P < .001$) and **B**, severe preeclampsia ($P < .001$). In contrast, maternal plasma sVEGFR-1 concentration (dotted line) was of limited use in the prediction of **A**, early onset preeclampsia (area under the curve [AUC], 0.49; $P = .9$) and **B**, severe preeclampsia (area under the curve, 0.54; $P = .5$).

Figure 2.

Flow diagram for the identification of patients at risk for early onset and/or severe preeclampsia (PE), with the use of, sequentially, UADV measurement and the determination of maternal plasma PIGF concentration.

Figure 3.

Simplified flow diagram for the identification of patients at risk for early onset and/or severe preeclampsia (PE), with the use of UADV followed by maternal plasma PIGF determinations. The *asterisk* denotes that data for patients with normal UADV were combined, regardless of the plasma PIGF concentration.

Figure 4.

Flow diagram for the identification of patients at risk for early onset and/or severe preeclampsia (PE), with the use of maternal plasma PIGF determination followed by UADV.

Figure 5.

Cumulative hazard ratios for the clinical presentation of preeclampsia (*PE*) according to the UADV and maternal plasma PIGF concentrations among study groups (only patients with preeclampsia were included in this analysis). The examination-to-diagnosis interval in patients with abnormal UADV and plasma PIGF of ≤ 280 pg/mL (median, 69 days; interquartile range, 58-88 days) was significantly shorter than in patients with abnormal UADV and plasma PIGF concentration of >280 pg/mL (median, 94 days; interquartile range, 84-106 days) and patients with normal UADV (median, 99 days; interquartile range, 91-107 days) who had preeclampsia (log rank, 37.9; $P < .001$)

Table 1

Diagnostic indices of abnormal UADV in the identification of patients destined to develop preeclampsia, early onset preeclampsia, and/or severe preeclampsia and patients whose condition required delivery at ≤ 34 weeks of gestation

Outcome	Sensitivity (%) [*]	Specificity (%) [*]	PPV (%) [*]	NPV (%) [*]	Likelihood ratio (+) (95% CI)	Likelihood ratio (-) (95% CI)
Preeclampsia	35.5 (39/110)	89.6 (2721/3036)	11 (39/354)	97.5 (2721/2792)	3.42 (2.60-4.49)	0.72 (0.55-0.95)
Early onset Preeclampsia (≤ 34 weeks of gestation)	72 (18/25)	89.6 (2721/3036)	5.4% (18/333)	99.7 (2721/2728)	6.94 (5.32-9.05)	0.31 (0.24-0.41)
Severe preeclampsia	72.7 (24/33)	89.4 (2783/3113)	6.8 (24/354)	99.7 (2783/2792)	6.86 (5.44-8.66)	0.31 (0.24-0.38)
Preeclampsia that required delivery at ≤ 34 weeks of gestation	86.7 (13/15)	89.5 (2726/3046)	3.9 (13/333)	99.9 (2726/2728)	8.25 (6.59-10.32)	0.15 (0.12-0.19)

^{*} Data in parentheses represents proportions.

Table 2

Logistic regression analysis of abnormal UADV or maternal plasma PIGF concentration of <280 pg/mL, for the prediction of the outcomes adjusted for maternal age, previous preeclampsia, nulliparity, smoking status, body mass index, and sample storage time.

Outcome	Abnormal UADV		PIGF concentration of <280 pg/mL	
	Odds ratio	95% CI	Odds ratio	95% C.I
Preeclampsia	4.3	2.82-6.66	2.6	1.67-3.94
Preeclampsia ≤34 weeks of gestation	24.1	9.61-60.44	5.5	1.98-15.08
Severe preeclampsia	21.1	9.47-47.14	6.5	2.59-16.34
SGA without preeclampsia	1.7	1.16-2.35	1.6	1.20-2.04

Diagnostic indices of a maternal PlGF concentration of <280 pg/mL in the identification of patients destined to develop preeclampsia, early onset preeclampsia, and/or severe preeclampsia and patients whose condition required delivery at ≤34 weeks of gestation

Table 3

Outcome	Sensitivity (%) [*]	Specificity(%) [*]	PPV(%) [*]	NPV(%) [*]	Likelihood ratio [±] (95% CI)	Likelihood ratio [±] (95% CI)
Preeclampsia	69.1 (76/110)	51.4 (1536/2988)	5 (76/1528)	97.8 (1536/1570)	1.42 (1.25-1.62)	0.60 (0.53-0.68)
Early onset preeclampsia (≤34 weeks of gestation)	80 (20/25)	51.4 (1527/2971)	1.4 (20/1464)	99.7 (1527/1532)	1.65 (1.35-2.01)	0.39 (0.32-0.48)
Severe preeclampsia that required delivery at ≤34 weeks of gestation	81.8(27/33)	51.0 (1555/3048)	1.8 (27/1520)	99.6 (1555/1561)	1.67 (1.42-1.97)	0.36 (0.30-0.42)
	86.7 (13/15)	51.3% (1530/2981)	0.9 (13/1464)	99.9 (1530/1532)	1.78 (1.46-2.18)	0.26 (0.21-0.32)

^{*} Data in parentheses represents proportions.

Diagnostic indices of a combination of abnormal UADV and maternal plasma PIGF concentration of <280 pg/mL in the identification of patients destined to develop preeclampsia, early onset preeclampsia, and/or severe preeclampsia and patients whose condition required delivery at ≤34 weeks of gestation

Table 4

Outcome	Sensitivity (%) [*]	Specificity (%) [*]	Positive predictive value (%) [*]	Negative predictive value (%) [*]	Likelihood ratio (+) (95% CI)	Likelihood ratio (-) (95% CI)
Preeclampsia	27.3 (30/110)	96.4 (2926/3036)	21.4 (30/140)	97.3 (2926/3006)	7.53 (5.27-10.75)	0.75 (0.53-1.08)
Early onset preeclampsia (≤34 weeks of gestation)	64 (16/25)	96.5 (3066/3176)	12.7 (16/126)	99.7 (3066/3075)	18.48 (13.07-26.13)	0.37 (0.26-0.53)
Severe preeclampsia	63.6 (21/33)	96.3 (3136/3255)	15.0 (21/140)	99.6 (3136/3148)	17.41 (12.74-23.79)	0.38 (0.28-0.52)
Preeclampsia that required delivery at ≤34 weeks of gestation	73.3 (11/15)	96.4 (3071/3186)	8.7 (11/126)	99.9 (3071/3075)	20.32 (14.26-28.95)	0.28 (0.19-0.40)

^{*} Data in parentheses represents proportions

Table 5

Logistic regression analysis of a combination of abnormal UADV and maternal plasma PIGF concentration of <280 pg/mL for the prediction of the outcomes that are adjusted for maternal age, previous preeclampsia, nulliparity, smoking status, body mass index, and sample storage time.

Outcome	Abnormal UADV + PIGF concentration of <280 pg/mL	
	Odds ratio	95% CI
Preeclampsia	8.6	5.35-13.74
Preeclampsia ≤34 weeks of gestation	43.8	18.48-103.89
Severe preeclampsia	37.4	17.64-79.07
SGA without preeclampsia	2.7	1.73-4.26

Table 6

Demographic and clinical characteristics of the study population according to UADV and maternal plasma PIGF concentration.

Variable	Normal UADV* (n=2792)	Abnormal UADV + PIGF concentration of ≥ 280 pg/mL (n=207)	Abnormal UADV + PIGF concentration of < 280 pg/mL (n=140)	P value
Maternal age (y) [†]	27 (14-46)	23 (14-43)	24 (16-42)	<.001
Nulliparity (%) [‡]	33.9 (947/2792)	57 (118/207)	54.3 (76/140)	<.001
Preeclampsia in a previous pregnancy (%) [‡]	2 (56/2792)	1.9 (4/207)	3.6 (5/140)	NS
Body mass index (kg/m ²) [†]	24.5 (16.2-89)	23.1 (15.9-37.1)	24.6 (18.0-44.0)	<.001
Smokers (%) [‡]	7.4 (207/2792)	8.7 (18/207)	3.6% (5/140)	NS
Gestational age at ultrasound (wk) [§]	24.1 $\pm$ 0.64	24.1 $\pm$ 0.60	24.1 $\pm$ 0.64	NS
Maternal plasma PIGF (pg/mL) [†]	279.1 (8.9-2572)	456.8 (282.3-2040)	172.9 (0-279.8)	<.001
Maternal plasma sVEGFR-1 (pg/mL) [†]	-	844 (0-2550)	735 (0-9450)	NS
Mean uterine artery pulsatility index [†]	0.77 (0.3-1.4)	1.10 (0.6-2.7)	1.36 (0.6-2.8)	<.001

* Regardless of the maternal plasma PIGF concentrations.

[†] Data are given as median (range)

[‡] Data in parentheses represents proportions

[§] Data are given as mean $\pm$ SD

Table 7

Clinical outcomes of the population according to UADV and maternal plasma PIGF concentration.

Variable	Normal UADV (n=2792)	Abnormal UADV + PIGF concentration of ≥280 pg/mL (n=207)	Abnormal UADV + PIGF concentration of <280 pg/mL (n=140)	P value
Preeclampsia	2.5 (71/2792)	4.3 (9/207)	21.4 (30/140)	<.001
Early onset preeclampsia (≥34 weeks of gestation)	0.3 (7/2728)	1 (2/200)	12.7 (16/126)	<.001
Severe preeclampsia	0.3 (9/2792)	1.4 (3/207)	15 (21/140)	<.001
Birthweight <10 th percentile*	8.1 (225/2786)	8.7 (18/207)	20 (28/140)	<.001
Birthweight <5 th percentile*	3.5 (97/2786)	4.8 (10/207)	10.7 (15/140)	<.001
Abruptio placentae	0.9 (24/2792)	1.9 (4/207)	3.6 (5/140)	.001
Eclampsia	0.03 (1/2792)	0	0.7 (1/140)	.01
Spontaneous preterm delivery (≥35 weeks of gestation)	1.5 (41/2787)	1.9 (4/207)	3.6 (5/140)	NS
Spontaneous preterm delivery (≥32 weeks of gestation)	0.4 (10/2787)	0.5 (1/207)	2.2 (3/140)	NS
Composite of severe neonatal morbidity	0.4 (12/2792)	1.9 (4/207)	3.6 (5/140)	<.001

The results are expressed as percentages and proportions. NS, not significant

* In the absence of preeclampsia