OBSTETRICS

Toward a new taxonomy of obstetrical disease: improved performance of maternal blood biomarkers for the great obstetrical syndromes when classified according to placental pathology

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BACKGROUND: The major challenge for obstetrics is the prediction and prevention of the great obstetrical syndromes. We propose that defining obstetrical diseases by the combination of clinical presentation and disease mechanisms as inferred by placental pathology will aid in the discovery of biomarkers and add specificity to those already known.

OBJECTIVE: To describe the longitudinal profile of placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and the PlGF/sFlt-1 ratio throughout gestation, and to determine whether the association between abnormal biomarker profiles and obstetrical syndromes is strengthened by information derived from placental examination, eg, the presence or absence of placental lesions of maternal vascular malperfusion.

STUDY DESIGN: This retrospective case cohort study was based on a parent cohort of 4006 pregnant women enrolled prospectively. The case cohort of 1499 pregnant women included 1000 randomly selected patients from the parent cohort and all additional patients with obstetrical syndromes from the parent cohort. Pregnant women were classified into six groups: 1) term delivery without pregnancy complications (n=540; control); 2) preterm labor and delivery (n=203); 3) preterm premature rupture of the membranes (n=112); 4) preeclampsia (n=230); 5) small-for-gestational-age neonate (n=334); and 6) other pregnancy complications (n=182). Maternal plasma concentrations of PlGF and sFlt-1 were determined by enzyme-linked immunosorbent assays in 7560 longitudinal samples. Placental pathologists, masked to clinical outcomes, diagnosed the presence or absence of placental lesions of maternal vascular malperfusion. Comparisons between mean biomarker concentrations in cases and controls were performed by utilizing longitudinal generalized additive models. Comparisons were made between controls and each obstetrical syndrome with and without subclassifying cases according to the presence or absence of placental lesions of maternal vascular malperfusion.

RESULTS: 1) When obstetrical syndromes are classified based on the presence or absence of placental lesions of maternal vascular malperfusion, significant differences in the mean plasma concentrations of PlGF, sFlt-1, and the PlGF/sFlt-1 ratio between cases and controls emerge earlier in gestation; 2) the strength of association between an abnormal PlGF/sFlt-1 ratio and the occurrence of obstetrical syndromes increases when placental lesions of maternal vascular malperfusion are present (adjusted odds ratio [aOR], 13.6 vs 6.7 for preeclampsia; aOR, 8.1 vs 4.4 for small-for-gestational-age neonates; aOR, 5.5 vs 2.1 for preterm premature rupture of the membranes; and aOR, 3.3 vs 2.1 for preterm labor (all $P<0.05$); and 3) the PlGF/sFlt-1 ratio at 28 to 32 weeks of gestation is abnormal in patients who subsequently delivered due to preterm labor with intact membranes and in those with preterm premature rupture of the membranes if both groups have placental lesions of maternal vascular malperfusion. Such association is not significant in patients with these obstetrical syndromes who do not have placental lesions.

CONCLUSION: Classification of obstetrical syndromes according to the presence or absence of placental lesions of maternal vascular malperfusion allows biomarkers to be informative earlier in gestation and enhances the strength of association between biomarkers and clinical outcomes. We propose that a new taxonomy of obstetrical disorders informed by placental pathology will facilitate the discovery and implementation of biomarkers as well as the prediction and prevention of such disorders.

Key words: angiogenic index-1, classification of disease, fetal death, liquid biopsy, omics, placental growth factor, placental lesions of maternal vascular malperfusion, preeclampsia, pregnancy, preterm birth, preterm labor, preterm premature rupture of the membranes, small for gestational age, soluble fms-like tyrosine kinase-1, soluble vascular endothelial growth factor receptor-1

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Introduction

The outcome of pregnancy has improved through advances in the treatment of hemorrhage, infection, and hypertension.[1–3] Such progress has depended on leveraging knowledge generated in other fields of medicine; however, this approach has not been helpful in addressing obstetrical syndromes such as spontaneous premature labor, preeclampsia, fetal growth restriction, and fetal death. Obstetrical disorders are unique in nature and represent the clinical manifestations of adaptive responses to insults inflicted during pregnancy. Yet, when biomarkers in early pregnancy are coupled with specific interventions, prevention has been effective in spontaneous preterm labor and preeclampsia.[4–13] We have argued that a body of knowledge, specific to pregnancy and to the pathophysiology of obstetrical complications, needs to be generated for progress to occur.

A major obstacle to progress has been the classification of obstetrical diseases.

AJOG at a Glance

Why was this study conducted?

The current taxonomy of obstetrical disease, eg, preeclampsia, preterm labor, preterm premature rupture of the membranes, small-for-gestational-age fetus, fetal death, etc., is exclusively based on symptoms and signs and does not provide etiological information. Understanding the causes of disease is essential for progress. Biomarkers that reflect the pathophysiology of a disease process have become powerful tools to predict, diagnose, and monitor response to therapy. We propose that a new taxonomy of obstetrical syndromes, which incorporates information derived from placental pathology, would facilitate the discovery of biomarkers. This study was conducted to examine whether subclassification of obstetrical syndromes, according to the presence or absence of placental lesions, would improve the performance of biomarkers.

Key findings?

When obstetrical syndromes are defined according to the combination of clinical signs and the presence or absence of placental lesions of maternal vascular malperfusion, rather than by clinical signs alone, a maternal blood biomarker (eg, placental growth factor, soluble fms-like tyrosine kinase-1, or the PlGF/sFlt-1 ratio) (1) becomes informative earlier in gestation; (2) has a higher adjusted odds ratio, indicating a stronger magnitude of association with the development of each obstetrical syndrome; and (3) becomes significantly associated with the development of preterm labor and preterm premature rupture of the membranes, conditions not previously thought to be predicted by such biomarkers.

What does this add to what is known?

We present evidence to support the formulation of a new taxonomy of obstetrical syndromes in which conditions are defined by the combination of clinical symptoms and signs as well as indicators of disease pathophysiology, ie, the presence or absence of placental lesions. Such taxonomy would improve the characterization of obstetrical disorders by including information about the mechanisms of disease as reflected in placental pathology. We propose that this approach creates more homogeneous syndrome-subsets, which can facilitate the discovery and implementation of biomarkers, and provides a new framework to test therapeutic interventions and preventive strategies.

The current taxonomy of obstetrical disorders is largely based on symptoms and signs, which are inadequate descriptors of disease because they are nonspecific and, importantly, represent the late manifestations of pathologic processes. The terms "preterm labor," "preterm premature rupture of the membranes" (PPROM), "preeclampsia," "small for gestational age" (SGA), "fetal death," "macrosomia," etc., provide no information about the causes of these conditions[14,15]; therefore, it is not surprising that therapeutic approaches in obstetrics have not focused on the underlying causes of disease but rather on symptoms, eg, tocolysis to decrease uterine contractility in spontaneous preterm birth or antihypertensive agents in preeclampsia.[14,15]

A rational approach to obstetrical disorders should be based on the understanding of the etiological explanations and the mechanisms of disease. The roadmap used by other disciplines can provide insights for obstetrics. Indeed, progress in other fields of medicine has been made largely through knowledge derived from anatomical pathology by analyzing tissues obtained from autopsies and biopsies.[16–19] For example, molecular profiling of tissues allows the classification of tumors and identification of biomarkers.[20–27] In obstetrics, insights into the mechanisms of disease responsible for pregnancy complications can be derived from examining the placenta.[28–41] This organ contains fetal and maternal components,[42] has been considered "a diary of intrauterine life,"[43,44] is universally available after delivery, and represents a large human biopsy.[45] Liquid biopsies in pregnancy promise to allow minimally invasive assessment of placental function and pathology.

The two major placental pathologic processes responsible for adverse pregnancy outcomes are of inflammatory[46–55] and of vascular nature.[56-68] This is not surprising given that successful reproduction requires profound adaptations of two maternal systems, cardiovascular and immunologic. Indeed, hemochorial placentation demands remodeling of the uterine circulation and, in particular, of the spiral arteries. Failure of transformation of the spiral arteries predisposes to placental lesions of maternal vascular malperfusion (MVM),[69-72] which have been identified in virtually all major obstetrical syndromes.[57–60,73–76] Similarly, pregnancy imposes fundamental changes in the immune system to manage the risk of infection[77–79] in the context of a semiallogenic pregnancy. Abnormal microbial-host interactions or sterile inflammation can lead to acute and chronic inflammatory lesions of the placenta, often observed in spontaneous preterm labor,[46,48,51,52,80–84] and in other obstetrical syndromes such as fetal death.[85,86] Biomarkers, in the form of liquid biopsies, are now available to diagnose patients with placental acute and chronic inflammatory lesions, eg, interleukin (IL)-6 for acute and CXCL-10 for chronic inflammatory lesions.[47,87–90] Yet, a major unmet need is the antenatal diagnosis of placental vascular pathology.

Ananth and Vintzileos have coined the term "ischemic placental disease" to refer to a cluster of conditions, including preeclampsia, SGA, and placental

abruption,[91−94] which account for 25% of all preterm births.[95] Lesions of maternal vascular malperfusion are the pathologic hallmark of ischemic placental disease and are present in 70% of unexplained fetal deaths,[86,96−100] and with lesser frequency in preeclampsia,[56,101−104] SGA,[102,105,106] preterm labor,[60] and PPROM.[59] Maternal plasma concentrations of placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and the PlGF/sFlt-1 ratio are biomarkers for the presence of these placental lesions[95] and the resulting syndromes: unexplained fetal death,[107−110] preeclampsia,[111−117] and fetal growth restriction.[112,118]

The current study was conducted to compare the profile of PlGF, sFlt-1, and the PlGF/sFlt-1 ratio in maternal blood in patients who present with preeclampsia, SGA, preterm labor, and PPROM and in a control group, taking into account the presence or absence of placental lesions of MVM. To accomplish this goal, we first examined the change in the longitudinal profile of these analytes in obstetrical syndromes defined only by clinical presentation; then, we subclassified cases according to the presence or absence of placental lesions. We hypothesized that differences in the mean concentrations of biomarkers between cases and controls would be larger and that the strength of association would be greater when information derived from anatomic pathologic examination of the placenta is considered.

The motivation for this work is our belief that a new taxonomy of obstetrical disorders, based on the underlying causes and mechanisms of diseases and informed by the results of pathologic examination of the placenta, will contribute to the progress of obstetrics.

Materials and Methods

Study design and participants

This retrospective case-cohort study was based on a parent cohort of 4006 pregnant women enrolled prospectively.[95] The median gestational age (GA) at enrollment was 12.3 weeks (interquartile range [IQR], 9.0−16.9). The case-cohort study (n=1499) included 1000 randomly selected patients from the parent cohort and all additional patients with obstetrical syndromes from the parent cohort. The large fraction (1000 of 4006 [25%]) of patients selected at random from the parent cohort surpassed the minimum recommended (15%) so that the risk estimates based on the case-cohort study would be similar to those obtained based on the full parent cohort.[117,118] Patients were classified into the following groups: (1) term delivery without pregnancy complications (control), (2) preterm labor and delivery (PTL), (3) PPROM, (4) preeclampsia, and (5) delivery of an SGA neonate; some of these pregnancy complications co-occurred (see below). Co-occurrence of syndromes included instances of preeclampsia with SGA (n=68), PTL with SGA (n=19), and PPROM with SGA (n=9), among others. Of note, the cohort also included 24 cases of fetal death. Of these cases, 13 were assigned to the group of other obstetrical complications (n=182) (Table 1), whereas the remainder of 11 cases was complicated by one or more obstetrical syndromes that are the primary focus of this study.

The exclusion criteria included multiple gestation, active vaginal bleeding, severe maternal morbidity (eg, renal insufficiency, congestive heart disease, and chronic respiratory insufficiency), active hepatitis, or known fetal chromosomal abnormalities and major congenital anomalies.

All study participants provided written informed consent before study enrollment and were observed until delivery. The use of clinical data and biological specimens obtained from these women for research purposes was approved by the Human Investigation Committee of Wayne State University and the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, US Department of Health and Human Services.

Clinical definitions

Preclampsia was defined as new-onset hypertension and proteinuria developing after 20 weeks of gestation.[119−121] "Hypertension" was defined as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg, measured at least on two occasions, 4 hours to 1 week apart.[120] "Proteinuria" was defined as a urine protein level ≥300 mg in a 24-hour urine collection, or two random urine specimens, obtained 4 hours to 1 week apart, showing ≥1+ by dipstick.[120] "Early preeclampsia" was defined as preeclampsia resulting in a preterm delivery <34 weeks of gestation.[122−124] "Late preeclampsia" was defined as preeclampsia resulting in delivery ≥34 weeks of gestation.[124] SGA neonates included in this study had a birthweight <5th percentile for GA at delivery, according to a US reference population.[125] The diagnosis of "PPROM" was determined by a sterile speculum examination with documentation of either vaginal pooling or a positive nitrazine or ferning test. "Spontaneous preterm labor" was defined as the spontaneous onset of labor with intact membranes and delivery <37 weeks of gestation.

Sample collection and immunoassays for placental growth factor and soluble fms-like tyrosine kinase-1

Blood samples from women at enrollment and during subsequent examinations scheduled every four weeks until the 24th week of gestation, and biweekly thereafter until delivery, were collected in tubes containing ethylenediaminetetraacetic acid solution. Samples were centrifuged and stored at −70°C. Maternal plasma concentrations of PlGF and soluble vascular endothelial growth factor (VEGF) receptor-1 (also known as sFlt-1) were determined by immunoassays, as described previously (R&D Systems, Minneapolis, MN).[112] The

inter-assay and intra-assay coefficients of variation of the assays were 1.4% and 3.9% for sFlt-1 and 6.02% and 4.8% for PlGF, respectively. The sensitivity of the assays was 16.9 pg/mL for sFlt-1 and 9.5 pg/mL for PlGF. Laboratory personnel performing the assays were masked to clinical information.

Placental pathology

Placentas were examined histologically according to standardized protocols by four perinatal pathologists who were masked to clinical diagnoses and obstetrical outcomes, as described elsewhere.[48,126–128] Placental features consistent with MVM were diagnosed by using criteria established by the Perinatal Section of the Society for Pediatric Pathology[126] and the Amsterdam Placental Workshop Group Consensus[129] and included at least one of the following: (1) villous changes, which are further subdivided into abrupt onset (remote villous infarcts, recent villous infarcts), gradual onset with intermediate duration (increased syncytial knots, villous agglutination, increased intervillous fibrin), or gradual onset with prolonged duration (distal villous hypoplasia) and (2) vascular lesions (persistent muscularization of the basal plate arteries, mural hypertrophy of the decidual arterioles, acute atherosis of the basal plate arteries and/or of the decidual arterioles).

Statistical analysis

Longitudinal analysis of biomarker concentrations

PlGF, sFlt-1, and the PlGF/sFlt-1 ratio were first log-transformed[130] to stabilize variance along the GA span. To estimate the mean biomarker concentrations in groups of patients and the differences between cases and controls, we utilized generalized additive models to fit the data as a function of spline transformation terms of GA at blood draw. These models allowed for a smooth relationship between the mean biomarker plasma concentrations and GA while accounting for the within-subject correlated observations. Nonoverlapping 95% confidence intervals (CI) of the mean biomarkers between cases and controls were considered to represent significant differences in mean values at a given GA. Generalized additive models were fit and evaluated with the *mgcv* and *itsadug* packages for the R statistical language and environment (www.r-project.org).

Calculation of multiples of the mean for gestational age and maternal characteristics

Multiple of the mean (MoM) values for PlGF and sFlt-1 were calculated by using data from the random set of 1000 pregnancies, taking into account maternal characteristics and GA.[131] MoM models were previously described for several intervals throughout gestation, yet the choice of the interval of 28 to 32 weeks was informed by the results of the longitudinal analysis. In this analysis, missing information on nulliparity, maternal weight, and smoking status was imputed for 11% of the cases. MoM values were determined as the observed biomarker value for a given sample divided by the mean predicted by multiple regression. MoM cutoff values that define abnormal biomarker values for all conditions were selected as the 20th percentile (for PlGF or the PlGF/sFlt-1 ratio) or the 80th percentile (for sFlt-1) of a normal distribution with mean 0 and standard deviation estimated from the sample. The 20th and 80th percentile cutoff values were chosen to provide a sufficient number of cases with an abnormal PlGF/sFlt-1 ratio for each syndrome and their respective subgroups defined by the presence of placental lesions.

Cross-sectional analysis of biomarker multiple of the mean values

The magnitude of association between obstetrical syndromes and abnormal biomarker profiles was determined by using as reference the pooled group of controls and those with "other complications" who had a live birth and a sample available at the interval of 28 to 32 weeks. The resulting adjusted odds ratios (aOR) and 95% CIs obtained by logistic regression account for possible differences in GA at sampling and in maternal risk factors because the effects of these variables on biomarker data were removed during the calculation of MoM values. In addition, relative risk (RR) estimates were determined by robust Poisson regression,[132] weighting data to reflect the parent cohort as previously described for this case-cohort study.[95] All analyses were carried out by using the R statistical language and environment (www.r-project.org).

Results

Demographic and clinical characteristics of the study population

The frequency of major obstetrical syndromes in the parent cohort (n=4006) was as follows: preeclampsia, 6% (n=230); SGA neonate <5th percentile in birthweight, 8.3% (n=334); PTL, 5% (n=203); and PPROM, 3% (n=112). The demographic characteristics of the case-cohort study (n=1499) are described in Table 1.

Women who presented with PTL or PPROM had a higher mean age than those in the control group ($P<.05$, for both). Maternal weight was lower in women who had PTL or delivered an SGA neonate and higher in those who developed preeclampsia, compared to those in the control group (all, $P<.05$). Moreover, women who developed preeclampsia or SGA had a higher rate of nulliparity, whereas those who developed PTL were more likely to be parous than those in the control group (all, $P<0.05$). Smoking was more common in patients with PPROM or SGA than in those in the control group (all, $P<0.05$).

TABLE 1
Demographic characteristics of the study cohort (n=1499)

Characteristic	Term delivery controls (n=540) {491}	Preterm labor (n=203) {195}	PPROM (n=112) {109}	SGA (n=334) {312}	Preeclampsia (n=230) {216}	Other complications[a] (n=182) {173}
Age (y)	22 (20–26)	23 (21–27)[b]	25 (21–30)[b]	22 (19–28)	23 (19–28)	24 (20–29)[b]
African American	92.3 (453)	92.8 (181)	92.7 (101)	95.2 (297)	95.2 (219)	91.2 (166)
Nulliparity	36.5 (179)	27.2 (53)[b]	31.2 (34)	43.9 (137)[b]	46.1 (106)[b]	45.1 (82)[b]
Weight (kg)[c]	70 (59–82)	66 (57–82)[b]	71 (59–90)	64 (57–77)[b]	77 (64–98.2)[b]	81 (64–100)[b]
Height (cm)	162.6 (160.0–167.6)	162.6 (157.5–167.6)	162.6 (157.5–167.6)	161.3 (157.5–165.1)[b]	165.1 (157.5–167.6)	162.6 (158.1–170.2)
Body mass index (kg/m^2)	26.0 (22.5–31.3)	25.4 (21.7–31.9)	27.6 (22.9–32.4)	25.0 (21.8–30.1)[b]	28.9 (23.8–35.7)[b]	29.7 (24.6–36.5)[b]
Smoking	18.7 (92)	21.5 (42)	30.3 (33)[b]	31.1 (97)[b]	21.7 (50)	18.1 (33)
Chronic hypertension	0 (0)	7.2 (14)[b]	5.5 (6)[b]	10.6 (33)[b]	29.1 (67)[b]	18.1 (33)[b]
Gestational hypertension	0 (0)	4.6 (9)[b]	4.6 (5)[b]	10.6 (33)[b]	0 (0)[b]	40.1 (73)[b]
GA at delivery (wk)	39.6 (38.9–40.4)	35.0 (30.0–36.3)[b]	34.0 (30.0–35.4)[b]	38.5 (36.9–39.4)[b]	37.4 (35.9–39.0)[b]	39.0 (37.8–40.1)[b]
Birthweight (g)	3310 (3072.0–3565.0)	2330 (1377.0–2662.0)[b]	2080 (1340.0–2500.0)[b]	2387 (2100.0–2635.0)[b]	2805 (2141.2–3268.8)[b]	3280 (3005.0–3588.8)
Number of longitudinal samples per patient	5 (5–6)	4 (3–6)	4 (3–6)	5 (4–6)	5 (4–6)	5 (5–6)
GA at sample (wk)	28.1 (21.1–34.4)	24.4 (19.1–29.6)	24.7 (19.3–29.7)	26.7 (20.3–33.9)	26.6 (20.4–32.8)	26.9 (20.7–34.1)

Data are presented as median (interquartile range) or percentage (number). Of note, "n" indicates the number of women with biomarker data, and "{}" indicates the number of women with biomarker data for whom the complete set of demographics characteristics listed were available.

GA, gestational age; *PE*, preeclampsia; *PTL*, preterm labor; *PPROM*, preterm premature rupture of the membranes; *SGA*, small for gestational age.

[a] This group includes women with pregnancy complications other than PTL, PPROM, SGA, and PE; [b] Represents significant difference between the current group and term delivery controls ($P<.05$) assessed with the Wilcoxon test for continuous data and the Fisher's exact test for categorical data. Differences were not assessed for the last 2 variables; [c] Missing data for 96 women.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

Frequency of maternal vascular lesions of malperfusion

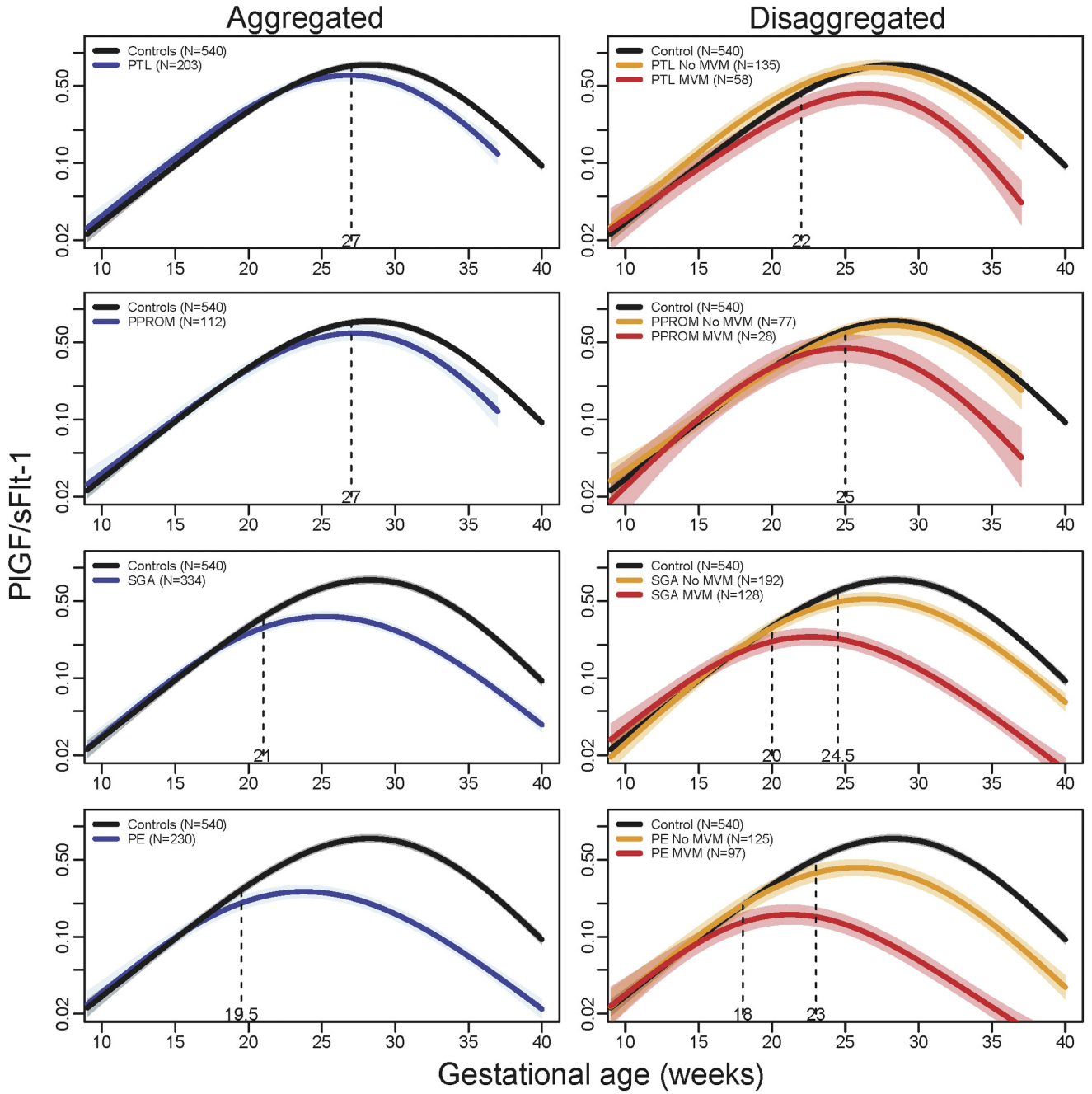
The frequency of placental lesions consistent with maternal vascular lesions of malperfusion was 44% (96/224) in preeclampsia, 40% (128/334) in pregnancies with an SGA neonate, 30% (58/203) in cases of PTL, 27% (28/112) in cases of PPROM, 26% (45/176) in the group labeled as having "other complications of pregnancy," and 15% (80/532) in the control group. Nulliparity was associated with an increase in the risk of delivery of placentas with lesions of MVM (OR, 1.3; 95% CI, 1.01–1.66; $P=.03$).

Biomarkers become informative earlier in gestation when placental lesions are present

PlGF and sFlt-1 were measured in 7560 maternal plasma samples collected throughout gestation from the 1499 women in the study cohort (Table 1). The mean PlGF/sFlt-1 ratio was estimated as a function of GA by using generalized additive models. Longitudinal profiles differed by both clinical presentation and the presence of placental lesions of MVM (Figure 1).

The mean PlGF/sFlt-1 ratio was lower in patients who presented with complications of pregnancy than in those in the control group. The magnitude and timing at which differences were observed between cases and controls varied with the specific obstetrical syndrome and the presence or absence of placental lesions of MVM (Figure 1). Patients who subsequently developed preeclampsia had a lower PlGF/sFlt-1 ratio, starting at 19.5 weeks of gestation; those with SGA at 21 weeks of gestation; and those with PPROM or PTL at 27 weeks of gestation ($P<0.05$, for all comparisons) (Figure 1, left column). However, when syndromes were classified according to the presence of placental lesions of MVM, differences in the mean PlGF/sFlt-1 ratio between the cases with placental lesions of MVM and the controls were detected earlier in

FIGURE 1
Maternal plasma PlGF/sFlt-1 ratio in normal and complicated pregnancies with and without placental lesions of maternal vascular malperfusion

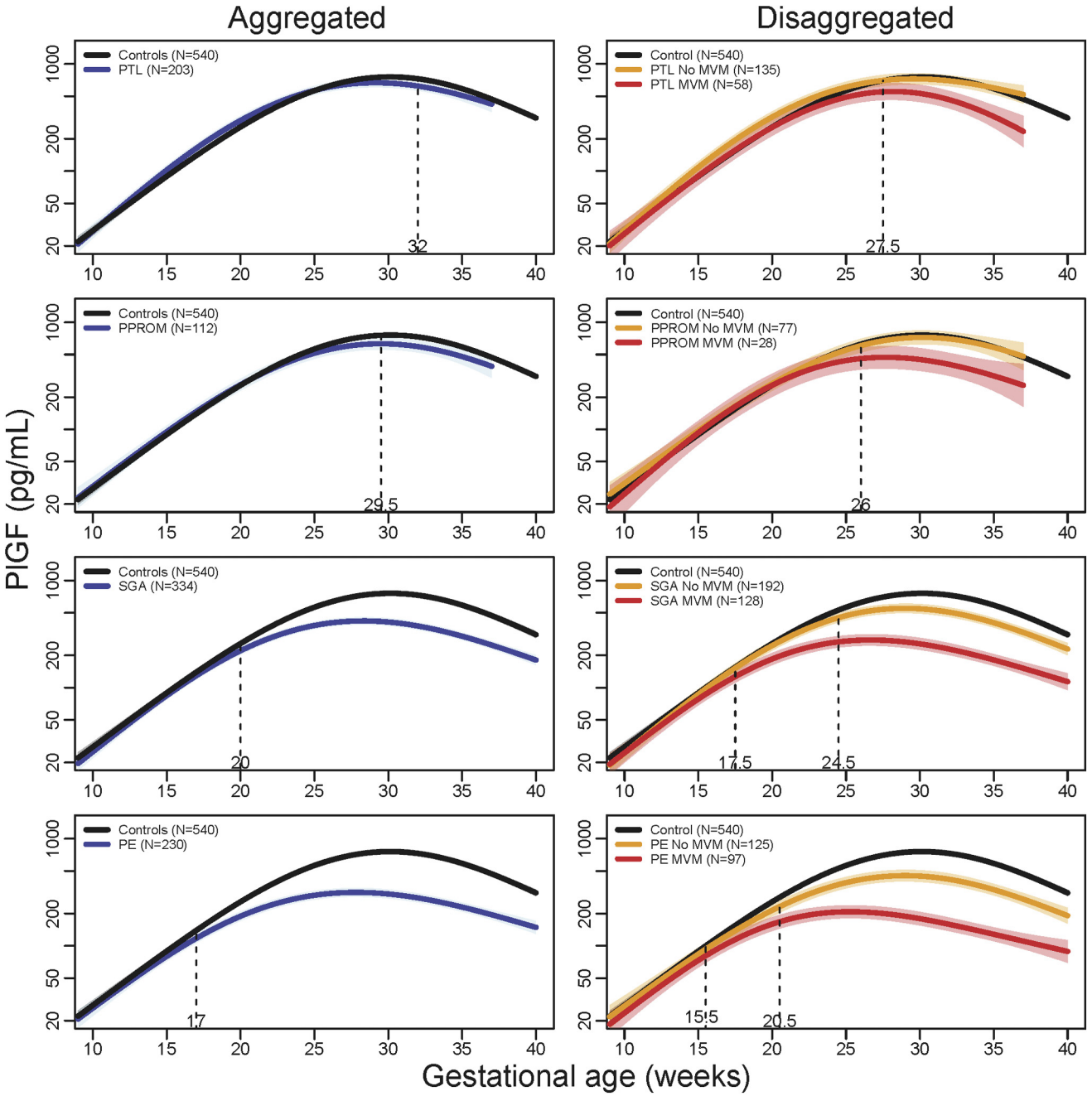


Mean and 95% confidence interval values were estimated by generalized additive models using spline transformations of GA in term delivery controls and complicated pregnancies. *Dotted vertical lines* represent the earliest GA when the PlGF/sFlt-1 ratio is significantly lower in cases than in controls.

GA, gestational age; *MVM*, maternal vascular malperfusion; *PE*, preeclampsia; *PlGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

FIGURE 2
Maternal plasma PIGF in normal and complicated pregnancies with and without placental lesions of maternal vascular malperfusion

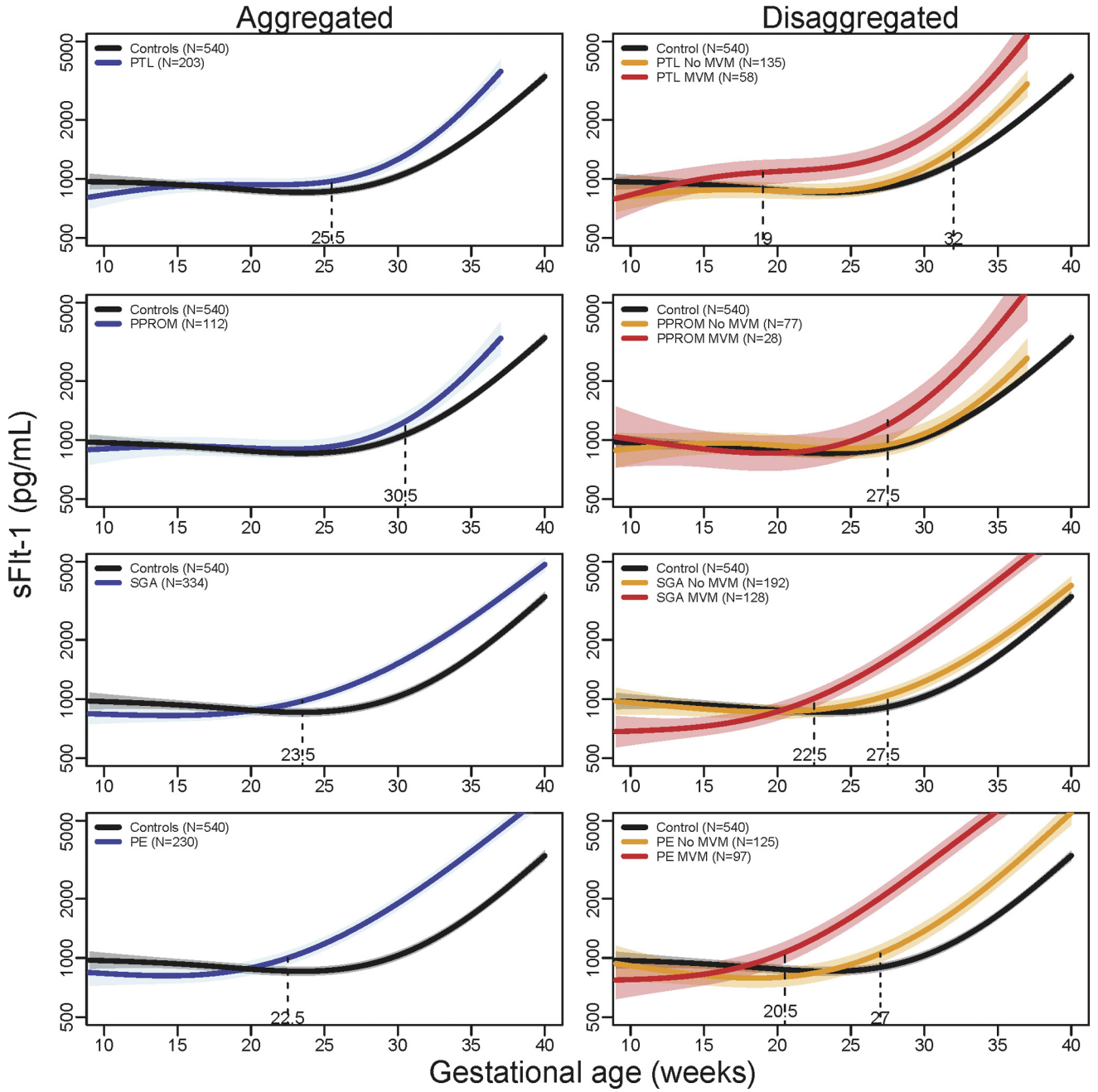


Mean and 95% confidence interval values were estimated by generalized additive models using spline transformations of GA in term delivery controls and complicated pregnancies. *Dotted vertical lines* represent the earliest GA when PIGF is significantly lower in cases than in controls.

GA, gestational age; *MVM*, maternal vascular malperfusion; *PE*, preeclampsia; *PIGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

FIGURE 3
Maternal plasma sFlt-1 in normal and complicated pregnancies with and without placental lesions of maternal vascular malperfusion



Mean and 95% confidence interval values were estimated by generalized additive models using spline transformations of GA in term delivery controls and complicated pregnancies. *Dotted vertical lines* represent the earliest GA when sFlt-1 is significantly higher in cases than in controls.

GA, gestational age; *MVM*, maternal vascular malperfusion; *PE*, preeclampsia; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

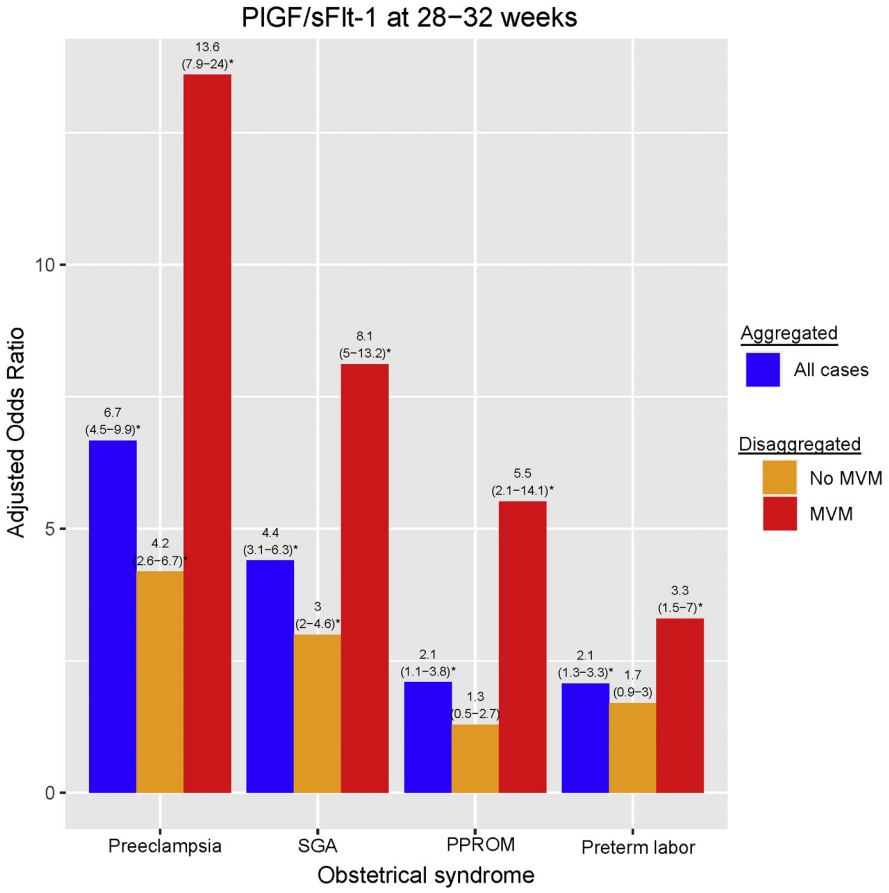
gestation: at 18 weeks for preeclampsia, 20 weeks for SGA, 22 weeks for PTL, and 25 weeks for PPROM ($P<.05$, for all comparisons) (Figure 1, right column).

Moreover, when placental lesions of MVM were not present, there was no difference between PTL or PPROM cases and controls in the profile of a PlGF/sFlt-1 ratio at any time during gestation (Figure 1, right column), nor in PlGF and sFlt-1 separately (Figures 2 and 3). For example, based on sFlt-1 analysis, differences between the PTL cases and the controls emerged at 25.5 weeks of gestation based on aggregated analysis but as early as 19 weeks of gestation in the disaggregated analysis (Figure 3). Moreover, only when SGA was disaggregated could we observe that a low first-trimester sFlt-1 concentration is a risk factor for future development of SGA, and a similar trend was noted for preeclampsia (Figure 3).

The magnitude of association between biomarkers and pregnancy complications is greater when placental lesions are present

The aORs for the association between a low PlGF/sFlt-1 ratio at 28 to 32 weeks of gestation and obstetrical syndromes in aggregate analysis were

FIGURE 4
Adjusted odds ratios for obstetrical complications conferred by an abnormal PlGF/sFlt-1 ratio at 28 to 32 weeks of gestation

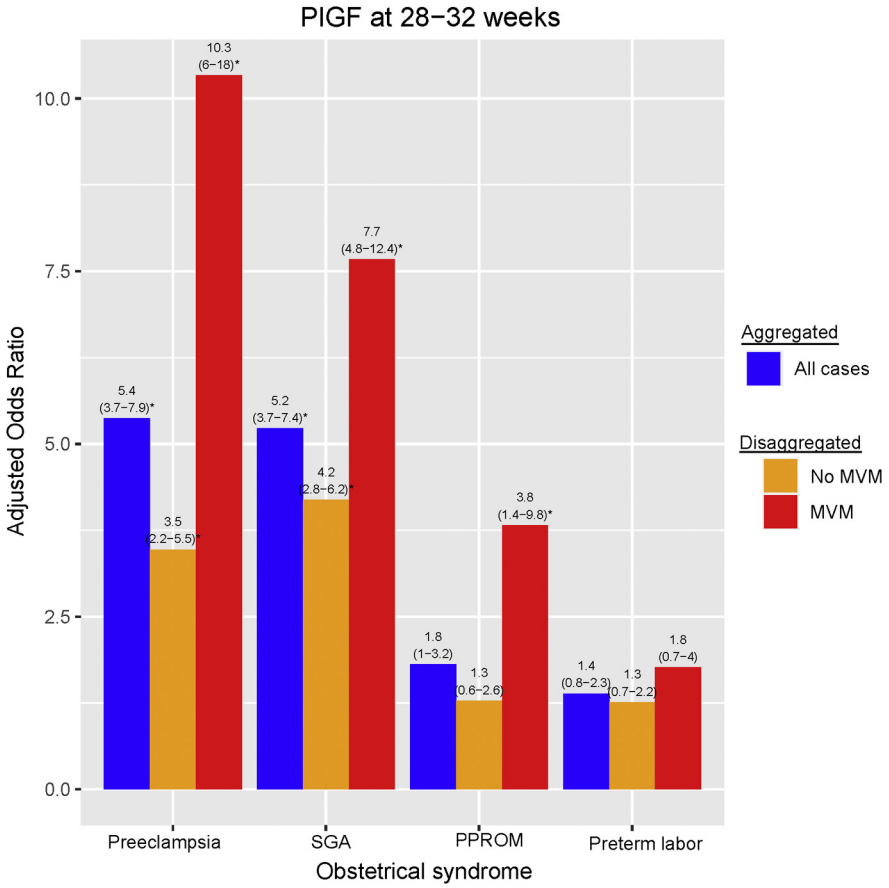


Results are presented for adverse outcomes in aggregated and disaggregated cases by the presence of placental lesions of MVM. A positive test was defined as MoM (PlGF/sFlt-1 ratio) <20th percentile. Significant associations ($P<.05$) obtained via logistic regression are shown with an *asterisk*. Moreover, 95% confidence intervals are listed under estimates.

MoM, multiple of the mean; *MVM*, maternal vascular malperfusion; *PlGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

FIGURE 5
Adjusted odds ratios for obstetrical complications conferred by abnormal PIGF at 28 to 32 weeks of gestation



Results are presented for adverse outcomes in aggregated and disaggregated cases by the presence of placental lesions of MVM. A positive test was defined as MoM PIGF <20th percentile. Significant associations ($P<.05$) obtained via logistic regression are shown with an *asterisk*. Moreover, 95% confidence intervals are listed under estimates.

MoM, multiple of the mean; *MVM*, maternal vascular malperfusion; *PIGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *SGA*, small for gestational age.

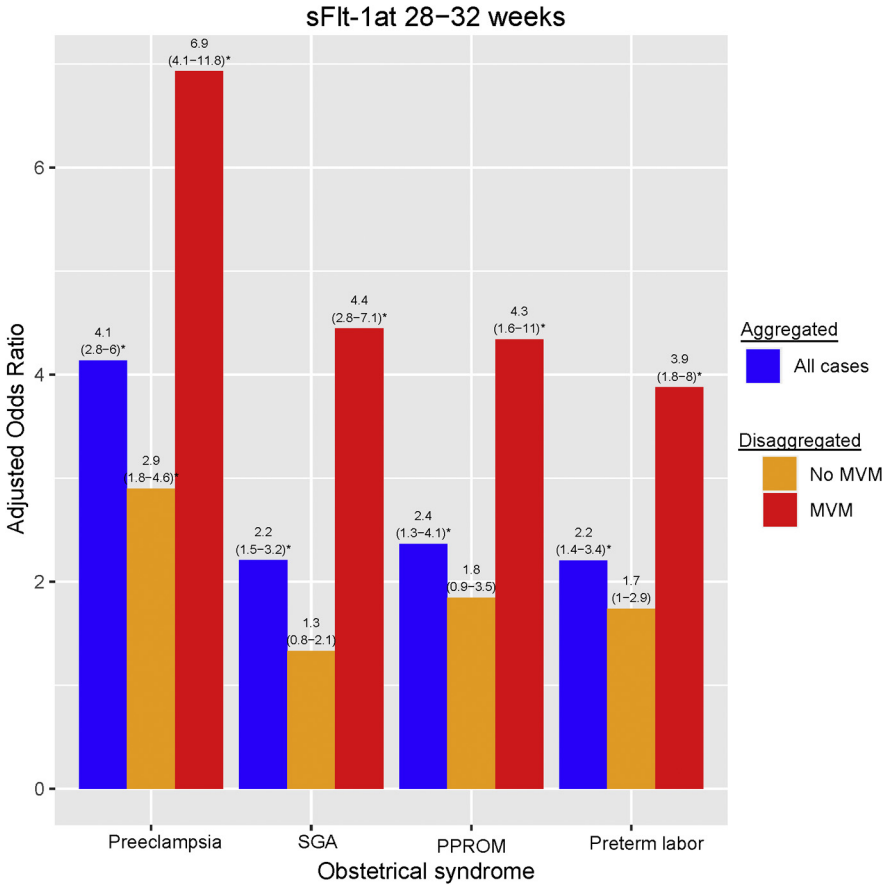
Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

6.7 for preeclampsia, 4.4 for SGA, and 2.1 for PPROM and PTL (all, $P<.05$) (Figure 4, blue bars). The strength of association (measured by the OR) doubled for obstetrical diseases with placental lesions of MVM, reaching 13.6 for preeclampsia, 8.1 for SGA, 5.5 for PPROM, and 3.3 for PTL (all, $P<.05$) (Figure 4, red bars). It is noteworthy, in the absence of placental lesions of MVM, that the associations between an abnormal PlGF/sFlt-1 ratio at 28 to 32 weeks of gestation and obstetrical syndromes were 2- to 3-fold weaker and did not reach significance for the PTL and PPROM groups (Figure 4, yellow bars). Similar findings were observed on the basis of analysis results of PlGF and sFlt-1 separately (Figures 5 and 6) and by using RR estimates and predictive performance metrics, such as the likelihood ratio of a positive or negative result (Table 2).

The profile of biomarkers throughout gestation in each obstetrical syndrome

The PlGF/sFlt-1 ratio in normal gestation reaches its peak at around 28 weeks of gestation and then decreases with advancing GA. The

FIGURE 6
Adjusted odds ratios for obstetrical complications conferred by abnormal sFlt-1 at 28 to 32 weeks of gestation



Results are presented for adverse outcomes in aggregated and disaggregated cases by presence of placental lesions of MVM. A positive test was defined as MoM sFlt-1 >20th percentiles. Significant associations ($P<.05$) obtained via logistic regression are shown with *asterisk*. Moreover, 95% confidence intervals are listed under estimates.

MoM, multiple of the mean; *MVM*, maternal vascular malperfusion; *PPROM*, preterm premature rupture of the membranes; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

profile is different for each obstetrical syndrome with MVM, as shown in Figure 7 for the PlGF/sFlt-1 ratio and in Figure 8 for the inverse ratio sFlt-1/PlGF. The most abnormal profiles of the PlGF/sFlt-1 ratio among cases with placental lesions of MVM were observed for fetal death, early preeclampsia, and SGA (<5th percentile), with 20% of the cases in this latter group also complicated by preeclampsia. However, in patients with late preeclampsia, PTL, and PPROM, abnormalities were detectable only in the late second and third trimesters of pregnancy. These findings have implications for the implementation of biomarkers. In other words, this knowledge can inform biomarker timing and threshold values for risk assessment of different syndromes and realistic expectations of their performance. Overall, these findings demonstrated that subclassification of obstetrical syndromes, according to placental pathology findings, has important consequences for the discovery and implementation of biomarkers in clinical obstetrics.

TABLE 2
Strength of the association between abnormal biomarkers and obstetrical syndromes in aggregate and disaggregated by presence of placental lesions of maternal vascular malperfusion

			Cases		Controls							
Predictor	Outcome	Outcome type	Total	Positive test	Total	Positive test	aOR	aRR	Sensitivity	Specificity	Positive LR	Negative LR
sFlt-1	PTL	All cases	126	34	627	90	2.2 (1.4–3.4)	2.1 (1.4–3.2)	0.27 (0.19–0.36)	0.86 (0.83–0.88)	1.9 (1.33–2.65)	0.9 (0.8–1.0)
sFlt-1	PTL	With MVM	33	13	627	90	3.9 (1.8–8.0)[a]	3.8 (1.8–7.7)[a]	0.39 (0.23–0.58)	0.86 (0.83–0.88)	2.7 (1.72–4.37)	0.7 (0.5–0.9)
sFlt-1	PTL	Without MVM	93	21	627	90	1.7 (1.0–2.9)	1.7 (1.0–2.8)	0.23 (0.15–0.32)	0.86 (0.83–0.88)	1.6 (1.03–2.40)	0.9 (0.8–1.0)
sFlt-1	PPROM	All cases	74	21	627	90	2.4 (1.3–4.1)	2.3 (1.4–3.9)	0.28 (0.19–0.40)	0.86 (0.83–0.88)	2.0 (1.31–2.98)	0.8 (0.7–1.0)
sFlt-1	PPROM	With MVM	19	8	627	90	4.3 (1.6–11.0)[a]	4.3 (1.7–10.7)[a]	0.42 (0.20–0.67)	0.86 (0.83–0.88)	2.9 (1.67–5.14)	0.7 (0.5–1.0)
sFlt-1	PPROM	Without MVM	55	13	627	90	1.8 (0.9–3.5)	1.8 (1.0–3.4)	0.24 (0.13–0.37)	0.86 (0.83–0.88)	1.6 (0.99–2.75)	0.9 (0.8–1.0)
sFlt-1	SGA	All cases	248	67	627	90	2.2 (1.5–3.2)	1.4 (1.0–1.9)	0.27 (0.22–0.33)	0.86 (0.83–0.88)	1.9 (1.42–2.49)	0.9 (0.8–0.9)
sFlt-1	SGA	With MVM	89	38	627	90	4.4 (2.8–7.1)[a]	2.7 (1.6–4.5)[a]	0.43 (0.32–0.54)	0.86 (0.83–0.88)	3.0 (2.19–4.04)	0.7 (0.6–0.8)
sFlt-1	SGA	Without MVM	159	29	627	90	1.3 (0.8–2.1)	0.9 (0.6–1.5)	0.18 (0.13–0.25)	0.86 (0.83–0.88)	1.3 (0.87–1.86)	1.0 (0.9–1.0)
sFlt-1	PE	All cases	171	70	627	90	4.1 (2.8–6.0)	3.4 (2.4–4.8)	0.41 (0.33–0.49)	0.86 (0.83–0.88)	2.9 (2.19–3.71)	0.7 (0.6–0.8)
sFlt-1	PE	With MVM	67	36	627	90	6.9 (4.1–11.8)[a]	6.4 (3.9–10.5)[a]	0.54 (0.41–0.66)	0.86 (0.83–0.88)	3.7 (2.79–5.02)	0.5 (0.4–0.7)
sFlt-1	PE	Without MVM	104	34	627	90	2.9 (1.8–4.6)	2.5 (1.6–4.1)	0.33 (0.24–0.43)	0.86 (0.83–0.88)	2.3 (1.63–3.19)	0.8 (0.7–0.9)
PlGF	PTL	All cases	126	22	627	83	1.4 (0.8–2.3)	1.4 (0.8–2.2)	0.17 (0.11–0.25)	0.87 (0.84–0.89)	1.3 (0.86–2.03)	1.0 (0.9–1.0)
PlGF	PTL	With MVM	33	7	627	83	1.8 (0.7–4.0)[a]	1.7 (0.7–4.1)[a]	0.21 (0.09–0.39)	0.87 (0.84–0.89)	1.6 (0.81–3.19)	0.9 (0.8–1.1)
PlGF	PTL	Without MVM	93	15	627	83	1.3 (0.7–2.2)	1.2 (0.7–2.2)	0.16 (0.09–0.25)	0.87 (0.84–0.89)	1.2 (0.74–2.02)	1.0 (0.9–1.1)
PlGF	PPROM	All cases	74	16	627	83	1.8 (1.0–3.2)	1.8 (1.0–3.1)	0.22 (0.13–0.33)	0.87 (0.84–0.89)	1.6 (1.01–2.63)	0.9 (0.8–1.0)
PlGF	PPROM	With MVM	19	7	627	83	3.8 (1.4–9.8)[a]	3.8 (1.5–9.7)[a]	0.37 (0.16–0.62)	0.87 (0.84–0.89)	2.8 (1.49–5.18)	0.7 (0.5–1.0)
PlGF	PPROM	Without MVM	55	9	627	83	1.3 (0.6–2.6)	1.3 (0.6–2.6)	0.16 (0.08–0.29)	0.87 (0.84–0.89)	1.2 (0.66–2.32)	1.0 (0.9–1.1)
PlGF	SGA	All cases	248	110	627	83	5.2 (3.7–7.4)	2.9 (2.1–3.8)	0.44 (0.38–0.51)	0.87 (0.84–0.89)	3.4 (2.62–4.28)	0.6 (0.6–0.7)
PlGF	SGA	With MVM	89	48	627	83	7.7 (4.8–12.4)[a]	3.9 (2.4–6.5)[a]	0.54 (0.43–0.65)	0.87 (0.84–0.89)	4.1 (3.09–5.38)	0.5 (0.4–0.7)
PlGF	SGA	Without MVM	159	62	627	83	4.2 (2.8–6.2)	2.8 (1.9–4.2)	0.39 (0.31–0.47)	0.87 (0.84–0.89)	2.9 (2.23–3.89)	0.7 (0.6–0.8)
PlGF	PE	All cases	171	77	627	83	5.4 (3.7–7.9)	3.9 (2.8–5.6)	0.45 (0.37–0.53)	0.87 (0.84–0.89)	3.4 (2.62–4.41)	0.6 (0.6–0.7)
PlGF	PE	With MVM	67	41	627	83	10.3 (6.0–18.0)[a]	9.3 (5.6–15.5)[a]	0.61 (0.49–0.73)	0.87 (0.84–0.89)	4.6 (3.51–6.10)	0.4 (0.3–0.6)
PlGF	PE	Without MVM	104	36	627	83	3.5 (2.2–5.5)	2.7 (1.7–4.2)	0.35 (0.26–0.45)	0.87 (0.84–0.89)	2.6 (1.88–3.64)	0.8 (0.7–0.9)
PlGF/sFlt-1 ratio	PTL	All cases	126	27	627	73	2.1 (1.3–3.3)	2.0 (1.3–3.1)	0.21 (0.15–0.30)	0.88 (0.86–0.91)	1.8 (1.24–2.74)	0.9 (0.8–1.0)
PlGF/sFlt-1 ratio	PTL	With MVM	33	10	627	73	3.3 (1.5–7.0)[a]	3.2 (1.5–6.9)[a]	0.3 (0.16–0.49)	0.88 (0.86–0.91)	2.6 (1.49–4.56)	0.8 (0.6–1.0)

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

(continued)

TABLE 2
Strength of the association between abnormal biomarkers and obstetrical syndromes in aggregate and disaggregated by presence of placental lesions of maternal vascular malperfusion *(continued)*

			Cases		Controls							
Predictor	Outcome	Outcome type	Total	Positive test	Total	Positive test	aOR	aRR	Sensitivity	Specificity	Positive LR	Negative LR
PlGF/sFlt-1 ratio	PTL	Without MVM	93	17	627	73	1.7 (0.9–3.0)	1.7 (1.0–2.9)	0.18 (0.11–0.28)	0.88 (0.86–0.91)	1.6 (0.97–2.54)	0.9 (0.8–1.0)
PlGF/sFlt-1 ratio	PPROM	All cases	74	16	627	73	2.1 (1.1–3.8)	2.0 (1.1–3.6)	0.22 (0.13–0.33)	0.88 (0.86–0.91)	1.9 (1.14–3.01)	0.9 (0.8–1.0)
PlGF/sFlt-1 ratio	PPROM	With MVM	19	8	627	73	5.5 (2.1–14.1)[a]	5.4 (2.1–13.6)[a]	0.42 (0.20–0.67)	0.88 (0.86–0.91)	3.6 (2.05–6.39)	0.7 (0.4–1.0)
PlGF/sFlt-1 ratio	PPROM	Without MVM	55	8	627	73	1.3 (0.5–2.7)	1.3 (0.6–2.8)	0.15 (0.06–0.27)	0.88 (0.86–0.91)	1.2 (0.64–2.46)	1.0 (0.9–1.1)
PlGF/sFlt-1 ratio	SGA	All cases	248	91	627	73	4.4 (3.1–6.3)	2.5 (1.8–3.4)	0.37 (0.31–0.43)	0.88 (0.86–0.91)	3.2 (2.40–4.13)	0.7 (0.6–0.8)
PlGF/sFlt-1 ratio	SGA	With MVM	89	46	627	73	8.1 (5.0–13.2)[a]	4.2 (2.5–6.9)[a]	0.52 (0.41–0.62)	0.88 (0.86–0.91)	4.4 (3.31–5.96)	0.5 (0.4–0.7)
PlGF/sFlt-1 ratio	SGA	Without MVM	159	45	627	73	3.0 (2.0–4.6)	2.2 (1.5–3.3)	0.28 (0.21–0.36)	0.88 (0.86–0.91)	2.4 (1.75–3.38)	0.8 (0.7–0.9)
PlGF/sFlt-1 ratio	PE	All cases	171	80	627	73	6.7 (4.5–9.9)	4.7 (3.3–6.7)	0.47 (0.39–0.55)	0.88 (0.86–0.91)	4.0 (3.07–5.26)	0.6 (0.5–0.7)
PlGF/sFlt-1 ratio	PE	With MVM	67	43	627	73	13.6 (7.9–24.0)[a]	12 (7.1–20.2)[a]	0.64 (0.52–0.76)	0.88 (0.86–0.91)	5.5 (4.17–7.29)	0.4 (0.3–0.6)
PlGF/sFlt-1 ratio	PE	Without MVM	104	37	627	73	4.2 (2.6–6.7)	3.1 (2–4.9)	0.36 (0.26–0.46)	0.88 (0.86–0.91)	3.1 (2.18–4.28)	0.7 (0.6–0.8)

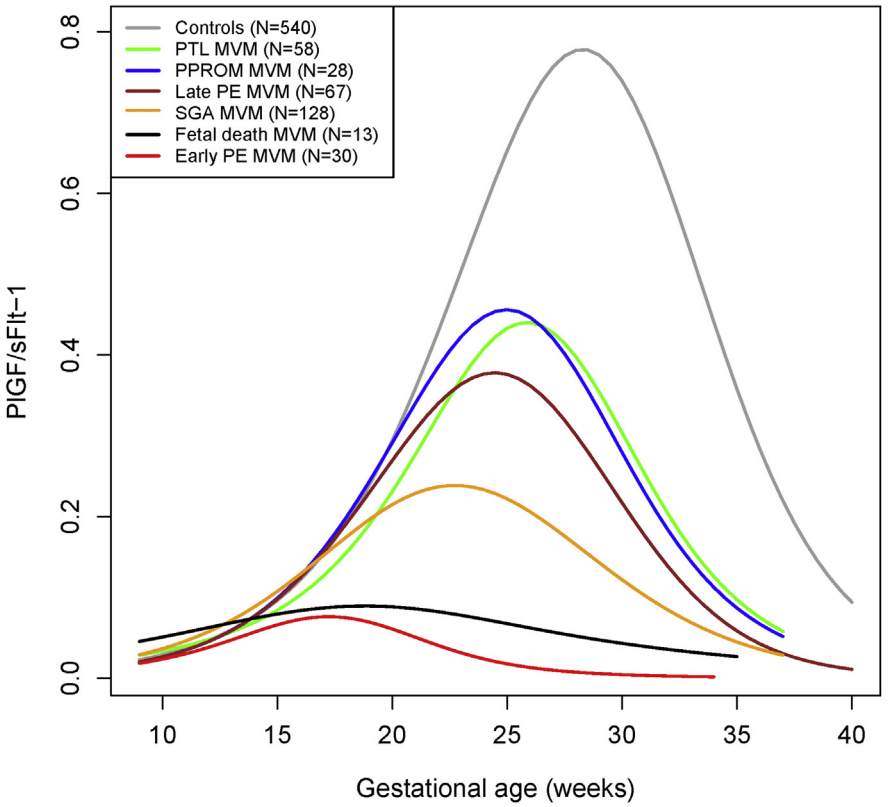
Estimates are provided with 95% confidence intervals.

aOR, adjusted odds ratio; *aRR*, adjusted relative risk; *LR*, likelihood ratio; *MVM*, maternal vascular malperfusion; *PE*, preeclampsia; *PlGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

[a] The largest estimates of aOR and aRR for a given biomarker and obstetrical syndrome are always for the subgroup with MVM lesions.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

FIGURE 7
Maternal plasma PlGF/sFlt-1 ratio in normal and complicated pregnancies with placental lesions of maternal vascular malperfusion



Mean values were estimated by generalized additive models using spline transformations of gestational age.

MVM, maternal vascular malperfusion; *PE*, preeclampsia; *PlGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

Discussion

Principal findings of the study

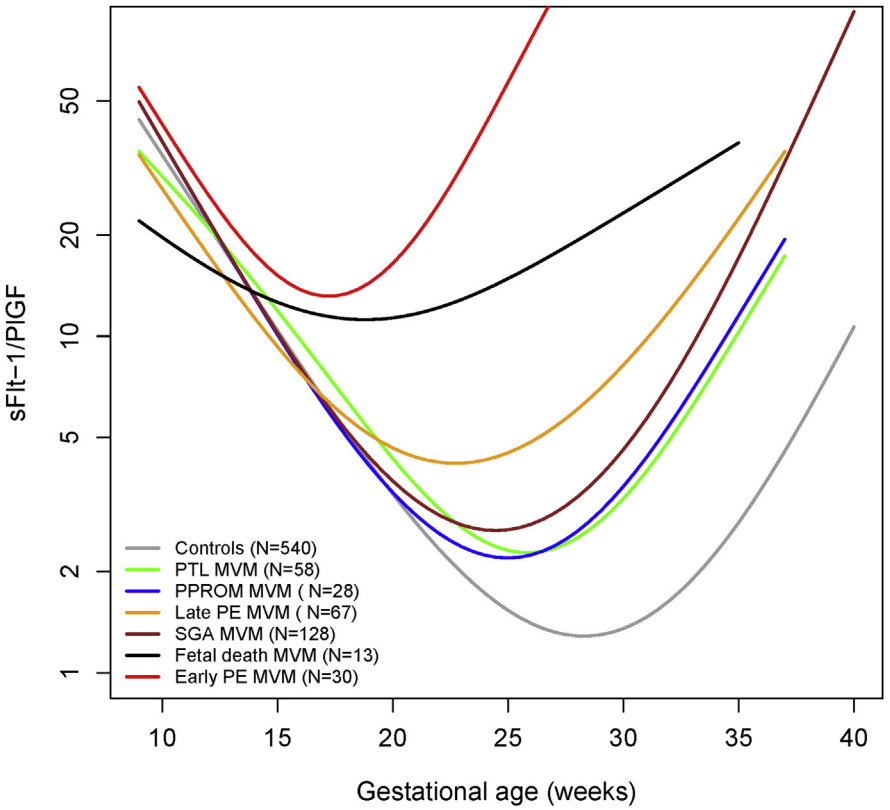
The subclassification of obstetrical syndromes according to the presence or absence of placental lesions of MVM (1) allows biomarkers in maternal blood (PlGF, sFlt-1, and the PlGF/sFlt-1 ratio) to become informative earlier in gestation; (2) enhances the strength of association between abnormal biomarkers and clinical outcomes; and (3) uncovers syndromes in which PlGF was previously not known to be informative (ie, PTL and PPROM) (See Video Files 1 and 2 for a summary of the findings). Collectively, these observations support the concept that a new nosology of obstetrical syndromes, which includes information about placental pathology findings, can enhance the discovery of biomarkers that, in turn, could improve the prediction and prevention of disease.

Results in the context of what is known

Maternal blood PlGF and sFlt-1 as biomarkers for preeclampsia

Pregnancy requires substantial changes in maternal vascular anatomy and physiology to provide adequate blood supply to the placenta and fetus, and this is partially accomplished through angiogenesis, which is regulated by a balance of growth factors (known as angiogenic and antiangiogenic factors). The prototypic angiogenic factor is VEGF, which binds to its receptors[133–135] and induces the proliferation of endothelial cells and tube formation.[136] PlGF is a member of the VEGF family that was isolated from a cDNA library of a placenta at term.[137] Several decades ago, investigators reported that the maternal plasma concentration of PlGF was lower in patients destined to develop preeclampsia than in patients with a normal pregnancy outcome[138]; however, the implementation of PlGF measurements to predict preeclampsia has been difficult because of false-positive and false-negative results.[139–141] A subset of patients with preeclampsia or even eclampsia do not have low concentrations of PlGF (or increased concentrations of the antiangiogenic factors sFlt-1 or soluble endoglin).[142,143] Moreover, patients with an abnormal profile of angiogenic or antiangiogenic factors may not develop preeclampsia; however, they could

FIGURE 8
Maternal plasma ratio of sFlt-1/PlGF in normal and complicated pregnancies with placental lesions of maternal vascular malperfusion



Mean values were estimated by generalized additive models using spline transformations of gestational age. A logarithmic axis was used to enhance visualization of differences between groups.

MVM, maternal vascular malperfusion; *PE*, preeclampsia; *PlGF*, placental growth factor; *PPROM*, preterm premature rupture of the membranes; *PTL*, preterm labor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

Romero. A new taxonomy of obstetrical syndromes: clinical presentation and placental pathology. Am J Obstet Gynecol 2022.

experience serious adverse pregnancy outcomes, such as fetal death,[105,106] fetal growth restriction,[110,144–147] PTL,[148,149] and severe perivillous fibrin deposition,[150] which often involve vascular lesions of the placenta.

sFlt-1 is a splice variant of the VEGF receptor, fms-like tyrosine kinase 1, which lacks the transmembrane and cytoplasmic domains and has important anti-angiogenic activity.[135,151,152] sFlt-1 binds free VEGF and PlGF in the maternal circulation. Syncytiotrophoblast and syncytial knots are sources of sFlt-1, and its concentration in the uterine vein in patients with preeclampsia is higher than in the peripheral circulation.[153] Previously, we reported that sFlt-1 concentrations in maternal plasma are elevated in patients with preeclampsia,[107,110–113,115,153–156] fetal death,[105,106] massive perivillous fibrin deposition,[150] SGA,[110,116,147] mirror syndrome,[157] and twin-to-twin transfusion syndrome.[158] Moreover, recent reports suggested that patients with fetal parvovirus[159] and cytomegalovirus infection[160] may also have high maternal blood concentrations of sFlt-1. This is also the case in patients with molar pregnancy.[161,162]

Maternal blood PlGF/sFlt-1 ratio is a biomarker for placental lesions of maternal vascular malperfusion

Vascular lesions of the placenta have been implicated in most obstetrical syndromes.[57–62,73–76,163] We previously reported that the ratio of PlGF/sFlt-1 (which we referred to as the "angiogenic index-1" in earlier publications[95,108]) is a biomarker for the presence of placental lesions of MVM.[95] Women who deliver <34 weeks of gestation with placental lesions of MVM have a lower maternal plasma PlGF/sFlt-1 ratio within 48 hours of delivery.[95] Moreover, an angiogenic index-1 below the 2.5th percentile at 20 to 23 weeks of gestation identifies 70% of women who deliver <34 weeks of gestation with placental lesions of MVM.[95] Therefore, angiogenic index-1 is the first prenatal biomarker for MVM. The lack of specificity of the PlGF/sFlt-1 ratio for an obstetrical syndrome, such as preeclampsia, merely reflects that placental lesions of MVM are also present in other obstetrical syndromes and are not specific to preeclampsia.[95]

PlGF/sFlt-1 ratio is a powerful predictor of unexplained fetal death, particularly in cases presenting placental lesions of maternal vascular malperfusion

A cross-sectional study showed that unexplained fetal death was associated with abnormally high concentrations of sFlt-1.[105] Subsequent longitudinal studies have shown that a PlGF/sFlt-1 ratio at 30 to 34 weeks of gestation could identify patients at risk of fetal death and that the positive likelihood of an abnormal PlGF/sFlt-1 ratio was 14.[107] Moreover, an abnormal ratio at 24 to 28 weeks of gestation predicted unexplained fetal death in the third trimester of pregnancy, and when the endpoint was fetal death with placental lesions of MVM, the likelihood ratio increased from 14 to 20.[108] In other words, the test performs better when the endpoint includes not only the clinical presentation and fetal death but also the presence of placental lesions of MVM. A logical question that we tested in the current study was whether similar findings would apply to other obstetrical syndromes, in particular, preeclampsia, SGA, and PTL with and without intact membranes.

The impact of classifying obstetrical syndromes according to placental pathology

In this report, we have shown that in the absence of placental lesions of MVM

there was no difference in the maternal plasma PlGF/sFlt-1 ratio between patients with PTL or PPROM and GA-matched controls at any time during pregnancy. However, differences in the PlGF/sFlt-1 profiles emerged as early as 22 weeks of gestation for PTL and 25 weeks of gestation for PPROM if the cases had placental lesions of MVM. This finding indicates that the association between an abnormal PlGF/sFlt-1 ratio and PTL and PPROM is mediated through the development of placental lesions of MVM. By contrast, for preeclampsia and severe SGA, differences between cases and controls were detected, regardless of the presence of placental lesions of MVM. However, when lesions were present, the abnormality in PlGF/sFlt-1 profiles emerged about five weeks earlier for both conditions (Figure 1). Furthermore, the findings for the ratio were also observed for the individual analytes, PlGF and sFlt-1, as shown in Figures 2 (PlGF) and 3 (sFlt-1).

The magnitude of the association between an abnormal PlGF/sFlt-1 ratio (after adjusting for maternal characteristics and obstetrical history) at 28 to 32 weeks of gestation and the occurrence of obstetrical syndromes were strengthened when the obstetrical disorders were disaggregated according to the presence of placental lesions of MVM (Figure 4). A MoM PlGF/sFlt-1 ratio <20th percentile was associated with PPROM (aOR, 2.1); however, the magnitude of association increased (aOR, 5.5) after subclassification of this syndrome by the presence of placental lesions of MVM. Moreover, this was the case for other syndromes: the association was strengthened when conditions were subclassified by the presence of placental lesions of MVM (SGA [aOR, 8.1 vs 4.4], preeclampsia [aOR, 13.6 vs 6.7], and PTL [aOR, 3.3 vs 2.1]). The findings were similar for PlGF and sFlt-1 alone (Figures 5 and 6) and also by using RR estimates rather than ORs (Table 2). Moreover, the results were consistent when using the 10th percentile instead of the 20th percentile (PlGF) and the 90th percentile instead of the 80th percentile (sFlt-1) as cutoff values to define an abnormal test result (data not shown).

Clinical implications

Obstetrical disorders are syndromic,[14,15,164,165] and each disorder has multiple etiologies. In the case of PTL, the causes include intra-amniotic infection,[166–169] sterile intra-amniotic inflammation,[170,171] cervical disorders,[172] and a breakdown of maternal-fetal tolerance,[51,52,173] all of which can lead to the activation of the common pathway of parturition (myometrial contractility, cervical remodeling, and decidual or membrane activation).[164,165,174] The terms "preterm labor with intact membranes," "PPROM," and "cervical insufficiency" merely describe the clinical presentation of asynchronous activation of the different components of the common pathway of parturition and therefore do not provide information about etiology.

Adequate blood supply to the placenta and fetus is essential for a successful pregnancy. Therefore, it is not surprising that vascular pathology is the most frequent mechanism of disease in obstetrics. Lesions of vascular pathology are most frequently found in cases of fetal death,[97,175–177] preeclampsia,[73,84,178,179] SGA/fetal growth restriction,[180–183] and abruptio placentae,[184,185] yet they have also been recognized in other obstetrical syndromes conventionally not considered to be predominantly of vascular origin (PTL,[60,186,187] PPROM,[59,186,188] and spontaneous abortion[175,189,190]). Biomarkers for placental vascular lesions can be expected to predict obstetrical syndromes caused by this pathologic process but not by other mechanisms of disease such as infection, sterile intra-amniotic inflammation, and maternal anti-fetal rejection. Other molecules, such as IL-6,[47,191,192] IL-8,[193–195] CXCL-10,[87,196] and CXCR-3,[88] are putative biomarkers under these circumstances.

Research implications

The observation that syndrome subclassification or disaggregation by placental pathology findings leads to stronger associations between biomarkers and obstetrical disease has several implications for research. First, studies of biomarker discovery require a precise endpoint. Most biomarker studies in obstetrics are aimed to predict obstetrical syndromes defined by symptoms and signs. These efforts have been largely unsuccessful or have led to claims that have not been replicated. We propose that defining obstetrical disorders by the combination of clinical presentation and placental pathology would generate more precise endpoints (often referred to as "phenotypes") and that this would enhance the likelihood of biomarker discovery and assessment of true performance. For example, rather than attempting to predict all cases of spontaneous PTL, future studies should focus on the discovery of biomarkers to predict PTL associated with acute histologic chorioamnionitis (most often because of intra-amniotic infection) or chronic chorioamnionitis (the pathologic hallmark of maternal antifetal rejection). Such an approach has been successful in other areas of medicine (eg, the likelihood of infection or sepsis can be assessed by inflammatory cytokines, such as IL-6,[197–201] transplant rejection with CXCL-10,[202–204] and tumor recurrence with alpha-fetoprotein[205–208] or human chorionic gonadotropin[209,210]). It is obvious that a universal biomarker for all pathologic processes does not exist and it is time that obstetrics, investigators, and funding agencies accept this reality.

Second, the conceptual framework advanced in this article can help to understand the apparent different behavior of biomarkers (eg, PlGF, sFlt-1, alpha-fetoprotein, and tumor necrosis factor alpha) among ethnic groups.[211–215] The different behavior of PlGF and sFlt-1 may reflect the frequency and burden of MVM among cohorts.

Third, the findings reported herein suggest that current biomarkers may be used in novel ways to predict disease. For example, we noted that a lower sFlt-1 concentration in the first trimester of pregnancy is a risk factor for the subsequent development of SGA and possibly for preeclampsia. However, this

observation was obscured when all cases (aggregated) were compared to controls, and this may apply to other biomarkers.

Fourth, the discovery of biomarkers using omics or more targeted approaches may be facilitated by syndrome subclassification. By comparing molecular profiles of cases with and, separately, without placental lesions of MVM to those of women with normal pregnancy, new subsets of biomarkers may emerge. For example, in a previously published longitudinal study that utilized proteomics in maternal blood, we found at 8 to 16 weeks of gestation that the maternal concentrations of matrix metalloproteinase-7 and glycoprotein IIb/IIIa were predictive of early preeclampsia.[216] However, only when preeclampsia was subclassified according to the presence of placental lesions of MVM, angiotensin-converting enzyme 2 emerged as a predictor for early preeclampsia. Similar observations have been made with other molecules, such as sialic acid-binding immunoglobulin-like lectin 6 (siglec-6) at 22.1 to 32.0 weeks of gestation.[216]

The concept that obstetrical disorders are syndromes caused by multiple mechanisms of disease has gained momentum.[217-221] The work herein demonstrates the value of placental pathology in the subclassification of obstetrical syndromes. However, a standardized approach (optimal sampling and quantitative assessment of placental lesions) is important.[222] Indeed, we have provided evidence that shows the higher the number of lesions of MVM, the greater the abnormality in angiogenic and antiangiogenic markers.[95]

Importantly, syndrome subclassification can be accomplished not only by placental pathology but through other tools such as molecular markers,[223-232] reflecting mechanisms of disease detectable even before pathologic changes can be observed in the placenta. Such molecular changes may be found not only in maternal blood but also in other biological fluids. Biophysical information such as that derived from imaging technologies can also serve for this purpose.

Liquid biopsies to diagnose placental health and disease

At first, the proposal to incorporate placental pathology findings in the classification of obstetrical syndromes could seem impractical for the index pregnancy, given the organ is available only after delivery.

However, we argue that the placental status can be assessed non-invasively in ongoing pregnancies through a liquid biopsy. Progress in the understanding of the causes, diagnosis, treatment, and prevention of disease has been made possible because of the contributions of anatomic pathology—first, through autopsies and, only more recently, through biopsies. Autopsies do not help the deceased, yet they play a fundamental role in the advancement of medicine. Placental pathology has been shown to be the most informative tool for understanding fetal death and also for providing information to counsel patients about the risk in future pregnancies. It is now well established that some lesions, for example, massive perivillous fibrin deposition[150,233,234] and acute chorioamnionitis,[235] tend to recur in subsequent pregnancies. Such is the case for the clinical cluster referred to as ischemic placental disease.[93]

A "liquid biopsy" is a test performed on a sample of blood or another biological specimen (eg, amniotic fluid,[236,237] cervicovaginal fluid,[238,239] saliva,[240] crevicular fluid,[241] or urine[242,243]) whose result can be used to infer pathologic changes in distant tissues (eg, cancer)[244,245] or other pathologic processes (eg, atherosclerosis).[246–248] We envision that liquid biopsies will provide a means to examine placental health and disease in real time, which is an unmet need in the field of reproduction. Gestational liquid biopsies can utilize omics techniques to interrogate differences in cell-free DNA,[249–251] coding and noncoding RNA,[225,252–260] proteins,[216,229,261–266] metabolites,[267–271] and extracellular vesicles.[272–280] The data presented herein suggest that a liquid biopsy based on the examination of maternal blood is imminently feasible.

Strengths and limitations

The major strengths of this study include the following: (1) the use of a longitudinal case-cohort design that allowed the inclusion of all cases of interest from a cohort of 4006 women, (2) all placentas underwent histologic examination by placental pathologists masked to clinical information and pregnancy outcome, (3) the large number of PlGF and sFlt-1 measurements in patients who were serially sampled, and (4) the information is derived largely from an African American population, which is often understudied, despite the higher burden of adverse pregnancy outcomes relative to other populations.[281]

Among the limitations of this study, we note the following:

1. Some patients had samples collected in less than 3 GA intervals; therefore, they were ineligible for the first sampling stage, which may have introduced a selection bias. However, we included a complete census of all patients from the parent cohort who developed any of the great obstetrical syndromes, regardless of the number of samples available, and the reference population was selected regardless of pregnancy outcome, greatly reducing the chance of differential selection bias.
2. Missing data for some patient characteristics.
3. Insufficient observations to interrogate the differences between preterm and term preeclampsia and gestational-age subgroups for other obstetrical syndromes.

Data and results herein are presented not only for PlGF and sFlt-1 separately but also for the combination of the two biomarkers via a ratio. For historical reasons, we featured the PlGF/sFlt-1 ratio,[110] whereas later reports by other investigators used the sFlt-1/PlGF ratio.[282–285] However, the findings herein based on the PlGF/sFlt-1 ratio will also hold if the sFlt-1/PlGF ratio were to be used instead, by considering that women are at risk when the PlGF/sFlt-1 ratio is

abnormally high. The assumption behind the use of either of the two ratios is that the decrease in PlGF will be as equally predictive of disease as the increase in sFlt-1. However, this assumption may not hold depending on the GA at measurement. Current risk prediction models involving maternal blood PlGF and sFlt-1 concentrations do not rely on ratios but use GA-dependent weighting of the evidence provided by each biomarker.[131,286]

Conclusion

The classification of obstetrical syndromes according to the presence and absence of placental lesions of MVM allows biomarkers to be informative earlier in gestation and enhances the strength of association between biomarkers and clinical outcomes. We propose that a new taxonomy of obstetrical disorders, which includes information derived from placental pathology, will facilitate the discovery and implementation of biomarkers in clinical practice to improve the prediction and prevention of obstetrical syndromes. ■

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