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Placental protein levels in maternal serum are associated with adverse pregnancy outcomes in nulliparous patients

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Abstract

Background: The NICHD Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-be (nuMoM2b) was established to investigate the underlying causes and pathophysiologic pathways associated with adverse pregnancy outcomes in nulliparous gravidas.

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Contributors statement

All authors are responsible for the research, and all made substantial contributions to study conception, analysis, interpretation, manuscript drafting, and critical revision of the final submitted version.

Disclosure statement

The authors report no conflicts of interest.

Objective: Our objectives were to study placental physiology and identify novel biomarkers in relation to adverse pregnancy outcomes, including preterm birth (medically-indicated and spontaneous), preeclampsia, small for gestational age (SGA) neonates, and stillbirth. We measured levels of placental proteins in the maternal circulation in the first two trimesters of pregnancy.

Materials and Methods: Maternal serum samples were collected at two study visits (6-13 weeks and 16-21 weeks), and levels of nine analytes were measured. The analytes we measured were vascular endothelial growth factor (VEGF), placental growth factor (PlGF), endoglin (Eng), soluble fms-like tyrosine kinase-1 (sFlt-1), A disintegrin and metalloproteinase domain-containing protein 12 (ADAM12), pregnancy-associated plasma protein A (PAPP-A), free β human chorionic gonadotropin (βhCG), inhibin A, and alpha-fetoprotein (AFP). The primary outcome was preterm birth between 20 weeks 0 days and 36 weeks 6 days gestation. Secondary outcomes were spontaneous preterm births, medically-indicated preterm births, preeclampsia, SGA neonates, and stillbirth.

Results: A total of 10,038 eligible gravidas were enrolled into the nuMoM2b cohort, from which a nested case-control study was performed comparing 800 cases with preterm birth (466 spontaneous preterm births, 330 medically-indicated preterm births, and 4 unclassified preterm births), 568 with preeclampsia, 406 with SGA birth, and 49 with stillbirth, with 911 controls who delivered at term without complications. Although levels of each analyte generally differed between cases and controls at one or both visits, the odds ratios revealed a less than two-fold difference between cases and controls in all comparisons. Receiver operating characteristic curves, generated to determine the relationship between analyte levels and preterm birth and the other adverse pregnancy outcomes, resulted in areas under the curves (AUCs) that were relatively low (range 0.50-0.64) for each analyte. Logistic regression modeling demonstrated that AUCs for predicting adverse pregnancy outcomes were greater using baseline clinical characteristics and combinations of analytes compared to baseline characteristics alone, but AUCs remained relatively low for each outcome (0.65-0.78).

Conclusion: We have found significant associations between maternal serum levels of analytes evaluated early in pregnancy and subsequent adverse pregnancy outcomes in nulliparous gravidas. However, the test characteristics for these analytes do not support their use as clinical biomarkers to predict adverse pregnancy outcomes, either alone or in combination with maternal clinical characteristics.

Condensation

Early pregnancy maternal serum levels of proteins that affect placental angiogenesis, implantation, and development are associated with subsequent adverse pregnancy outcomes in nulliparous gravidas.

Keywords

Preterm birth; preeclampsia; small for gestational age; stillbirth; vascular endothelial growth factor; placental growth factor; endoglin; soluble fms-like tyrosine kinase-1; A disintegrin and metalloproteinase domain-containing protein 12; pregnancy-associated plasma protein A

Introduction

Most reproductive biologists agree that failed invasion by extravillous trophoblast cells into the maternal spiral arteries early in pregnancy reduces maternal blood flow into the placenta and induces functional and pathological changes in the placenta that are observed frequently in those who have hypertensive disorders of pregnancy, small for gestational age (SGA) neonates, and stillbirth.[1–5] Many of these pregnancy complications result in medically-indicated preterm births. There also is evidence that impaired trophoblast invasion into the maternal uterine vasculature early in pregnancy can result in placental dysfunction that leads to spontaneous preterm birth (e.g., preterm labor, preterm premature rupture of membranes).[6–11] Unfortunately, reproductive biologists studying placental function have been stymied by the absence of animal models that recapitulate the human placenta and the inability to access placental tissue during human pregnancy, so the ability to identify placental dysfunction before these adverse outcomes occur has been limited.

The human placenta is characterized by a villous structure that provides a large surface area for gas and nutrient exchange, and placental products are secreted mostly from the villous surface of the syncytiotrophoblast into the intervillous space and maternal circulation.[12] In order to study placental physiology in relation to adverse pregnancy outcomes and to identify novel biomarkers of these outcomes, we measured levels of placental proteins in the maternal circulation in a large cohort of nulliparous gravidas during the first two trimesters of pregnancy. The nine proteins we studied fall into three broad physiological categories: 1) angiogenesis – vascular endothelial growth factor (VEGF), placental growth factor (PlGF), endoglin (Eng), and soluble fms-like tyrosine kinase-1 (sFlt-1);[13–20] 2) placental implantation and development – A disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) and pregnancy-associated plasma protein A (PAPP-A);[21,22] and 3) established clinical markers of fetal aneuploidy – free β human chorionic gonadotropin (βhCG), inhibin A, and alpha-fetoprotein (AFP).[23,24] Although AFP is not secreted by the placenta, elevated levels in second trimester maternal serum have been associated with adverse pregnancy outcomes, likely in association with excessive placental permeability.[25,26]

The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development established the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-be (nuMoM2b) to investigate the underlying causes and pathophysiologic pathways associated with adverse pregnancy outcomes (e.g., preterm birth, hypertensive disorders of pregnancy, SGA neonates) in nulliparous gravidas.27 More than 10,000 gravidas with singleton pregnancies were enrolled in nuMoM2b, which combined detailed demographic and medical information, clinical parameters, ultrasound measurements, genetics, biomarker measurements in biologic fluids, and psychosocial and behavioral measures in both prespecified and exploratory analyses to identify pregnant gravidas at risk for adverse pregnancy outcomes.[5,27,28] Maternal peripheral blood samples were collected from all nuMoM2b participants during the first and second trimesters of pregnancy. The objective of the current analysis was to determine the utility of placental analytes in maternal serum to predict adverse pregnancy outcomes, including preterm birth (medically-indicated and spontaneous), preeclampsia, SGA neonates, and stillbirth.

Methods

Nulliparous gravidas with a viable singleton pregnancy were recruited during their first trimester into the nuMoM2b observational prospective cohort at eight institutions across the United States. The data-coordinating and analysis center was RTI International (Research Triangle Park, NC). Each site's local governing institutional review board approved the nuMoM2b protocol and procedures (ClinicalTrials.gov identifier: NCT01322529).[27]

Participants were enrolled into the nuMoM2b cohort between 6 weeks 0 days and 13 weeks 6 days gestational age (first study visit). Gestational dating was based on a documented ultrasound crown-rump length measurement by a certified nuMoM2b sonographer at the first study visit, and potential participants were considered eligible for enrollment if they had no previous pregnancy that lasted ≥20 weeks based on self-report and review of available medical records.[27]

Participants were evaluated at three study visits during pregnancy and again at delivery. Peripheral maternal blood samples were collected at all three study visits: first study visit (6 weeks 0 days to 13 weeks 6 days), second study visit (16 weeks 0 days to 21 weeks 6 days), and third study visit (22 weeks 0 days to 29 weeks 6 days). We collected 4-8 cc maternal blood in serum separating tubes, centrifuged the samples, and stored 0.5 cc serum aliquots at −70C within two hours following collection. Samples were transported on dry ice to analytical laboratories (see below) for subsequent batch analyses. For the current study, we measured placental analyte levels in maternal serum samples collected at the first two study visits, because earlier pregnancy biomarkers are more likely to yield effective strategies for preventing adverse pregnancy outcomes.

Maternal serum levels of placental analytes were measured at two laboratories: Translational Core Laboratory at Children's Hospital of Philadelphia (Philadelphia, PA) and Eurofins NTD, LLC (Melville, NY). Two analytes were measured by enzyme-linked immunosorbent assays (ELISA) at the Children's Hospital laboratory: Eng (Human Endoglin Assay, R&D Systems, Minneapolis, MN), and ADAM12 (Human ADAM-12 ELISA Assay, R&D Systems, Minneapolis, MN). Two analytes were measured by electrochemiluminescence assays (ECL) at the Children's Hospital laboratory: VEGF (Human VEGF-A Electrochemiluminescence Assay, Merck Sharp & Dohme, Kenilworth, NJ), and sFlt-1 (Human Flt-1 Electrochemiluminescence Assay, Merck Sharp & Dohme). Five analytes were measured by lanthanide-based time resolved fluorometry (TRF) at the Eurofins NTD laboratory: PlGF, PAPP-A, inhibin, βHCG, and AFP. The TRF assays are New York State Department of Health-approved and utilize the AutoDELFIA system (PerkinElmer). Briefly, the TRF assays are "sandwich-type" immunoassays utilizing a monoclonal antibody (mAb) immobilized on the bottom of a microtitration strip plate. As the analyte is captured, mAb labeled with Europium (tracer) binds to different sites on the molecules. The addition of inducer causes the release of the respective tracer conjugated Europium into solution, and the fluorescent-specific signals are proportional to the concentration of analyte targeted in the sample. Levels of each analyte were measured once by ELISA, ECL, or TRF – 97.53 percent of assays yielded numerical results in range,

2.42 percent of assays yielded results above or below standard ranges, and only 0.05 percent of assays did not yield numerical results.

The primary outcome of this analysis was preterm birth between 20 weeks 0 days and 36 weeks 6 days gestation. Secondary outcomes were subsets of preterm births, including spontaneous and medically-indicated preterm birth, and adverse pregnancy outcomes that frequently are associated with preterm birth, including preeclampsia, SGA birth, and stillbirth. Definitions for preeclampsia, SGA birth, stillbirth, and spontaneous preterm birth in the nuMoM2b cohort have been published.[27] Preeclampsia included eclampsia, preeclampsia with and without severe features, and super-imposed preeclampsia.[27] Those with preeclampsia or non-proteinuric gestational hypertension followed by preterm birth were analyzed as an additional outcome group. Antepartum gestational hypertension was defined as new-onset hypertension ≥140 mm Hg systolic or ≥90 mm Hg diastolic on two occasions at least six hours apart >20 weeks 0 days' gestation and prior to labor and delivery. Small for gestational age births were defined as neonates with birth weight less than the fifth percentile for gestational age at delivery based on Alexander birth weight curves.[5,27,29] Stillbirth was a fetal death at an estimated gestational age of ≥20 weeks 0 days with Apgar scores of 0 at 1, 5, and 10 minutes with no other signs of life by direct observation. Spontaneous preterm births were defined as deliveries at 20 weeks 0 days to 36 weeks 6 days resulting from preterm labor or preterm premature rupture of membranes. Medically-indicated preterm births were those resulting from labor induction or cesarean delivery for maternal or fetal indication in the absence of preterm labor or preterm premature rupture of membranes.

Biospecimen sample selection for analysis was performed using a case/sub-cohort design. Specifically, available biospecimens from the first and second study visits from all participants delivered at or after 20 weeks of gestation with preterm birth, preeclampsia, stillbirth, or SGA birth were selected for laboratory analysis as "case" samples. "Control" samples were then selected from available biospecimens on the subset of participants delivered at or after 20 weeks without any of these adverse pregnancy outcomes. Geographic region and institution were not considered when selecting controls. The sample size for the controls was determined by the available funds remaining for the Visit 1 and Visit 2 assays of the biomarkers. The budget allowed for assay of 4,800 samples across the two visits. The control samples were randomly selected using a sampling frequency, $f=12\%$, after selecting the adverse pregnancy outcome samples. Finally, a random selection of the adverse pregnancy outcome cases was identified using the sampling frequency, $f=12\%$. This random selection of cases combined with the controls defined each random sub-cohort.

Demographic and baseline clinical characteristics were described using means and standard deviations or counts and percentages. Comparisons were performed using chi-square tests for categorical variables and analysis of variance methods for continuous variables.

The median and interquartile range for each analyte on the original scale were assessed for each adverse pregnancy outcome group and for the controls by study visit. Comparison of analyte distributions between an adverse pregnancy outcome group and controls was performed using Wilcoxon rank-sum tests.

Analyte results were then assessed for normality at each visit. Those analyte results that showed a lack of normality were transformed using an optimal power transformation, across visits, that was determined using the Box-Cox algorithm. The transformed analyte results from both visits were then regressed onto gestational age to generate Studentized residuals. The Studentized residuals allow for study of the associations between adverse pregnancy outcomes and analyte results while accounting for changes in analyte levels depending on gestational age.

The transformed and Studentized analyte results were then independently assessed for association with each adverse pregnancy outcome at each visit using appropriate controls from the random sub-cohort (e.g., preterm births compared to no preterm birth = random sub-cohort minus preterm birth cases). Results were summarized using odds ratios and areas under receiver operating characteristic curves (AUCs) from logistic regression, each with 95 percent confidence intervals.

To determine which analytes were most important in predicting adverse pregnancy outcomes and whether the most important analytes provide prediction beyond baseline clinical characteristics, we then performed model selection for each adverse pregnancy outcome using cross-validation regression with the least absolute shrinkage and selection operator (LASSO) method.[30] This was repeated for visit 1, visit 2, and visit 1 and visit 2 together. Potential variables included the transformed and Studentized analyte results that were allowed as independent selections or through interactions with gestational age. To determine which combination of analytes best predicted an adverse pregnancy outcome above baseline characteristics, each LASSO model included the baseline characteristic prediction as an offset. The baseline characteristics in the prediction were maternal age, self-identified race/ethnicity, early pregnancy body mass index (BMI), smoking status during the three months prior to pregnancy, pregestational diabetes, and chronic hypertension. LASSO model results were summarized using AUCs from logistic regression for the baseline characteristics and for the baseline characteristics plus the selected analyte variables.

Finally, consideration was given to sonographic findings of cervical length ≤25 mm and bilateral resistance index in the uterine arteries ≥0.59, both measured at visit 2.[5,28] Data were summarized using AUCs from logistic regression for the baseline characteristics, plus the selected analyte variables plus cervical length; the baseline characteristics, plus the selected analyte variables plus resistance index; and the baseline characteristics, plus the selected analyte variables plus both sonographic measures.

All tests were performed using a nominal significance level of α=0.05 (two-sided). No correction was made for multiple comparisons. Analyses were performed using SAS 9.4 (Cary, NC) and R Version 3.6.2.

Results

A total of 10,038 eligible gravidas were consented and enrolled into the nuMoM2b cohort between October 2010 and September 2013, and delivery outcomes after 20 weeks 0 days and adequate maternal serum for analyses were available for 9,222 participants (Figure 1).

There were 800 preterm births (466 spontaneous preterm births, 330 medically-indicated preterm births, and 4 unclassified preterm births), 568 cases of preeclampsia, 406 SGA births, and 49 stillbirths. In addition, there were 62 cases of gestational hypertension among those who also had a preterm birth. There was modest overlap among these adverse pregnancy outcomes – 57 participants had both preeclampsia and SGA births, 25 had both spontaneous preterm births and preeclampsia, and 13 had both spontaneous preterm births and SGA births. Overall in the nested case-control study, there was a total of 1,502 cases (some cases had more than one adverse pregnancy outcome, while a total of 800 delivered preterm) and 911 controls who delivered at term without complications.

Demographic characteristics were compared between the 1,502 participants with adverse pregnancy outcomes (cases) and the 911 with no adverse pregnancy outcomes (controls, Table 1). Controls were more likely to be non-Hispanic White and have more years of education. Cases were more likely to smoke and have a higher BMI, chronic hypertension, and pregestational diabetes.

Levels of analytes in maternal serum at study visits 1 and 2 are presented in Table 2. Test characteristics for each assay (sensitivity, intra-assay coefficient of variability, inter-assay coefficient of variability) are provided in Supplemental Table 1. The only analytes in maternal serum that were associated with preterm birth at both study visits ($P<0.05$) were AFP (elevated levels compared to controls) and PlGF (decreased levels compared to controls). At both study visits, elevated maternal serum levels of AFP were associated with spontaneous preterm birth and SGA births. At both study visits, decreased levels of maternal serum PlGF were associated with medically-indicated preterm birth and common causes for medically-indicated preterm birth, including preeclampsia and SGA growth. Similarly, decreased maternal serum levels of PAPP-A at both study visits were associated with medically-indicated preterm birth, preeclampsia, and SGA births. In contrast, decreased maternal serum levels of PAPP-A were not associated with overall preterm birth (the primary outcome).

At both study visits, the odds ratios for association of the transformed assay results with preterm birth and other adverse pregnancy outcomes were relatively modest (point estimate range 0.62-1.67, Table 3). Similarly, AUCs for receiver operating characteristic curves used to measure the accuracy of transformed assay results in predicting preterm birth and other adverse pregnancy outcomes demonstrated weak predictive accuracy across discrimination thresholds (point estimate range: 0.50-0.64, Table 3). The only analytes that yielded more than one AUC >0.60 were PAPP-A at study visit 1 (medically-indicated preterm birth and SGA birth) and PlGF at study visit 2 (medically-indicated preterm birth, preeclampsia, preeclampsia/gestational hypertension, and SGA birth).

Analyses were conducted to determine if placental analytes plus baseline clinical characteristics could predict adverse pregnancy outcomes. Analyses were based on logistic regression models for cases of the adverse pregnancy outcome versus controls from a random sub-cohort. Baseline characteristics included: age, self-identified race/ethnicity, BMI, smoking status, pregestational diabetes, and chronic hypertension. Overall, AUCs for placental analytes plus baseline characteristics were relatively modest (point estimate range,

0.68-0.78, Figure 2), but AUCs for predicting adverse pregnancy outcomes were stronger for placental analytes plus baseline characteristics compared to baseline characteristics alone. Placental analytes at both study visits plus baseline characteristics most strongly predicted preeclampsia and medically-indicated preterm birth (AUCs 0.71-0.78, Figure 2). Finally, adding indicators from visit 2 ultrasounds for cervical length ≤0.25 mm and bilateral resistance index in the uterine arteries ≥0.59 did not improve any of the predictions (results not shown).

Discussion

Principal Findings

Levels of placental proteins in maternal serum at 6-13 weeks and 16-21 weeks of gestation were significantly different between nulliparous cases with adverse pregnancy outcomes and controls with uncomplicated pregnancies. In particular, decreased levels of PlGF and PAPP-A were associated with medically-indicated preterm birth and common causes for medically-indicated preterm birth, including preeclampsia and SGA birth. However, the placental analytes have only modest value as clinical biomarkers for adverse pregnancy outcomes, with odds ratios ranging from 0.5 to 2.0. In addition, AUCs for receiver operating characteristic curves used to measure the accuracy of the assay results in predicting adverse pregnancy outcomes were relatively low, even when placental analytes were used in combination with baseline clinical characteristics to predict adverse pregnancy outcomes.

Results

Circulating levels of placental/fetal proteins, particularly angiogenic factors produced by the placenta, are associated with adverse pregnancy outcomes.[14,31,32,33] Investigators also have attempted to combine circulating levels of placental proteins, maternal clinical factors, and sonographic markers (i.e., fetal biometry, uterine artery flow) to predict adverse pregnancy outcomes.[18,34] For many of these studies, the addition of biomarkers of impaired placentation only marginally improved the predictive values for adverse pregnancy outcomes compared to maternal clinical factors.[18,34] In the current study of nulliparous antibacterial mouth wash, dental floss, antibacterial toothpaste, a power toothbrush, and educational material gravidas enrolled for prenatal care at eight centers across the United States, circulating levels of ten different placental/fetal proteins also were associated with adverse pregnancy outcomes attributed to placental dysfunction, but the addition of these biomarkers of impaired placentation again only marginally improved the predictive values for adverse pregnancy outcomes compared to maternal clinical factors alone.

Clinical Implications

The relationship between adverse pregnancy outcomes and low levels of circulating placental proteins is strongest for PlGF (low levels at first and second study visits), which promotes angiogenesis within the placenta,[13,14,19] and proteins that promote placental implantation and development (PAPP-A, ADAM12)[21,22] at the first study visit. Among the individual adverse pregnancy outcomes we studied, the association was strongest between circulating levels of placental proteins and the most common causes of medically-indicated preterm births, including preeclampsia and abnormal fetal growth (SGA neonates). The

correlation was weakest between circulating levels of placental proteins and spontaneous preterm births. Overall, our findings indicate that circulating levels of placental proteins have low predictive ability to be useful in guiding therapies to prevent preterm birth and other adverse pregnancy outcomes in nulliparas, and that the combination of these biomarkers with clinical characteristics only marginally improved their predictive value. Importantly, our results were obtained from maternal serum samples collected at <22 weeks gestation in healthy nulliparous patients, which is in contrast to recent studies that demonstrated more clinical utility for sFlt-1 and PlGF assays performed later in pregnancy in patients with suspected preeclamapsia.[32,35]

Research Implications

Although the predictive capability of the studied biomarkers is limited, opportunities exist to study novel placental proteins in different groups of patients that will yield clinically useful biomarkers for patients at highest risk of adverse outcomes attributed to placental dysfunction. For example, PAPP-A is produced almost exclusively by placental trophoblast cells, cleaves insulin-like growth factor binding proteins, and is directly involved in placental function and fetal growth.[36,37] We observed low levels of circulating PAPP-A at the first study visit in those who underwent medically-indicated preterm births later in pregnancy. In contrast, PAPP-A2 is 45 percent homologous with PAPP-A, appears to downregulate trophoblast differentiation and migration, and is expressed at increased levels in maternal serum and placental samples from cases of preeclampsia.[38–40] It is possible that the ratio between PAPP-A and PAPP-A2 levels might yield a more discriminatory biomarker for adverse pregnancy outcomes among low-risk patients. In addition, future studies should focus on higher risk groups of patients, including those with medical complications of pregnancy, obesity, and advanced maternal age.

Strengths and Limitations

The nuMoM2b Network recruited and followed a large, multi-center cohort whose clinical phenotypes were recorded accurately. Hence, results from this cohort probably are generalizable for nulliparous gravidas across the United States. In addition, the current study employed sophisticated regression analyses that allowed us to validate interactions between the placental analytes and baseline clinical characteristics.[30] A case/sub-cohort design was employed for selection of maternal serum specimens for assay of placental protein levels. All participants with adverse pregnancy outcomes and a 12 percent random sample of those without any adverse pregnancy outcome were selected for bioassay. Then, a 12 percent random sample of the participants with an adverse pregnancy outcome were combined with those without an adverse pregnancy outcome to constitute a sub-cohort for selection of controls for different case sets. The ratios of controls to cases in the analyses were 1.2 for preterm birth, 1.8 for preeclampsia, 2.6 for SGA births, and 22.1 for stillbirth, reflecting efficient use of resources and minimal loss of power over the use of bioassay results from all participants without any adverse pregnancy outcome for case-control analyses. Importantly, our results reveal associations but do not imply causation of adverse pregnancy outcomes by placental dysfunction and aberrant expression of placental proteins secreted into the maternal circulation.

Conclusions

Maternal serum levels of proteins produced primarily in the placenta are different early in pregnancy in nulliparous gravidas who later have preterm births and other adverse pregnancy outcomes. However, the test characteristics for these analytes do not support their use as clinical biomarkers to predict adverse pregnancy outcomes in nulliparas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance

Why was this study conducted?

- This study was conducted to identify novel biomarkers of placental function in relation to adverse pregnancy outcomes, including preterm birth, preeclampsia, small for gestational age births, and stillbirth.

What are the key findings?

- Maternal serum levels of each analyte at first and second trimester study visits differed between cases with adverse pregnancy outcomes and controls. In particular, decreased levels of placental growth factor and pregnancy-associated plasma protein A were associated with medically-indicated preterm birth, preeclampsia, and small for gestational age births.
- Test characteristics (eg., odds ratios, areas under receiver operating characteristic curves) for these analytes do not support their use as clinical biomarkers to predict adverse pregnancy outcomes.

What does this study add to what is already known?

- Although this study confirms the relationship between maternal serum levels of placental proteins and adverse pregnancy outcomes, the findings do not support the use of these proteins in clinical protocols for identifying nulliparous patients at high-risk for adverse pregnancy outcomes.

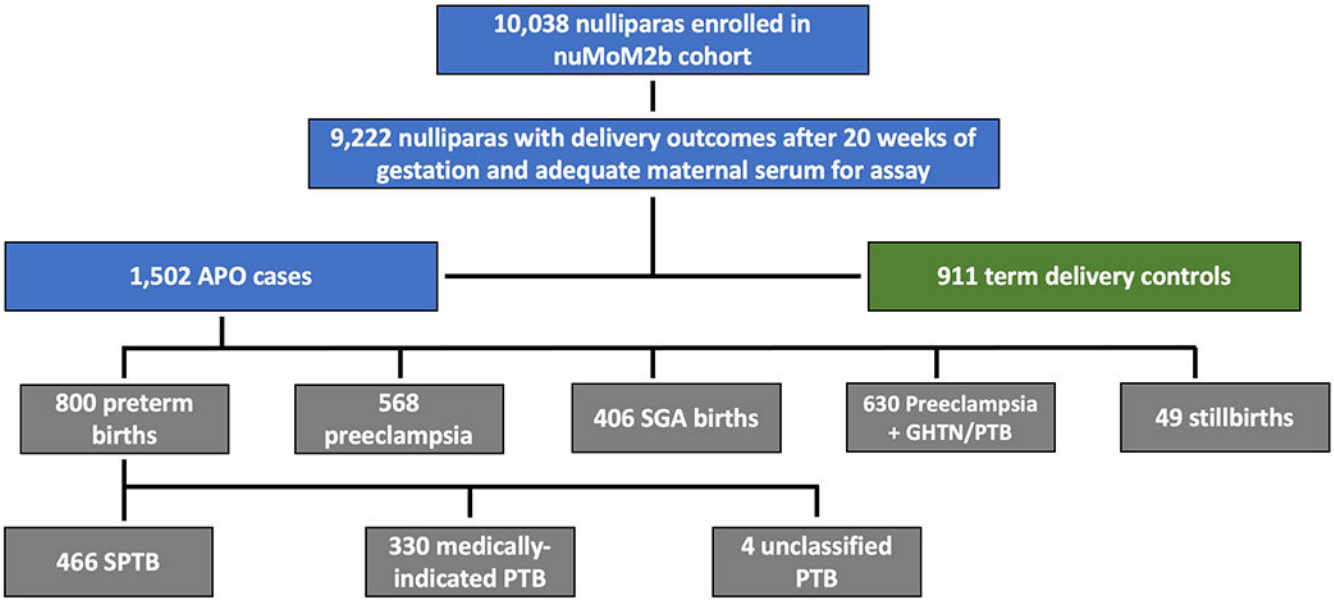

Figure 1. Flow chart of nuMoM2b participants who were included in the nested case-control study.

Levels of analytes in maternal serum samples were compared between term delivery controls (green box) and cases with adverse pregnancy outcomes (APO, gray boxes). SGA = small for gestational age; SPTB = spontaneous preterm birth; PTB = preterm birth; preeclampsia + GHTN/PTB = preeclampsia cases (all gestational ages) plus preterm births complicated by gestational hypertension

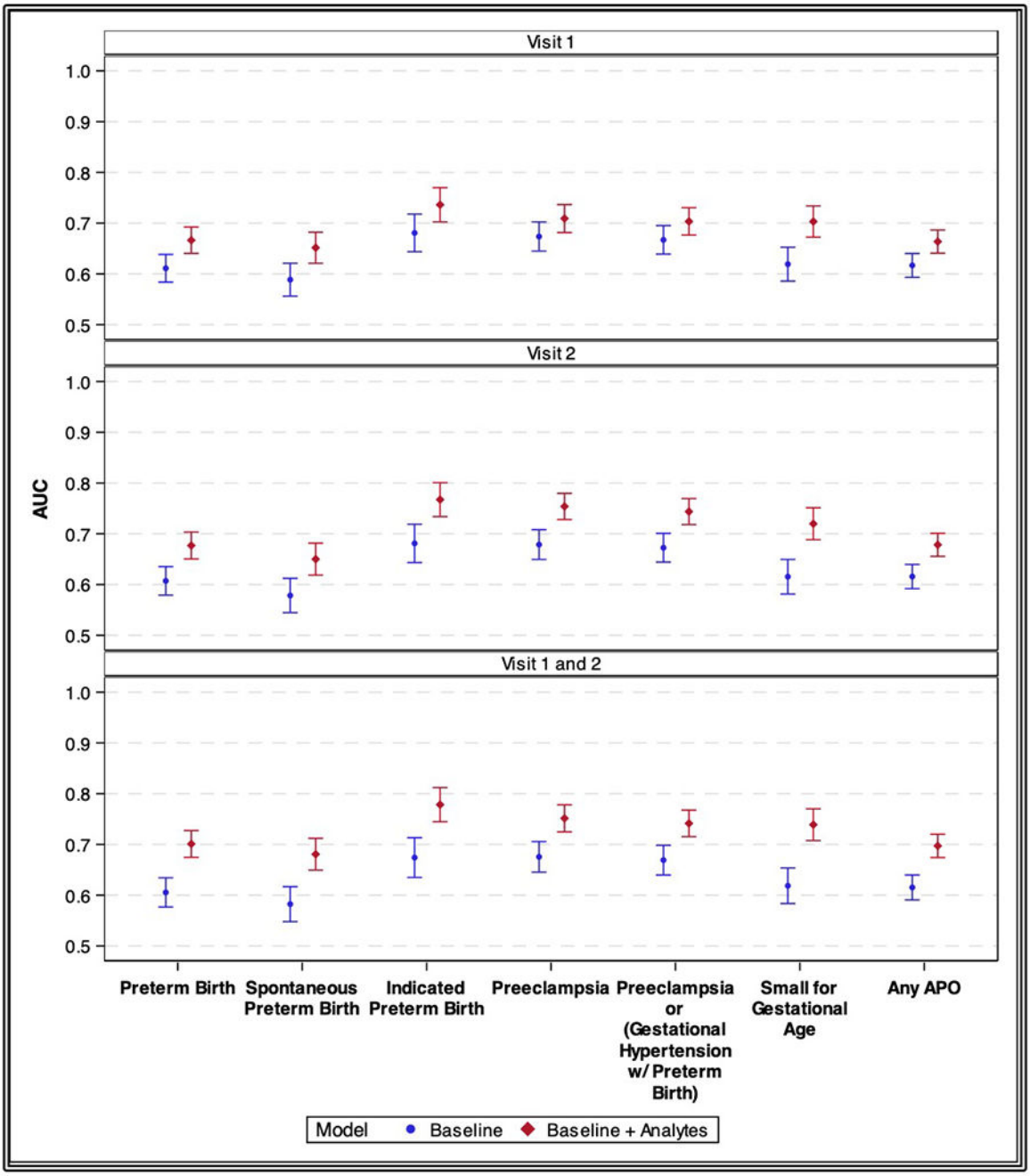

Figure 2. Areas under receiver operating characteristic curves (95% confidence intervals) for prediction of adverse pregnancy outcome using baseline characteristics and selected placental analytes by study visit.

Analyses are based on logistic regression models for cases of adverse pregnancy outcome versus controls from a random sub-cohort. Baseline characteristics included: age, race/ethnicity, BMI, smoking status, pregestational diabetes, and chronic hypertension. A lasso cross-validation method was used for selection of placental analytes for inclusion in the logistic regression models, allowing for interaction terms with gestational age, and offset for baseline characteristics. The placental analytes were transformed prior to implementation of

the cross-validation. Power transformations were used to normalize the data, followed by regression on gestational age to generate studentized residuals.

Table 1.

Descriptive characteristics by sample population.

Descriptive Characteristics	Sample Population		p-value[1]
	Case (N = 1502)	Control (N = 911)	
Maternal age, in years			
Mean (standard deviation)	26.6 (6.0)	27.0 (5.6)	0.0664
Category: n (%)			0.0111
13-21	375 (25.0)	191 (21.0)	
22-35	1004 (66.9)	662 (72.7)	
>35	122 (8.1)	58 (6.4)	
Maternal race: n (%)			<.0001
White Non-Hispanic	798 (53.2)	583 (64.0)	
Black Non-Hispanic	302 (20.1)	112 (12.3)	
Hispanic	258 (17.2)	146 (16.0)	
Asian	53 (3.5)	28 (3.1)	
Other	90 (6.0)	42 (4.6)	
Early Pregnancy BMI, in kg/m^2			
Mean (standard deviation)	27.6 (7.3)	25.9 (5.9)	<.0001
Category: n (%)			<.0001
<25	673 (45.9)	498 (55.6)	
25 to <30	366 (24.9)	229 (25.6)	
≥30	428 (29.2)	169 (18.9)	
Smoked during 3 months prior to pregnancy: n (%)	324 (21.6)	135 (14.8)	<.0001
Chronic hypertension: n (%)	86 (5.7)	12 (1.3)	<.0001
Pre-gestational diabetes: n (%)	68 (4.5)	6 (0.7)	<.0001
Education status attained: n (%)			<.0001
Less than high school	179 (11.9)	69 (7.6)	
Completed high school or GED	217 (14.5)	107 (11.7)	
Some college	327 (21.8)	167 (18.3)	
Associate or technical degree	169 (11.3)	82 (9.0)	
Completed college	339 (22.6)	259 (28.4)	
Degree work beyond college	269 (17.9)	227 (24.9)	
Gestational Age at V1, in weeks			
Mean (standard deviation)	11.6 (1.5)	11.6 (1.4)	0.2382
Gestational Age at V2, in weeks			
Mean (standard deviation)	18.6 (1.6)	18.5 (1.5)	0.2838
Gestational Age at Birth, in weeks[2]			
Mean (standard deviation)	35.9 (3.9)	39.3 (1.2)	-
Birth weight, in gm[2]			
Mean (standard deviation)	2571.9 (753.5)	3421.5 (409.8)	-
Small for gestational age[2] (<5th percentile): n (%)	406 (27.3)	0 (0)	-

Descriptive Characteristics	Sample Population		p-value[1]
	Case (N = 1502)	Control (N = 911)	
Small for Gestational Age[2] (<10th percentile): n (%)	523 (35.2)	47 (5.2)	-

[1] P-values are shown for chi-square tests and from ANOVA F-tests.

[2] P-values not shown since preterm birth and small for gestational age birth are defined as cases.

Table 2.

Median (25th, 75th percentiles) for placental analytes by pregnancy outcome group and study visit.

Placental analyte measurements are summarized on their original scale. Two analytes were measured by enzyme-linked immunosorbent assays (ELISA): Eng and ADAM12. Two analytes were measured by electrochemiluminescence assays (ECL): VEGF and sFlt-1. Five analytes were measured by lanthanide-based time resolved fluorometry (TRF): PlGF, PAPP-A, inhibin, βHCG, and AFP.

		Adverse Pregnancy Outcome (N=1,502)						
Analyte	**No Adverse Pregnancy Outcome (N=911)**	**Preterm Birth (N=800)**	**Spontaneous Preterm Birth (N=466)**	**Indicated Preterm Birth (N=330)**	**Preeclampsia (N=568)**	**Preeclampsia or (Gestational Hypertension w/ Preterm Birth) (N=630)**	**Small for Gestational Age (N=406)**	**Stillbirth (N=49)**
Visit 1	**(N=866)**	**(N=768)**	**(N=449)**	**(N=315)**	**(N=540)**	**(N=599)**	**(N=386)**	**(N=49)**
sFlt-1 (pg/mL)	931 (706, 1203)	830 (622, 1133)*	883 (646, 1198)*	805 (602, 1092)*	838 (626, 1114)*	833 (615, 1129)*	793 (615, 1159)*	900(711, 1163)
Adam-12 (ng/mL)	4.9 (3.7, 6.2)	4.5 (3.3, 5.8)*	4.6 (3.5, 6.0)	4.2 (2.9, 5.5)*	4.5 (3.3, 5.7)*	4.5 (3.2, 5.7)*	4.4 (3.4, 5.7)*	4.5 (2.9, 5.5)*
Endoglin (ng/mL)	6.3 (5.4, 7.4)	6.3 (5.4, 7.6)	6.4 (5.5, 7.5)	6.2 (5.2, 7.7)	6.3 (5.3, 7.7)	6.3 (5.2, 7.7)	6.5 (5.4, 7.6)	5.9 (4.8, 7.8)
VEGF (pg/mL)	0.72 (0.51, 1.09)	0.80 (0.54, 1.33)*	0.77 (0.53, 1.22)	0.87 (0.54, 1.66)*	0.88 (0.57, 1.57)*	0.88 (0.57, 1.64)*	0.84 (0.57, 1.50)*	0.81 (0.54, 1.38)
BhCG (ng/mL)	20.9 (13.8, 31.9)	19.9 (13.0, 31.0)	20.2 (13.4, 31.4)	19.6 (12.4, 30.7)	19.5 (12.1, 29.9)*	19.3 (11.9, 29.7)*	20.9 (13.3, 30.4)	22.1 (14.7, 40.2)
AFP (IU/mL)	12.9 (8.1, 18.9)	14.3 (8.2, 20.8)*	15.0 (8.6, 21.9)*	13.4 (7.4, 19.8)	13.5 (8.4, 19.3)	13.7 (8.4, 19.4)	15.4 (9.4, 22.5)*	13.0 (8.4, 21.3)
PlGF (pg/mL)	41.9 (29.6, 58.0)	38.1 (27.3, 55.1)*	40.4 (29.5, 57.2)	34.3 (25.1, 50.9)*	37.4 (26.9, 52.6)*	37.2 (27.0, 51.9)*	36.9 (24.1, 55.2)*	39.7 (27.2, 61.7)
Inhibin A (pg/mL)	306 (224, 424)	314 (227, 432)	313 (231, 430)	320 (223, 440)	313 (225, 453)	311 (221, 446)	317 (219, 450)	307 (206, 441)
PAPP-A (mU/mL)	1012 (485, 1973)	819 (328, 1661)*	926 (406, 1846)	692 (249, 1430)*	776 (335, 1604)*	774 (322, 1629)*	801 (327, 1418)*	781 (287, 1430)*
sFlt-1/PlGF Ratio	22.4 (14.4, 32.1)	22.0 (14.0, 32.7)	21.2 (13.6, 31.6)	22.6 (14.9, 33.9)	22.0 (15.3, 33.1)	21.9 (15.2, 33.2)	21.6 (13.8, 34.1)	20.9 (15.9, 33.4)
Visit 2	**(N=839)**	**(N=713)**	**(N=418)**	**(N=291)**	**(N=514)**	**(N=571)**	**(N=370)**	**(N=42)**
sFlt-1 (pg/mL)	895 (648, 1213)	868 (614, 1224)	866 (605, 1239)	887 (625, 1217)	848 (594, 1178)*	845 (589, 1178)*	829 (611, 1191)	947 (776, 1364)
Adam-12 (ng/mL)	9.9 (7.9, 12.1)	9.9 (7.6, 12.5)	10.1 (7.6, 12.6)	9.7 (7.3, 12.3)	9.9 (7.7, 12.4)	9.8 (7.7, 12.4)	9.8 (7.8, 12.3)	8.9 (6.4, 12.8)

Analyte	No Adverse Pregnancy Outcome (N=911)	Adverse Pregnancy Outcome (N=1,502)						
		Preterm Birth (N=800)	Spontaneous Preterm Birth (N=466)	Indicated Preterm Birth (N=330)	Preeclampsia (N=568)	Preeclampsia or (Gestational Hypertension w/ Preterm Birth) (N=630)	Small for Gestational Age (N=406)	Stillbirth (N=49)
Endoglin (ng/mL)	5.3 (4.6, 6.1)	5.6 (4.8, 6.8) *	5.4 (4.7, 6.4) *	6.0 (4.9, 7.4) *	5.6 (4.7, 6.8) *	5.6 (4.7, 6.8) *	5.7 (4.9, 7.0) *	5.5 (4.9, 6.4)
VEGF (pg/mL)	0.91 (0.64, 1.46)	0.96 (0.66, 1.70)	0.97 (0.70, 1.61) *	0.94 (0.60, 1.79)	1.09 (0.67, 1.92) *	1.07 (0.68, 1.94) *	0.95 (0.61, 1.67)	0.92 (0.58, 1.34)
BhCG (ng/mL)	4.1 (2.7, 6.4)	4.2 (2.6, 6.5)	4.0 (2.4, 6.3)	4.3 (2.9, 7.0)	3.9 (2.4, 6.2)	3.8 (2.4, 6.4)	4.1 (2.4, 6.9)	4.7 (2.5, 7.2)
AFP (IU/mL)	44.2 (34.1, 57.7)	47.3 (35.9, 62.7) *	49.1 (37.2, 63.8) *	45.2 (34.7, 61.3)	45.3 (34.7, 61.4)	45.3 (34.9, 60.9)	49.6 (37.2, 65.9) *	42.4 (30.7, 58.4)
PlGF (pg/mL)	195.2 (133.8, 282.7)	179.9 (115.4, 284.3) *	209.2 (137.2, 320.9) *	145.2 (80.6, 223.5) *	161.1 (104.1, 240.7) *	162.6 (103.7, 240.7) *	152.3 (95.5, 241.5) *	166.2 (90.9, 313.6)
Inhibin A (pg/mL)	196 (155, 254)	214 (166, 293) *	204 (154, 273)	236 (178, 330) *	215 (163, 294) *	215 (161, 293) *	212 (158, 294) *	235 (173, 299)
PAPP-A (mU/mL)	9418 (5530, 14615)	9165 (4986, 16029)	9463 (5578, 16703)	7660 (4077, 15443) *	8232 (4350, 14907) *	8292 (4417, 14918) *	8459 (4833, 14099) *	7516 (4035, 15416)
sFlt-1/PlGF Ratio	4.5 (2.9, 7.1)	4.7 (2.9, 7.8)	4.1 (2.6, 6.4) *	5.8 (3.7, 10.1) *	5.1 (3.1, 8.9) *	5.1 (3.1, 8.8) *	5.2 (3.3, 9.0) *	5.1 (3.5, 8.9)

An asterisk (*) and shading indicates that the comparison between the adverse pregnancy group and the no adverse pregnancy group is significant at an alpha=0.05 level of significance based on Wilcoxon rank-sum test. Green shading is used for analytes that are higher for the adverse pregnancy group. Yellow shading is used for analytes that are lower for the adverse pregnancy group. Sample sizes vary by visit and by analyte. The table header gives the number of participants with one or more of the placental analyte measurements for each pregnancy outcome group across visits. The number of participants by visit for each group are given within the table.

Table 3.

Odds ratios (ORs) and areas under receiver operating characteristic curves (AUCs) for association of selected adverse pregnancy outcomes and placental analytes by study visit.

Analyses are based on logistic regression models for cases of adverse pregnancy outcome versus controls from a random sub-cohort. Power transformation were used to normalize the placental analytes and subsequently regress on gestational age to generate studentized residuals for inclusion in the logistic regression models for predicting the adverse pregnancy outcome. Odds ratios and AUCs are marked as significant at an alpha=0.05 based on Wald chi-square tests.

		Adverse Pregnancy Outcome						
Analyte	**Statistic**	**Preterm Birth**	**Spontaneous Preterm Birth**	**Indicated Preterm Birth**	**Preeclampsia**	**Preeclampsia or (Gestational Hypertension w/ Preterm Birth)**	**Small for Gestational Age**	**Stillbirth**
Visit 1								
sFlt-1 (pg/mL)	OR (95% CI)	0.80 (0.72, 0.89)†	0.87 (0.77, 0.98)†	0.73 (0.64, 0.84)†	0.80 (0.72, 0.90)†	0.80 (0.71, 0.89)†	0.76 (0.67, 0.86)†	0.88 (0.64, 1.20)
	AUC (95% CI)	0.56 (0.53, 0.58)≠	0.53 (0.50, 0.57)≠	0.58 (0.55, 0.62)≠	0.56 (0.53, 0.59)≠	0.56 (0.53, 0.59)≠	0.57 (0.54, 0.61)≠	0.53 (0.45, 0.61)
Adam-12 (ng/mL)	OR (95% CI)	0.86 (0.77, 0.96)†	0.97 (0.85, 1.10)	0.71 (0.61, 0.83)†	0.75 (0.66, 0.85)†	0.75 (0.67, 0.85)†	0.74 (0.64, 0.86)†	0.70 (0.49, 0.99)†
	AUC (95% CI)	0.55 (0.52, 0.57)≠	0.51 (0.48, 0.55)	0.59 (0.55, 0.63)≠	0.57 (0.54, 0.60)≠	0.57 (0.54, 0.60)≠	0.57 (0.53, 0.60)≠	0.58 (0.49, 0.67)
Endoglin (ng/mL)	OR (95% CI)	1.01 (0.92, 1.11)	1.04 (0.93, 1.18)	0.95 (0.84, 1.09)	0.97 (0.87, 1.08)	0.96 (0.87, 1.07)	1.03 (0.91, 1.16)	0.87 (0.64, 1.18)
	AUC (95% CI)	0.51 (0.48, 0.53)	0.51 (0.48, 0.54)	0.51 (0.47, 0.55)	0.51 (0.48, 0.54)	0.51 (0.48, 0.54)	0.51 (0.48, 0.55)	0.55 (0.46, 0.64)
VEGF (pg/mL)	OR (95% CI)	1.16 (1.05, 1.27)*	1.06 (0.95, 1.19)	1.29 (1.14, 1.45)*	1.32 (1.19, 1.47)*	1.35 (1.22, 1.49)*	1.27 (1.13, 1.43)*	1.14 (0.86, 1.51)
	AUC (95% CI)	0.54 (0.51, 0.57)≠	0.52 (0.48, 0.55)	0.57 (0.53, 0.60)≠	0.58 (0.55, 0.61)≠	0.58 (0.55, 0.61)≠	0.57 (0.53, 0.60)≠	0.53 (0.45, 0.62)
BhCG (ng/mL)	OR (95% CI)	0.81 (0.73, 0.91)†	0.84 (0.74, 0.95)†	0.76 (0.66, 0.88)†	0.81 (0.71, 0.91)†	0.79 (0.70, 0.89)†	0.96 (0.84, 1.10)	1.13 (0.80, 1.59)
	AUC (95% CI)	0.54 (0.52, 0.57)≠	0.53 (0.50, 0.56)	0.56 (0.53, 0.60)≠	0.55 (0.52, 0.58)≠	0.56 (0.53, 0.59)≠	0.51 (0.47, 0.54)	0.53 (0.43, 0.62)
AFP (IU/mL)	OR (95% CI)	1.37 (1.23, 1.52)*	1.48 (1.31, 1.67)*	1.20 (1.04, 1.38)*	0.97 (0.85, 1.09)	1.01 (0.90, 1.14)	1.39 (1.22, 1.59)*	1.44 (1.10, 1.88)*
	AUC (95% CI)	0.57 (0.55, 0.60)≠	0.60 (0.56, 0.63)≠	0.53 (0.49, 0.57)	0.50 (0.47, 0.53)	0.51 (0.48, 0.54)	0.58 (0.55, 0.62)†	0.56 (0.47, 0.65)

Analyte	Statistic	Adverse Pregnancy Outcome						
		Preterm Birth	**Spontaneous Preterm Birth**	**Indicated Preterm Birth**	**Preeclampsia**	**Preeclampsia or (Gestational Hypertension w/ Preterm Birth)**	**Small for Gestational Age**	**Stillbirth**
PlGF (pg/mL)	OR (95% CI)	0.99 (0.87, 1.13)	1.16 (0.99, 1.35)	0.79 (0.65, 0.95)†	0.74 (0.63, 0.86)†	0.74 (0.64, 0.86)†	0.77 (0.66, 0.92)†	1.05 (0.68, 1.63)
	AUC (95% CI)	0.51 (0.48, 0.54)	0.52 (0.49, 0.56)	0.56 (0.52, 0.59)≠	0.56 (0.53, 0.59)≠	0.56 (0.53, 0.59)≠	0.55 (0.52, 0.59)†	0.52 (0.44, 0.60)
Inhibin A (pg/mL)	OR (95% CI)	0.97 (0.88, 1.06)	0.96 (0.86, 1.07)	0.97 (0.86, 1.10)	0.99 (0.89, 1.10)	0.97 (0.88, 1.07)	1.03 (0.91, 1.16)	0.94 (0.71, 1.24)
	AUC (95% CI)	0.50 (0.48, 0.53)	0.50 (0.47, 0.53)	0.51 (0.47, 0.54)	0.49 (0.46, 0.52)	0.50 (0.47, 0.53)	0.51 (0.47, 0.54)	0.53 (0.44, 0.62)
PAPP-A (mU/mL)	OR (95% CI)	0.88 (0.77, 0.99)†	1.08 (0.93, 1.25)	0.64 (0.54, 0.77)†	0.69 (0.60, 0.80)†	0.70 (0.61, 0.81)†	0.62 (0.52, 0.73)†	0.75 (0.51, 1.12)
	AUC (95% CI)	0.54 (0.51, 0.57)≠	0.51 (0.48, 0.54)	0.61 (0.57, 0.64)≠	0.59 (0.56, 0.62)≠	0.59 (0.56, 0.62)≠	0.60 (0.57, 0.64)≠	0.58 (0.50, 0.66)
sFlt-1/PlGF Ratio	OR (95% CI)	0.84 (0.73, 0.96)†	0.78 (0.67, 0.91)†	0.92 (0.77, 1.10)	1.07 (0.93, 1.24)	1.06 (0.92, 1.22)	0.95 (0.81, 1.12)	0.84 (0.55, 1.28)
	AUC (95% CI)	0.53 (0.50, 0.56)≠	0.54 (0.51, 0.57)≠	0.51 (0.48, 0.55)	0.51 (0.48, 0.54)	0.51 (0.48, 0.54)	0.51 (0.48, 0.55)	0.55 (0.46, 0.63)
Visit 2								
sFlt-1 (pg/mL)	OR (95% CI)	0.96 (0.87, 1.05)	0.93 (0.83, 1.04)	0.97 (0.86, 1.10)	0.88 (0.79, 0.97)†	0.88 (0.79, 0.97)†	0.91 (0.81, 1.02)	1.26 (0.93, 1.70)
	AUC (95% CI)	0.51 (0.49, 0.54)	0.52 (0.49, 0.55)	0.51 (0.47, 0.55)	0.54 (0.50, 0.57)≠	0.54 (0.51, 0.57)≠	0.53 (0.50, 0.57)	0.56 (0.48, 0.65)
Adam-12 (ng/mL)	OR (95% CI)	1.01 (0.93, 1.11)	1.02 (0.92, 1.13)	0.98 (0.87, 1.10)	0.92 (0.84, 1.02)	0.93 (0.85, 1.02)	0.96 (0.86, 1.07)	0.85 (0.65, 1.13)
	AUC (95% CI)	0.50 (0.47, 0.53)	0.50 (0.47, 0.54)	0.51 (0.47, 0.55)	0.52 (0.49, 0.56)	0.52 (0.49, 0.55)	0.52 (0.48, 0.55)	0.56 (0.46, 0.66)
Endoglin (ng/mL)	OR (95% CI)	1.30 (1.18, 1.44)*	1.14 (1.01, 1.28)*	1.51 (1.33, 1.72)*	1.29 (1.16, 1.44)*	1.28 (1.16, 1.43)*	1.38 (1.22, 1.56)*	1.21 (0.88, 1.66)
	AUC (95% CI)	0.57 (0.54, 0.60)≠	0.53 (0.50, 0.56)	0.61 (0.57, 0.65)≠	0.56 (0.53, 0.60)≠	0.56 (0.53, 0.59)≠	0.59 (0.55, 0.62)≠	0.54 (0.45, 0.63)
VEGF (pg/mL)	OR (95% CI)	1.07 (0.97, 1.19)	1.11 (0.98, 1.25)	1.05 (0.92, 1.20)	1.21 (1.08, 1.35)*	1.22 (1.10, 1.36)*	1.03 (0.91, 1.17)	0.95 (0.69, 1.31)
	AUC (95% CI)	0.52 (0.49, 0.55)	0.53 (0.50, 0.56)	0.51 (0.47, 0.55)	0.56 (0.52, 0.59)≠	0.56 (0.53, 0.59)≠	0.51 (0.47, 0.54)	0.51 (0.42, 0.60)
BhCG (ng/mL)	OR (95% CI)	0.97 (0.89, 1.06)	0.91 (0.82, 1.02)	1.04 (0.92, 1.18)	0.93 (0.84, 1.03)	0.93 (0.85, 1.03)	1.02 (0.91, 1.14)	1.00 (0.74, 1.35)

Analyte	Statistic	Adverse Pregnancy Outcome: Preterm Birth	Spontaneous Preterm Birth	Indicated Preterm Birth	Preeclampsia	Preeclampsia or (Gestational Hypertension w/ Preterm Birth)	Small for Gestational Age	Stillbirth
	AUC (95% CI)	0.50 (0.47, 0.53)	0.52 (0.48, 0.55)	0.52 (0.48, 0.56)	0.52 (0.49, 0.55)	0.52 (0.49, 0.55)	0.50 (0.46, 0.53)	0.50 (0.41, 0.59)
AFP (IU/mL)	OR (95% CI)	1.24 (1.12, 1.37)*	1.25 (1.11, 1.41)*	1.20 (1.05, 1.37)*	1.03 (0.92, 1.14)	1.04 (0.93, 1.15)	1.36 (1.20, 1.54)*	1.14 (0.83, 1.55)
	AUC (95% CI)	0.55 (0.52, 0.58)≠	0.56 (0.52, 0.59)≠	0.53 (0.49, 0.57)	0.49 (0.46, 0.52)	0.50 (0.47, 0.53)	0.57 (0.54, 0.61)≠	0.49 (0.39, 0.58)
PlGF (pg/mL)	OR (95% CI)	0.92 (0.85, 1.00)†	1.17 (1.06, 1.30)*	0.67 (0.60, 0.75)†	0.69 (0.63, 0.77)†	0.70 (0.64, 0.77)†	0.71 (0.64, 0.78)†	0.80 (0.64, 1.01)
	AUC (95% CI)	0.54 (0.51, 0.56)≠	0.54 (0.51, 0.57)≠	0.64 (0.60, 0.68)≠	0.62 (0.59, 0.65)≠	0.62 (0.59, 0.65)≠	0.62 (0.59, 0.66)≠	0.54 (0.44, 0.64)
Inhibin A (pg/mL)	OR (95% CI)	1.29 (1.15, 1.45)*	1.11 (0.98, 1.26)	1.67 (1.41, 1.97)*	1.33 (1.16, 1.51)*	1.29 (1.13, 1.46)*	1.30 (1.13, 1.49)*	1.43 (0.98, 2.09)
	AUC (95% CI)	0.57 (0.54, 0.60)≠	0.53 (0.49, 0.56)	0.62 (0.58, 0.66)≠	0.56 (0.53, 0.59)≠	0.56 (0.53, 0.59)≠	0.55 (0.51, 0.58)≠	0.58 (0.48, 0.68)
PAPP-A (mU/mL)	OR (95% CI)	0.98 (0.90, 1.06)	1.04 (0.94, 1.15)	0.88 (0.79, 0.98)†	0.85 (0.78, 0.94)†	0.86 (0.79, 0.94)†	0.84 (0.75, 0.93)†	0.85 (0.65, 1.11)
	AUC (95% CI)	0.51 (0.48, 0.54)	0.51 (0.47, 0.54)	0.54 (0.50, 0.58)	0.56 (0.52, 0.59)≠	0.55 (0.52, 0.58)≠	0.56 (0.52, 0.59)≠	0.54 (0.44, 0.64)
sFlt-1/PlGF Ratio	OR (95% CI)	1.04 (0.96, 1.13)	0.83 (0.75, 0.92)†	1.39 (1.25, 1.55)*	1.24 (1.13, 1.36)*	1.23 (1.13, 1.35)≠	1.25 (1.13, 1.39)*	1.32 (1.05, 1.67)*
	AUC (95% CI)	0.51 (0.48, 0.54)	0.55 (0.52, 0.59)≠	0.60 (0.56, 0.64)≠	0.57 (0.54, 0.60)≠	0.57 (0.54, 0.60)≠	0.57 (0.54, 0.61)≠	0.55 (0.46, 0.65)

*Indicates increased odds for higher values of the analyte (shaded green);

†indicates increased odds for lower values of the analyte (shaded yellow); and

≠indicates a significant AUC (shading consistent with the direction of the odds ratio)