Published in final edited form as:

Obstet Gynecol. 2012 June ; 119(6): 1234–1242. doi:10.1097/AOG.0b013e3182571669.

First-Trimester Prediction of Preeclampsia in Low-Risk Nulliparous Women

Leslie Myatt, Ph.D., Rebecca G. Clifton, Ph.D., James M. Roberts, M.D., Catherine Y. Spong, M.D., John C. Hauth, M.D., Michael W. Varner, M.D., John M. Thorp Jr., M.D., Brian M. Mercer, M.D., Alan M. Peaceman, M.D., Susan M. Ramin, M.D., Marshall W. Carpenter, M.D., Jay D. Iams, M.D., Anthony Sciscione, D.O., Margaret Harper, M.D., M.Sc., Jorge E. Tolosa, M.D., M.S.C.E., George Saade, M.D., Yoram Sorokin, M.D., and Garland D. Anderson, M.D. for the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network (MFMU)

Department of Obstetrics and Gynecology of University of Cincinnati, Cincinnati, OH (L.M.); University of Pittsburgh, Pittsburgh, PA (J.M.R.); University of Alabama at Birmingham, Birmingham, AL (J.C.H.); University of Utah, Salt Lake City, UT (M.W.W.); University of North Carolina at Chapel Hill, Chapel Hill, NC (J.M.T.); Case Western Reserve University-MetroHealth Medical Center, Cleveland, OH (B.M.M.); Northwestern University, Chicago, IL (A.M.P.); University of Texas Health Science Center at Houston, Houston, TX (S.M.R.); Brown University, Providence, RI (M.W.C.); The Ohio State University, Columbus, OH (J.D.I.); Drexel University, Philadelphia, PA (A.S.); Wake Forest University Health Sciences, Winston-Salem, NC (M.H.); Oregon Health & Science University, Portland, OR (J.E.T.); University of Texas Medical Branch, Galveston, TX (G.S.); Wayne State University, Detroit, MI (Y.S.); University of Texas Medical Center, Galveston, TX (G.D.A.); and The George Washington University Biostatistics Center, Washington, DC (R.G.C.); and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, MD (C.Y.S.)

Abstract

Objective—To identify clinical characteristics and biochemical markers in first-trimester samples that would possibly predict the subsequent development of preeclampsia.

Methods—We conducted a multicenter observational study in 2,434 low-risk nulliparous women to identify biomarkers that possibly predict preeclampsia. Clinical history, complete blood count, and biochemical markers were assessed in the first trimester. The trophoblast and angiogenesis markers ADAM-12 (a disintegrin and metalloprotease 12), pregnancy-associated plasma protein-A (PAPP-A), PP13, placental growth factor (PIGF), soluble fms-like tyrosine kinase-1, and endoglin were measured in a case-control subset of 174 women with preeclampsia and 509 controls.

Results—Univariable analysis revealed maternal age, race, marital status, years of education, source of medical payment, prenatal caregiver, body mass index (BMI), and systolic blood pressure at enrollment were significantly associated with preeclampsia. Mean platelet volume was greater at enrollment in women who later developed preeclampsia (median 9.4 vs 9.0 fl, $p=0.02$). First-trimester concentrations (multiples of the median) of ADAM-12 (1.14 vs 1.04, $p=0.003$),

Corresponding Author: Leslie Myatt, PhD, University of Texas Health Science Center San Antonio, Mail Code 7836, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, Myattl@uthscsa.edu, tel 210 567 7044, fax 210 567 5033.

Dr. Spong, Associate Editor of *Obstetrics & Gynecology*, was not involved in the review or decision to publish this article.

Presented at the 57th Annual Meeting of the Society for Gynecologic Investigation, Orlando FL, March 24–27, 2010.

Financial Disclosure:

The authors did not report any potential conflicts of interest.

PAPP-A (0.94 vs 0.98, $p=0.04$), and PlGF (0.83 vs 1.04, $p<0.001$) were significantly different in women who developed preeclampsia compared with controls. The optimal multivariable model included African American race, systolic blood pressure, BMI, education level, ADAM-12, PAPP-A and PlGF, and yielded an area under the curve of 0.73 (95% CI 0.69–0.77) and a sensitivity of 46.1% (95% CI 38.3–54.0) for 80% specificity.

Conclusion—A multivariable analysis of clinical data and biochemical markers in the first trimester did not identify a model that had clinical utility for predicting preeclampsia in a low-risk nulliparous population.

Introduction

Preeclampsia is the leading cause of fetal growth restriction, indicated premature delivery, and is responsible for over 50,000 maternal deaths annually worldwide (1–2). A screening test that could identify women early in pregnancy who would later develop preeclampsia would allow increased surveillance of those at risk, and reduce surveillance for those unlikely to develop the syndrome. Identification of an at-risk population would foster investigative studies and clinical trials.

Although defined by hypertension and proteinuria, preeclampsia involves multiple organ systems (e.g. renal, liver, brain, vascular, coagulation, placenta) that may define different pathophysiological phenotypes. Thus phenotype-specific (3) panels of biomarkers may be necessary to identify those at risk prior to appearance of overt disease (hypertension and proteinuria).

Here we describe an observational study in low-risk nulliparous women. Biomarkers chosen are based on the potential different underlying pathophysiologies of preeclampsia. We measured maternal blood concentrations of the syncytiotrophoblast proteins disintegrin and metalloproteinase 12 (ADAM12) (4), that cleaves insulin-like growth factor-binding protein (IGFBP) -3 and -4, pregnancy-associated plasma protein-A (PAPP-A) (5), which cleaves IGFBP-4 (6) and may regulate trophoblast invasion, together with the galectin Placental protein 13 (PP13) (7), a marker of trophoblast function. Preeclampsia is also associated with altered expression of placental derived pro- and anti-angiogenic proteins placental growth factor (PlGF), a vascular endothelial growth factor (VEGF) family member, soluble fms-like tyrosine kinase-1 (sFlt-1), the soluble form of Flt-1 VEGF receptor, and soluble endoglin, a transforming growth factor β (TGF β) co-receptor. Disturbances in the coagulation system are reported in preeclampsia, particularly decreased platelet number and increased platelet volume (8–9), hence we performed a CBC including platelet parameters. The primary objective was to identify clinical characteristics and biochemical markers in first-trimester samples that would possibly predict the subsequent development of preeclampsia.

Methods

Study design

The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network conducted this study as a planned observational cohort of a previously reported larger randomized controlled trial to estimate whether antioxidant supplementation (1000mg vitamin C and 400IU of vitamin E) prevented preeclampsia in nulliparous women at low risk for developing the syndrome(10). Women in the randomized clinical trial were eligible to participate in this cohort if their gestational age at enrollment was between 9 weeks, 0 days and 12 weeks, 6 days. Written informed consent was obtained from every participant and the study was approved by the institutional review board at each clinical site and the data coordinating center.

Clinical information including demographics, medical, obstetrical, family, social and sexual history was obtained at the time of enrollment by personal interview and chart review. Anthropometric measurements were taken for determination of body mass index and body fat by the waist/hip ratio at enrollment. Four measures of blood pressure were assessed: 1) systolic blood pressure, 2) diastolic blood pressure, 3) mean arterial pressure [diastolic + 1/3(systolic-diastolic)], and 4) pulse pressure (systolic-diastolic). A complete blood count (CBC) was performed and additional blood collected and stored for future evaluation of biochemical assays.

Biochemical assays

After enrollment but before study drug initiation blood was collected into EDTA and serum tubes, plasma or serum separated, aliquoted and stored at -70°C until analysis. For the biochemical marker analysis, a case-control study was performed: 174 women diagnosed with preeclampsia and an approximately 3:1 random sample of 509 normotensive, non-proteinuric women matched by center and gestational age at enrollment.

Three biomarkers, ADAM12, PAPP-A and PP-13, were measured in serum by NTD Laboratories, (Huntington Station NY). ELISA kits were used with inter-assay coefficients of variation of 7% at 146ng/ml, 4% at 2243mU/ml and 3% at 115pg/ml respectively. Three biomarkers, sFlt-1, endoglin, and PlGF, were measured in EDTA plasma using Luminex assays developed by Rules Based Medicine (Austin, TX). The inter-assay coefficients of variation were 9% at 1.378ng/ml for sFlt-1, 8% at 3.91ng/ml for Endoglin, and 10% at 476pg/ml for PlGF.

Primary Outcome

The primary outcome was the development of preeclampsia including mild and severe preeclampsia, HELLP syndrome and eclampsia. Mild preeclampsia was defined as mild pregnancy-associated hypertension (140–159 systolic or 90–109 diastolic on 2 occasions, 2–240 hours apart) and proteinuria (300–4,999 mg total protein/24 hours, 2+ or higher on dipstick testing, or a protein-to-creatinine ratio of 0.35 or higher). Severe preeclampsia was defined as preeclampsia with either severe pregnancy-associated hypertension (≥ 160 systolic or ≥ 110 diastolic on 2 occasions, 2–240 hours apart, or a single occurrence treated with anti-hypertensives) or protein excretion of 5g or more in a 24-hour urine sample or as mild pregnancy-associated hypertension with oliguria (<500 ml), pulmonary edema, or thrombocytopenia (platelet count of $<100,000$ per mm^3). For this analysis, severe preeclampsia, HELLP syndrome and eclampsia were combined as severe preeclampsia. Early-onset preeclampsia was defined as the development of preeclampsia prior to 34 weeks' gestation. Preeclampsia was confirmed through central review, using a standardized protocol by 3 reviewers not associated with the clinical site of origin, of de-identified medical records of all women with pregnancy-associated hypertension.

Statistical Analysis

For any given biomarker, the sample size of 683 with approximately 3:1 controls to cases yields 95% power to detect an odds ratio of 2 if approximately 25% of the controls are exposed (i.e. above the 75th percentile). This sample size also yields more than 80% power to detect as little as a 0.25 standard deviation in difference between cases and controls when treating a biomarker as a continuous variable.

Categorical variables were compared using the chi-square test and continuous variables using the Wilcoxon rank-sum test. Multiples of the median (MoM) were computed for each biomarker by dividing the observed measurement by the expected median which was derived from multiple regression of gestational age at sample collection, maternal weight in

kilograms, racial group, and smoking during pregnancy in the women that did not have an elevated blood pressure, proteinuria or a small for gestational age infant. All variables that were significant with a p-value less than 0.1 were included in the expected median model. The 75th percentile cutoff was defined using the women that did not have an elevated blood pressure, proteinuria or a small for gestational age infant.

Logistic regression analysis was used to determine which factors were significantly associated with preeclampsia. The final model was selected using backward elimination in which all variables were initially included in the model and then selectively removed if not significant ($p < 0.10$). The performance of screening for individual markers and the final multivariable model was determined by receiver operating characteristics (ROC) curves and calculating the sensitivity for a fixed 80% specificity. The specificity was set at 80% to maintain a reasonable false positive rate.

Unless specifically noted, a nominal p value less than 0.05 was considered to indicate statistical significance and no adjustments were made for multiple comparisons. Analyses were performed using SAS software (Cary, NC).

Results

Figure 1 shows the enrollment and follow-up of the women who participated in the original trial and the observational cohort in 16 clinical centers between April 2004 and February 2008. Of the 2,434 women enrolled into the observational cohort, 40 (1.6%) were lost to follow-up, resulting in a final cohort of 2,394. The median gestational age at enrollment was 11.6 (range 8.7–13.9) weeks. The overall incidence of preeclampsia (7.4%) and was not different between those allocated to antioxidants or to placebo (7.9% vs 6.8% respectively, $p = 0.32$). Data were available for 176 women who developed preeclampsia of whom 72 developed severe preeclampsia. Only 18 women developed early onset preeclampsia, a number too small to do multivariable analysis.

Clinical characteristics

Clinical characteristics obtained at their first-trimester enrollment visit are reported in Table 1. Univariable analysis revealed that maternal age, race, marital status, years of education, primary source of medical payment, prenatal caregiver, body mass index (BMI) and systolic blood pressure at enrollment were significantly associated with the development of preeclampsia (Table 1). These clinical characteristics were evaluated in a logistic regression model to predict preeclampsia. Of the four measures of blood pressure evaluated, systolic blood pressure was the most significant ($p = 0.001$) and had the highest area under the curve (AUC=0.58, 95% CI [0.53–0.62]). Backward elimination resulted in a final model that included African American race (aOR; 95% CI; 1.7, 1.1–2.6), Hispanic race (1.5, 1.0–2.5), systolic blood pressure (1.1, 1.1–1.2 per five unit increase) and BMI (1.1, 1.0–1.3 per five unit increase) at enrollment, and education level (0.9, 0.9–1.0 per one unit increase). This model had a sensitivity of 36% (95% CI 29–44) and specificity of 80% for development of preeclampsia; the area under the curve was 0.65 (95% CI, 0.61–0.69). When development of severe preeclampsia was considered, pulse pressure showed the most significant association (AUC 0.60, 95% CI 0.53–0.66) of the four blood pressure measures. Prenatal caregiver, infection, the use of prenatal vitamins, pulse pressure, years of education and number of sex partners were found to be significantly associated with development of severe preeclampsia. The final clinical model for prediction of severe preeclampsia yielded an AUC of 0.67, 95% CI 0.61–0.74) with a sensitivity of 42% (95% CI 30–54) at 80% specificity.

Complete Blood Count

Mean platelet volume (MPV) was significantly higher in the first trimester in women who later developed preeclampsia; however, there was no difference in white blood count, red blood count, hemoglobin, hematocrit, and platelet count (Table 2). MPV differed among the racial groups (Hispanic median 10.6fl [range 6.7–14.1], African American median 9.0fl (range 6.3–14.0) and Caucasian median 8.6fl (range 6.4–13.4) respectively, $p<0.001$) and was slightly higher with increasing BMI. The proportion of women with a MPV MoM at or above the 75th percentile was higher among preeclamptics compared with normotensive, non-proteinuric women (31.6% vs 24.3%, $p=0.03$). However, the area under the ROC curve for MPV MoM as a continuous measure was 0.54 (95% CI 0.49–0.58) with a sensitivity of 25% (95% CI 19–32) for 80% specificity. MPV MoM was not found to be associated with development of severe preeclampsia ($p=0.68$).

Biochemical markers

Biomarker concentrations were measured in 174 women who later developed preeclampsia and 509 normotensive and non-proteinuric women. Comparison of first-trimester concentrations (MoM) revealed that while ADAM-12 was significantly higher and PAPP-A significantly lower, there was no difference for PP13 in those patients who went on to develop preeclampsia compared with women who remained normotensive and non-proteinuric (Table 3). The proportion of women with an ADAM-12 MoM at or above the 75th percentile was higher among preeclamptics compared with normotensive, non-proteinuric women (35.5% vs 25.0%, $p=0.009$).

Among the angiogenic markers, PlGF were significantly lower in women who went on to develop preeclampsia compared with women who remained normotensive and non-proteinuric; however, sFlt and endoglin were not different (Table 3). PlGF had the highest area under the curve (0.61) with a sensitivity of 32% (95% CI 25–39) for 80% specificity (Table 3). The proportion of women with a PlGF MoM below the 25th percentile was higher among preeclamptics compared with normotensive, non-proteinuric women (38.5% vs 25.0%, $p<0.001$).

When all six first trimester biomarkers were combined, the area under the curve increased to 0.66 (0.62–0.71). However this yielded a sensitivity of 38% (95% CI 31–46) for a fixed 80% specificity.

When development of severe preeclampsia was considered, only ADAM-12, PAPP-A and PlGF were found to be significantly different in the first trimester in those women who developed severe preeclampsia (all $p < 0.001$) compared with normotensive women. When all 6 biomarkers were combined an AUC of 0.72 (95% CI 0.65–0.78) with a sensitivity of 48% (95% CI 35–60) for a fixed 80% specificity for predicting severe preeclampsia was found.

Multivariable Model

Clinical and biochemical factors individually identified as significant predictors of preeclampsia were initially included in the multivariable model. In combination, two factors were no longer significant: Hispanic race and MPV MoM. The final predictive model that included African American race, systolic blood pressure and BMI at enrollment, education level, ADAM-12 MoM, PAPP-A MoM and PlGF MoM had an area under the curve of 0.73 (95% CI 0.69–0.77) with a sensitivity of 46% (95% CI 38–54) for 80% specificity (Table 4, Figure 2). ADAM-12 MoM and PAPP-A MoM were highly correlated ($r=0.46$, $p<0.001$). Removal of ADAM-12 MoM and PAPP-A individually from the final model resulted in a slight decrease in the AUC (0.72 (0.67–0.76) for ADAM-12 and 0.72 (0.68–0.76) for PAPP-

A). The AUC when both biochemical markers were removed was 0.71 (0.67–0.76) with a sensitivity of 46% (95% CI 38–54) for 80% specificity.

A final predictive model was also constructed for development of severe preeclampsia. Variables remaining in the final model included clinical (obstetric caregiver and pulse pressure) and biomarker (ADAM12, PAPP-A and PlGF) data. This gave an AUC of 0.75 (95% CI 0.68–0.81) with a sensitivity of 55% (95% CI 43–67) at a fixed 80% specificity.

Discussion

This study was performed in a nulliparous population, with collection of a comprehensive clinical data set and with a standardized definition of preeclampsia that allowed for a rigorous evaluation. Despite evaluation of multiple first trimester clinical and biochemical parameters, we were unable to identify an algorithm that could predict subsequent preeclampsia with clinically useful sensitivity and specificity. When calculating sensitivity with specificity set at 80% we correctly identified less than half of those women who developed preeclampsia, i.e. less than the flip of a coin, and just more than half of those who developed severe preeclampsia. The small number of patients with early onset preeclampsia unfortunately precluded analysis for this group with a high incidence of maternal and fetal morbidity.

Multivariable analysis demonstrated that African American or Hispanic race, systolic blood pressure, BMI at enrollment, and education level were the strongest clinical predictors of development of preeclampsia but with only 36% sensitivity at 80% specificity. In contrast to previous findings diastolic blood pressure at enrollment (11) was not significantly higher. Hispanic ethnicity is not traditionally recognized as a risk factor for preeclampsia but is related to an increased incidence of obesity, metabolic syndrome and diabetes (12). Recent reports utilizing clinical variables collected at less than 15 or 16 weeks gestation similarly noted their low sensitivity for prediction of preeclampsia (13–14). When predicting severe preeclampsia the multivariable analysis of clinical predictors was improved to a sensitivity of 42% at 80% specificity, although still not clinically useful.

Preeclampsia is proposed to have an immune component with a protective effect of exposure to paternal antigens (15–16) but we found no relationship between number of partners or the duration of the sexual relationship and development of preeclampsia. Nor was there an association of smoking, a family history of preeclampsia or cardiovascular disease and development of the syndrome. This contrasts with the reports from Chesley (17) and more recently that a positive family history was associated with a 20–30% risk of developing preeclampsia (18). Our failure to find such associations may reflect our racially and ethnically heterogeneous population with a smaller proportion of smokers (16.9%) and a larger number of obese (22.5%) and overweight (26.0%) women.

Of the blood parameters only MPV, a sensitive indicator of platelet activation and consumption (19) was different at enrollment, being modestly greater in those who subsequently developed preeclampsia compared with those who remained normotensive. MPV is higher in patients with hypertension and pre-hypertension (20), in insulin resistant, non-obese, non-diabetic patients with coronary artery disease (21) and in those with diabetes mellitus, hypertension, hypercholesterolemia, smoking and obesity, which are all reported risk factors for preeclampsia. Cross-sectional studies of MPV in women with established preeclampsia revealed high sensitivity and specificity (8). We found that MPV MoM was significantly higher in the first trimester in women destined to develop preeclampsia preceding the clinical onset by 16–18 weeks, but with poor sensitivity and specificity. MPV MoM was however not significantly higher in women who later developed severe

preeclampsia, suggesting platelet activation is not common to all cases of preeclampsia. Unlike previous reports (22) the ratio of platelet volume/number did not relate to development of preeclampsia (data not shown). The association of platelet volume with African American and Hispanic race, first-trimester BMI and systolic blood pressure indicates we may be identifying susceptible women with subclinical vascular dysfunction (23).

Microarray analysis revealed ADAM12 to be the most highly upregulated gene transcript in placental tissue (24) from women with established preeclampsia, with corresponding increases in ADAM12 in maternal serum. Our finding of significantly increased serum concentrations of ADAM12 in the first trimester contradicts reports of decreased ADAM12 (25–26) in women who subsequently developed preeclampsia. This may be due to differing patient populations; however we agree with previous studies, that another proteinase, PAPP-A, was significantly lower in the first trimester (27–28) in women who developed preeclampsia compared with those who did not. Although significantly different at 9–12 weeks of gestation, the sensitivity, either singly or combined of ADAM12 or PAPP-A, for prediction of subsequent preeclampsia did not have clinical utility in agreement with previous smaller studies (26).

In contrast to the reports that first trimester PP13 is significantly reduced (29–31) in those patients who went on to develop preeclampsia we find no significant difference. A smaller retrospective study reported similar negative findings for PP13 although it found, as we did, a positive association for PAPP-A (32). Our finding of no increase in predictive power by combined measurement of PP13 and PAPP-A again agrees with recent studies (33)–(34).

Significant changes in the ratio of pro-angiogenic (PIGF) to antiangiogenic (sFlt-1 and endoglin) markers precede the clinical presentation of preeclampsia by several weeks (35). PIGF has the best predictive power in the first trimester but with low sensitivity and combination of angiogenic and antiangiogenic markers i.e. PIGF/sFlt and PIGF/endoglin ratios did not appreciably increase the sensitivity (data not shown). The low predictive capability of suites of trophoblast function or placental pro-angiogenic/antiangiogenic markers indicates that either the involvement of these agents in the pathophysiology of preeclampsia is a late event or that there are several pathologic phenotypes leading to the syndrome of preeclampsia (3, 36).

The low sensitivity (46.1%) from this multivariable analysis of clinical risk factors and biochemical markers points to the heterogeneity of preeclampsia, the difficulty of identifying at-risk patients from among a low-risk group and of defining an enriched population for study. When new biomarkers are advanced they may similarly lack sensitivity if the syndrome has several pathologic phenotypes. Defining the clinical outcomes of patients identified by abnormal biomarker values in the first trimester may prove useful in elucidating if preeclampsia has several different underlying pathologies. Phenotyping and predicting disease based on biomarkers may prove more useful for diagnosis of disease and outcome in individuals rather than a clinical diagnosis of the preeclampsia syndrome.

Acknowledgments

The project described was supported by grants from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) [HD34208, HD27869, HD40485, HD40560, HD40544, HD34116, HD40512, HD21410, HD40545, HD40500, HD27915, HD34136, HD27860, HD53118, HD53097, HD27917, and HD36801]; the National Heart, Lung, and Blood Institute; and the National Center for Research Resources [M01 RR00080, UL1 RR024153, UL1 RR024989] and its contents do not necessarily represent the official view of NICHD, NHLBI, NCRR or NIH.

The authors thank Sabine Bousleiman, R.N.C., M.S.N. and Margaret Cotroneo, R.N. for protocol development and coordination between clinical research centers; Elizabeth Thom, Ph.D, for protocol development and statistical analysis; and Kenneth J. Leveno, M.D. and Gail D. Pearson, M.D., Sc.D, for protocol development and oversight.

Literature Cited

1. World Health Organization. Estimates of maternal mortality: a new approach by WHO and UNICED. Geneva: World Health Organization; 1996.
2. Which anticonvulsant for women with eclampsia? Evidence from the Collaborative Eclampsia Trial. *Lancet*. 1995; 345:1455–63. [PubMed: 7769899]
3. Myatt, L.; Carpenter, L. Prediction of Preeclampsia. In: Lyall, F.; Belfort, M., editors. *Preeclampsia Etiology and Clinical Practice*. Cambridge: Cambridge University Press; 2007.
4. Irwin JC, Suen LF, Cheng BH, Martin R, Cannon P, Deal CL, et al. Human placental trophoblasts secrete a disintegrin metalloproteinase very similar to the insulin-like growth factor binding protein-3 protease in human pregnancy serum. *Endocrinology*. 2000 Feb; 141(2):666–74. [PubMed: 10650948]
5. Tornehave D, Chemnitz J, Teisner B, Folkersen J, Westergaard JG. Immunohistochemical demonstration of pregnancy-associated plasma protein A (PAPP-A) in the syncytiotrophoblast of the normal placenta at different gestational ages. *Placenta*. 1984 Sep-Oct;5(5):427–31. [PubMed: 6084247]
6. Irwin JC, Suen LF, Martina NA, Mark SP, Giudice LC. Role of the IGF system in trophoblast invasion and pre-eclampsia. *Hum Reprod*. 1999 Dec; 14(Suppl 2):90–6. [PubMed: 10690804]
7. Bohn H, Kraus W, Winckler W. Purification and characterization of two new soluble placental tissue proteins (PP13 and PP17). *Oncodev Biol Med*. 1983; 4(5):343–50. [PubMed: 6856484]
8. Howarth S, Marshall LR, Barr AL, Evans S, Pontre M, Ryan N. Platelet indices during normal pregnancy and pre-eclampsia. *Br J Biomed Sci*. 1999; 56(1):20–2. [PubMed: 10492911]
9. Norris LA, Sheppard BL, Burke G, Bonnar J. Platelet activation in normotensive and hypertensive pregnancies complicated by intrauterine growth retardation. *Br J Obstet Gynaecol*. 1994 Mar; 101(3):209–14. [PubMed: 8193094]
10. Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, et al. Vitamins C and E to prevent complications of pregnancy-associated hypertension. *N Engl J Med*. 2010 Apr 8; 362(14):1282–91. [PubMed: 20375405]
11. Thadhani R, Ecker JL, Kettyle E, Sandler L, Frigoletto FD. Pulse pressure and risk of preeclampsia: a prospective study. *Obstet Gynecol*. 2001 Apr; 97(4):515–20. [PubMed: 11275020]
12. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. *Arch Intern Med*. 2003 Feb 24; 163(4):427–36. [PubMed: 12588201]
13. North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. *BMJ*. 2011; 342:d1875. [PubMed: 21474517]
14. Nijdam ME, Janssen KJ, Moons KG, Grobbee DE, van der Post JA, Bots ML, et al. Prediction model for hypertension in pregnancy in nulliparous women using information obtained at the first antenatal visit. *J Hypertens*. 2010 Jan; 28(1):119–26. [PubMed: 19907344]
15. Dekker GA, Robillard PY, Hulsey TC. Immune maladaptation in the etiology of preeclampsia: a review of corroborative epidemiologic studies. *Obstet Gynecol Surv*. 1998 Jun; 53(6):377–82. [PubMed: 9618714]
16. Kho EM, McCowan LM, North RA, Roberts CT, Chan E, Black MA, et al. Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. *J Reprod Immunol*. 2009 Oct; 82(1):66–73. [PubMed: 19679359]
17. Chesley LC, Annitto JE, Cosgrove RA. The familial factor in toxemia of pregnancy. *Obstet Gynecol*. 1968 Sep; 32(3):303–11. [PubMed: 5742111]
18. Cincotta RB, Brennecke SP. Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. *Int J Gynaecol Obstet*. 1998 Jan; 60(1):23–7. [PubMed: 9506410]

19. Tygart SG, McRoyan DK, Spinnato JA, McRoyan CJ, Kitay DZ. Longitudinal study of platelet indices during normal pregnancy. *Am J Obstet Gynecol.* 1986 Apr; 154(4):883–7. [PubMed: 3963077]
20. Varol E, Akcay S, Icli A, Yucel H, Ozkan E, Erdogan D, et al. Mean platelet volume in patients with prehypertension and hypertension. *Clin Hemorheol Microcirc.* 2010; 45(1):67–72. [PubMed: 20571231]
21. Varol E, Akcay S, Ozaydin M, Erdogan D, Dogan A, Altinbas A. Mean platelet volume is associated with insulin resistance in non-obese, non-diabetic patients with coronary artery disease. *J Cardiol.* 2010 Apr 27.
22. von Dadelszen P, Magee LA, Devarakonda RM, Hamilton T, Ainsworth LM, Yin R, et al. The prediction of adverse maternal outcomes in preeclampsia. *J Obstet Gynaecol Can.* 2004 Oct; 26(10):871–9. [PubMed: 15507197]
23. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. *Placenta.* 2009 Mar; 30(Suppl A):S32–7. [PubMed: 19070896]
24. Gack S, Marme A, Marme F, Wrobel G, Vonderstrass B, Bastert G, et al. Preeclampsia: increased expression of soluble ADAM 12. *J Mol Med.* 2005 Nov; 83(11):887–96. [PubMed: 16247621]
25. Laigaard J, Sorensen T, Placing S, Holck P, Frohlich C, Wojdemann KR, et al. Reduction of the disintegrin and metalloprotease ADAM12 in preeclampsia. *Obstet Gynecol.* 2005 Jul; 106(1):144–9. [PubMed: 15994630]
26. Spencer K, Cowans NJ, Stamatopoulou A. ADAM12s in maternal serum as a potential marker of pre-eclampsia. *Prenat Diagn.* 2008 Mar; 28(3):212–6. [PubMed: 18264967]
27. Poon LC, Stratieva V, Piras S, Piri S, Nicolaides KH. Hypertensive disorders in pregnancy: combined screening by uterine artery Doppler, blood pressure and serum PAPP-A at 11–13 weeks. *Prenat Diagn.* 2010 Mar; 30(3):216–23. [PubMed: 20108221]
28. Spencer K, Cowans NJ, Nicolaides KH. Low levels of maternal serum PAPP-A in the first trimester and the risk of pre-eclampsia. *Prenat Diagn.* 2008 Jan; 28(1):7–10. [PubMed: 18000943]
29. Gonen R, Shahar R, Grimpel YI, Chefetz I, Sammar M, Meiri H, et al. Placental protein 13 as an early marker for pre-eclampsia: a prospective longitudinal study. *BJOG.* 2008 Nov; 115(12):1465–72. [PubMed: 19035985]
30. Chefetz I, Kuhnreich I, Sammar M, Tal Y, Gibor Y, Meiri H, et al. First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol.* 2007 Jul; 197(1):35, e1–7. [PubMed: 17618748]
31. Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. Longitudinal determination of serum placental protein 13 during development of preeclampsia. *Fetal Diagn Ther.* 2008; 24(3):230–6. [PubMed: 18753763]
32. Stamatopoulou A, Cowans NJ, Matwejew E, von Kaisenberg C, Spencer K. Placental Protein-13 and Pregnancy-Associated Plasma Protein-A as First Trimester Screening Markers for Hypertensive Disorders and Small for Gestational Age Outcomes. *Hypertens Pregnancy.* 2010 Aug 11.
33. Spencer K, Cowans NJ, Chefetz I, Tal J, Meiri H. First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia. *Ultrasound Obstet Gynecol.* 2007 Feb; 29(2):128–34. [PubMed: 17149788]
34. Akolekar R, Syngelaki A, Beta J, Kocylowski R, Nicolaides KH. Maternal serum placental protein 13 at 11–13 weeks of gestation in preeclampsia. *Prenat Diagn.* 2009 Dec; 29(12):1103–8. [PubMed: 19777530]
35. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med.* 2006 Sep 7; 355(10):992–1005. [PubMed: 16957146]
36. Myatt L, Miodovnik M. Prediction of preeclampsia. *Semin Perinatol.* 1999 Feb; 23(1):45–57. [PubMed: 10102170]

Figure 1.
Enrollment and follow-up of study participants.

Figure 2.

Receiver operating characteristic curve for final predictive model. Clinical and biochemical factors individually identified as significant predictors of preeclampsia (African American race, systolic blood pressure and body mass index [BMI] at enrollment, education level, a disintegrin and metalloprotease 12 [ADAM-12] multiple of the mean [MoM], pregnancy-associated plasma protein-A [PAPP-A] MoM, and placental growth factor [PlGF] MoM) were included in the final multivariable predictive model. This model had an area under the curve of 0.73 (95% CI 0.69–0.77).

Table 1

Clinical Characteristics Assessed at Enrollment

Characteristic	Preeclampsia (n=176)	No Preeclampsia (n=2,218)	Relative Risk (95% CI)	P
Maternal age	22 [19–25]	23 [20–26]	--	0.02
Race			--	<0.001
African American	61 (34.7)	531 (23.9)	2.0 (1.4–2.8)	
Hispanic	53 (30.1)	551 (24.8)	1.7 (1.2–2.4)	
Caucasian or other	62 (35.2)	1,136 (51.2)	Referent	
Married	62 (35.2)	1,034 (46.6)	0.6 (0.5–0.9)	0.004
Education (total years)	12 [11–14]	13 [12–16]		<0.001
Private insurance	52 (29.5)	917 (41.3)	0.6 (0.5–0.8)	0.002
Prenatal care by obstetrician	77 (43.8)	1,272 (57.3)	0.6 (0.5–0.8)	<0.001
Previous pregnancy	36 (20.5)	508 (22.9)	0.9 (0.6–1.2)	0.46
Family history of preeclampsia	26 (14.8)	294 (13.3)	1.1 (0.8–1.7)	0.57
Maternal family history cardiovascular disease	79 (44.9)	916 (41.3)	1.1 (0.9–1.5)	0.35
Maternal family history adult onset diabetes	81 (46.0)	948 (42.7)	1.1 (0.9–1.5)	0.40
Infections during pregnancy	33 (18.8)	347 (15.6)	1.2 (0.9–1.8)	0.28
Smoked during pregnancy	28 (15.9)	377 (17.0)	0.9 (0.6–1.4)	0.71
Alcohol during pregnancy	12 (6.8)	246 (11.1)	0.6 (0.3–1.1)	0.08
Multivitamins during pregnancy	131 (74.4)	1,768 (79.7)	0.8 (0.5–1.0)	0.10
Number of sex partners in lifetime	3 [1–5]	3 [1–5]	--	0.35
Duration of sexual relationship (months)	19 [9–42]	24 [10–45]	--	0.45
BMI at enrollment	27.3 [23.0–32.2]	24.6 [21.8–29.0]	--	<0.001
Waist to hip ratio *	0.9 [0.8–0.9]	0.8 [0.8–0.9]	--	0.08
Blood pressure				
Systolic	112 [108–120]	110 [102–118]	--	<0.001
Diastolic	68 [60–72]	66 [60–70]	--	0.33

BMI, body mass index.

Data are n (percent) or median [interquartile range].

* Data available on 2,325 women (171 preeclampsia, 2,154 no preeclampsia)

Table 2

Complete Blood Count Measured in the First Trimester

Biomarker	Preeclampsia (n=176)	No Preeclampsia (n=2,218)	P
White blood count ($\times 10^3/\text{mm}^3$) [*]	8.4 [7.2–10.1]	8.4 [7.0–9.8]	0.59
Red blood count ($\times 10^6/\text{mm}^3$) [*]	4.3 [4.1–4.5]	4.3 [4.0–4.5]	0.18
Hemoglobin (g/dl) [†]	12.7 [12.0–13.4]	12.8 [12.1–13.5]	0.11
Hematocrit (%) [*]	37.0 [35.0–39.0]	37.2 [35.4–39.2]	0.33
Platelet count ($\times 10^3/\text{mm}^3$) [‡]	267 [227–303]	261 [229–302]	0.94
Mean platelet volume (fl) [§]	9.4 [8.2–11.0]	9.0 [8.1–10.4]	0.02

Data are median [interquartile range].

^{*} Data available on 2,348 women (173 preeclampsia, 2175 no preeclampsia)[†] Data available on 2,349 women (173 preeclampsia, 2176 no preeclampsia)[‡] Data available on 2,345 women (172 preeclampsia, 2173 no preeclampsia)[§] Data available on 2,338 women (171 preeclampsia, 2167 no preeclampsia)

Table 3

First-Trimester Biochemical Concentrations and Preeclampsia

Biomarker	Preeclampsia (n=174)	Normotensive, Nonproteinuric (n=509)	P	Area Under the ROC Curve [95% CI]	Sensitivity at 80% Specificity % [95% CI]
ADAM-12 MoM *	1.14 [0.90–1.46]	1.04 [0.83–1.32]	0.003	0.58 [0.53–0.63]	29 [22–36]
PAPP-A MoM *	0.94 [0.61–1.31]	0.98 [0.67–1.53]	0.04	0.54 [0.49–0.59]	23 [17–31]
PP13 MoM *	1.02 [0.78–1.38]	0.99 [0.78–1.35]	1.00	0.51 [0.46–0.56]	21 [15–28]
sFlt-1 MoM	0.94 [0.63–1.36]	1.01 [0.67–1.40]	0.16	0.54 [0.48–0.59]	25 [19–32]
Endoglin MoM	1.01 [0.87–1.23]	1.00 [0.83–1.17]	0.20	0.53 [0.48–0.58]	26 [20–34]
PlGF MoM	0.83 [0.63–1.15]	1.04 [0.75–1.33]	<0.001	0.61 [0.56–0.66]	32 [25–39]

ADAM-12, a disintegrin and metalloprotease 12, MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A; s-Flt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor. Data are median [interquartile range]. MoMs were adjusted for gestational age, maternal weight, race and smoking.

* ADAM-12 available on 166 preeclamptic and 488 normotensive, PAPP-A available on 171 preeclamptic and 502 normotensive, PP13 available on 170 preeclamptic and 503 normotensive.

Table 4

Final Multivariable Model for Preeclampsia

Predictor	Odds Ratio	95% CI	P
African American	1.53	1.00–2.35	0.051
Systolic blood pressure *	1.04	1.02–1.07	<0.001
Education level *	0.93	0.86–1.00	0.052
BMI at enrollment *	1.05	1.02–1.09	0.002
ADAM-12 MoM *	2.61	1.56–4.38	<0.001
PAPP-A MoM *	0.73	0.58–0.90	0.004
PIGF MoM *	0.52	0.34–0.79	0.002

BMI, body mass index; ADAM-12, a disintegrin and metalloprotease 12; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A; PIGF, placental growth factor.

* Per one unit increase.