OBSTETRICS

Maternal serum glycosylated fibronectin as a point-of-care biomarker for assessment of preeclampsia

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OBJECTIVE: We assessed the association of glycosylated fibronectin (GlyFn) with preeclampsia and its performance in a point-of-care (POC) test.

STUDY DESIGN: GlyFn, placental growth factor (PlGF), and soluble vascular endothelial growth factor receptor 1 (sFlt1) levels were determined in serum samples from 107 pregnant women. In all, 45 were normotensive and 62 were diagnosed with preeclampsia. The ability of GlyFn to assess preeclampsia status and relationships between GlyFn and maternal characteristics and pregnancy outcomes were analyzed.

RESULTS: GlyFn serum levels in the first trimester were significantly higher in women with preeclampsia ($P < .01$) and remained higher throughout pregnancy ($P < .01$). GlyFn, sFlt1, PlGF, and the sFlt1/PlGF ratio were significantly associated ($P < .01$) with preeclampsia status, and the classification performance of these analytes represented by area under the receiver operating characteristic curve was 0.99, 0.96, 0.94, and 0.98, respectively, with 95% confidence intervals of 0.98–1.00, 0.89–1.00, 0.86–1.00, and 0.94–1.00, respectively. Increased GlyFn levels were significantly associated with gestational age at delivery ($P < .01$), blood pressure ($P = .04$), and small-for-gestational-age neonates. Repeated-measures analysis of the difference in weekly GlyFn change in the third trimester demonstrated that mild preeclampsia was associated with a weekly change of 81.7 μg/mL (SE 94.1) vs 195.2 μg/mL (SE 88.2) for severe preeclampsia. The GlyFn POC demonstrated similar performance to a plate assay with an area under the receiver operating characteristic curve of 0.93 and 95% confidence interval of 0.85–1.00.

CONCLUSION: GlyFn is a robust biomarker for monitoring of preeclampsia in both a standard and POC format, which supports its utility in diverse settings.

Key words: biomarker, glycosylated fibronectin, point-of-care, preeclampsia

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Preeclampsia is a potentially life-threatening complication unique to pregnancy that occurs in up to 7% of all pregnancies.[1] Hypertensive disorders, including preeclampsia, are the second leading cause of maternal mortality worldwide, responsible for 10-25% of all maternal deaths.[2] Unfortunately, clinical manifestations of preeclampsia may occur late in the course of the disease and may be associated with adverse maternal and neonatal outcomes. Robust biomarkers for screening, diagnosis, and monitoring, particularly with respect to severe preeclampsia, are necessary to appropriately manage preeclampsia and to mitigate adverse outcomes.[3,4] This is particularly the case in developing countries, where the burden of disease is greatest, and where medical intervention is often ineffective due to late presentation.[5,6] Furthermore, the incidence of preeclampsia has been increasing since 1990, which may be directly related to the increase in obesity.[7] Early and effective diagnostic tests are urgently needed to provide for appropriate triage to skilled medical facilities and management of preeclamptics.

No currently available biomarkers perform sufficiently well to justify replacement of the current clinical diagnosis of preeclampsia,[8,9] although various angiogenic/antiangiogenic markers such as soluble vascular endothelial growth factor receptor 1 (sFlt1), placental growth factor (PlGF), and soluble endoglin have been employed to develop single- or multi-analyte tests that may exhibit more robust performance.[10-16]

We[17] and others[18-23] have previously reported that total serum fibronectin

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(Fn) is strongly associated with preeclampsia. Fn is an abundant protein with a wide spectrum of functions. As a result of alternative splicing and proteolysis, the Fn gene encodes a collection of isoforms that differ in sequence and length.[24,25] The majority of the Fn present in serum or plasma is termed *plasma* Fn (pFn), which is produced and secreted in a soluble form by hepatocytes, while so-called cellular Fn (cFn) is produced by numerous cell types, including fibroblasts, endothelial cells, and smooth muscle cells.[26] A major distinction between pFn and cFn is the presence of alternatively spliced extra domains A and B that are absent in pFn but variably present in cFn.[27] It is increasingly clear that cFn is also found in the circulation, especially in various pathological conditions, including diabetes and inflammation.[28-32] Thus, specific Fn variants have the potential to serve as informative biomarkers, potentially reflecting the known involvement of Fn in vessel remodeling and inflammation.[33,34]

Both pFn and cFn exhibit complex patterns of glycosylation,[35] and we recently described the ability of elevated levels of a specific glycosylated version of Fn (Fn-Sambucus nigra [SNA]; characterized by its reactivity with SNA lectin) in maternal serum to predict gestational diabetes.[36] In light of the known associations between gestational diabetes and preeclampsia[37,38] and our previous finding that elevated total maternal serum Fn was associated with preeclampsia risk,[17] we evaluated the utility of maternal serum glycosylated Fn (GlyFn) as a potential biomarker for preeclampsia risk and for monitoring its progression. Additionally, we describe a point-of-care (POC) platform for analysis of GlyFn levels in blood that will facilitate rapid assessment of the progression of preeclampsia and that has potential as a method for screening.

Materials and Methods

Study population

Study participants were recruited from 2 patient populations as reported previously.[17] The longitudinal cohort was a nested case-control study of 60 women who were sampled serially throughout pregnancy, with the first sample taken between 6-14 weeks of gestation and an additional sample obtained in each trimester. In this longitudinal cohort, 15 women who developed preeclampsia at various gestational ages were matched with 45 women who remained normotensive and had measurements within approximately 2 weeks of their preeclampsia counterparts. A clinical preeclampsia cohort of 47 patients who were diagnosed with preeclampsia at various gestational ages was analyzed to measure the rate of change in GlyFn levels during the course of their preeclampsia. Preeclampsia status was defined having a systolic blood pressure $\geq$140 mm Hg or a diastolic blood pressure $\geq$90 mm Hg with proteinuria $\geq$300 mg/d.[39] In all, 207 serum samples were analyzed using a plate assay and 86 serum samples were also analyzed using a GlyFn POC device. A total of 26 participants included in the analysis had 1 measurement, 62 had 2 measurements, and 19 had 3 measurements. Severe and mild preeclampsia were defined based on National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy criteria.[40]

The study participants were recruited from the Department of Obstetrics and Gynecology, Oulu University Hospital, Oulu, Finland, and the Finnish maternity cohort serum bank at the National Institute for Health and Welfare from 2004 through 2006. The research protocol was approved by the Oulu University Hospital Ethics Committee, and all participants provided informed consent.

Analyte assays

Maternal serum was spun, aliquoted, and stored at −80°C until subjected to the assays described below.

GlyFn plate assay

Reacti-Bind plates (Thermo Scientific, Rockford, IL) were coated with an Fc fragment-specific goat antimouse IgG (catalog no. 115-005-071; Jackson ImmunoResearch Laboratories Inc, West Grove, PA) in carbonate buffer, pH 9.6, and incubated at 4°C overnight followed by washing with phosphate-buffered saline (PBS)-0.05% Tween 20. Plates were blocked with 3% bovine serum albumin in PBS, pH 7.2, for 1 hour at room temperature. Plates were then washed with PBS-0.05% Tween 20 buffer and an Fn monoclonal antibody was added and incubated for 45 minutes at room temperature. This Fn monoclonal antibody replaced our previous SNA lectin-coupled assay,[36] as we found that the reactivity of this monoclonal with a glycosylated fraction of Fn was similar to that exhibited by the SNA lectin, allowing a simplified assay protocol. Samples and standard protein (human Fn isolated from serum, catalog no. 1918-FN-02M; R&D Systems, Minneapolis, MN) were incubated for 45 minutes, washed, and a biotinylated antihuman Fn polyclonal antibody (catalog no. A0245; DAKO, Carpinteria, CA) was added. Labeling was performed using high-sensitivity streptavidin-horseradish peroxidase (catalog no. 21130; Thermo Scientific). After incubation for 45 minutes followed by washing the plate with PBS-0.05% Tween 20 buffer, the plate was developed with 100 μL of K-Blue TMB substrate (catalog no. 304177; Neogen, Glasgow, Scotland) and quenched by the addition of 2N sulfuric acid (H_2SO_4). The interassay coefficient of variation for the plate assay was 7.1% and the intra-assay coefficient of variation was 3.1%.

GlyFn POC assay

A fluorescence immunoassay, comprising an automated cassette reader (cPoC reader; LRE Medical, Oceanside, CA) and a disposable, single-use, plastic assay cartridge was developed that employs standard immunoassay techniques to specifically and quantitatively detect GlyFn in serum specimens. The polyclonal anti-Fn antibody employed in the plate assay described above was conjugated to a Tide Fluor 5WS succinimidyl ester fluorescent tag (catalog no. 2281; AAT Bioquest, Sunnyvale, CA) and served as the detection antibody. The monoclonal Fn antibody employed in the plate assay described above served as the capture antibody and was immobilized on a solid phase (test zone).

TABLE 1
Maternal characteristics by preeclampsia status and cohort

Clinical characteristic	Normotensive, n = 45	Longitudinal preeclampsia, n = 15	Clinical preeclampsia, n = 47	Group difference *P* value[a]
Maternal age at last menstrual period, y[b]	26.5 (19.0–35.0)	28.0 (21.0–34.0)	29.0 (20.0–40.0)	.14
Gestational age at delivery, wk[b]	40.1 (38.4–42.0)	40.2 (36.7–42.0)	36.3 (21.7–40.6)	< .01
Gestational age at diagnosis of preeclampsia, wk[b]	NA	38.9 (32.0–40.0)	32.7 (21.0–37.3)	< .01
Neonatal birthweight, g[b]	3510 (2690–4488)	3260 (2520–4100)	2250 (315–4200)	< .01
Nulliparity, n (%)[b]	29 (83)	11 (73)	32 (68)	.31

Data are median (range) or n (%). Gestational age at preeclampsia diagnosis was unknown for 8 clinical preeclampsia participants and birthweight was unknown for 1 clinical preeclampsia participant.

NA, not applicable.

[a] Group differences were determined using Kruskal-Wallis nonparametric analysis of variance for continuous variables and Fisher exact tests for categorical variable; [b] Maternal age, gestational age at delivery, birthweight, and parity data were unavailable for 13, 12, 12, and 10 normotensive patients, and for 2, 1, 1, and 0 longitudinal preeclampsia participants.

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Goat polyclonal antirabbit IgG, Fc antibody (catalog no. 111-045-046; Jackson ImmunoResearch Laboratories Inc) was immobilized in a separate capture zone to act as a reference for the test zone and to provide assurance that the device performed properly.

Serum was diluted in assay buffer and applied to the test strip. The serum flows down the diagnostic lane via capillary action, taking the fluorescent detection antibody into suspension. GlyFn in the specimen binds to the fluorescent antibody to form a multivalent complex that is captured by the antibody immobilized in the test zone. The cartridge is inserted into the cassette reader and quantitative measurements of GlyFn concentration in the range from 10–2000 μg/mL are displayed on the meter screen and/or printout after 10 minutes.

sFlt1 levels were determined by enzyme-linked immunosorbent assay as previously described.[17] Due to the large amount of serum needed for this assay, 13 participants were unable to be assayed for this analyte.

PlGF levels were determined using a commercial kit (human PlGF Quantikine enzyme-linked immunosorbent assay kit, catalog no. DPG00; R&D Systems). Due to inadequate serum sample, this analysis was subset to 57 subjects.

Plates were read using an Epoch plate reader at 450 nm, and data were processed using Gen5 software, version 1.10.8 (BioTek, Winooski, VT) and analyzed as described below.

Statistical analysis

Analyses were performed on the normotensive, longitudinal preeclampsia, and clinical preeclampsia samples separately, and were combined or compared only where indicated. Maternal characteristics were compared across study groups using Kruskal-Wallis nonparametric analysis of variance for continuous variables and Fisher exact test for categorical variables. Comparisons of GlyFn levels between longitudinal participants with and without preeclampsia were performed with parametric and nonparametric Wilcoxon *t* tests using measures matched by gestational age within approximately 2 weeks for each subject within a trimester. For analysis of sFlt1, PlGF, and the sFlt1/PlGF ratio, samples were subset to the third trimester and the normotensive group was compared to the clinical preeclampsia cohort via nonparametric Wilcoxon *t* tests.

To determine the average weekly change in GlyFn, linear regression with repeated measurements was used. Weekly change in controls was found to be stable across the span of pregnancy and was assessed by using a subset of patients with ≥2 repeated measures between 7-40 weeks. To determine the change in GlyFn in the progression of mild and severe preeclampsia, average change over time in GlyFn levels for these participants was assessed in a subset of participants with ≥2 repeated measurements between 33-38 weeks. All repeated measurements were taken within 2 weeks of the first measurement and measurements were on average 5 days after preeclampsia diagnosis, ranging between 5 days before to 19 days after preeclampsia diagnosis.

Receiver operating characteristic (ROC) curves for preeclampsia diagnosis were generated using predicted probabilities from simple logistic regression models using a single third-trimester measure for each subject from all cohorts. The area under the ROC curve (AUROC) and corresponding 95% confidence limits were calculated using simple logistic regression. Sensitivity and specificity were reported based on thresholds chosen, and 95% confidence limits calculated by the score method with a continuity correction are reported. Statistical tests of differences in ROC curves were calculated using contrast matrices of differences. Hypothetical predictive values and 95% confidence intervals (CIs) for diagnosis of preeclampsia were calculated using the standard logit method[41] using a population prevalence of 3%, 5%, or 7%.

TABLE 2
Serum biomarker concentration within longitudinal cohort by preeclampsia status and trimester

Biomarker concentrations	First trimester			Second trimester			Third trimester		
	Normotensive cohort (n = 24)	Longitudinal preeclampsia cohort (n = 11)	Group difference *P* value[a]	Normotensive cohort (n = 28)	Longitudinal preeclampsia cohort (n = 12)	Group difference *P* value[a]	Normotensive cohort (n = 34)	Longitudinal preeclampsia cohort (n = 13)	Group difference *P* value[a]
Gestational age at sample collection, wk	9.7 (6.4–13.0)	9.3 (7.3–11.0)	.79	23.3 (17.4–26.6)	22.4 (21.6–26.3)	.17	35.4 (27.0–39.7)	36.4 (28.1–38.7)	.13
GlyFn, μg/mL	62 (7–198)	184 (30–387)	< .01	41 (9–144)	161 (6–848)	< .01	54 (1–199)	239 (111–522)	< .01

Data are median (range).

GlyFn, glycosylated fibronectin.

[a] Group differences were determined using Wilcoxon nonparametric *t* tests.

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A post hoc analysis of maternal and fetal clinical characteristics and outcomes vs GlyFn values determined by the plate assay was performed. Within all cohorts, GlyFn was compared to gestational age at delivery, birthweight, systolic blood pressure, and diastolic blood pressure. Within clinical preeclampsia participants, comparative analyses were performed with GlyFn with respect to gestational age of preeclampsia start, uric acid, alanine transaminase, proteinuria, HELLP syndrome, small-for-gestational age, and placental insufficiency. For continuous variables, Pearson correlation coefficients and linear regression slopes were calculated. For interpretability, linear regression slopes were calculated to reflect a change in GlyFn of 100 μg. For categorical variables, Fisher exact tests were performed by categorizing participants as those with or without GlyFn levels ≥500 μg.

A comparison of the GlyFn plate assay to the GlyFn POC was performed on samples assayed by both methods to assess the POC test's ability to distinguish between participants with and without preeclampsia as well as to assess the ability of GlyFn to monitor progression of preeclampsia during the second and third trimesters. Correlation coefficients were calculated to compare the 2 measures. ROC curves were generated separately for GlyFn plate and POC data for classification of control vs preeclampsia, control vs mild preeclampsia, and mild vs severe preeclampsia, and the AUROC for each was compared between GlyFn plate and POC assays. Reported *P* values are 2-sided, and $P < .05$ was considered statistically significant. Statistical analysis was performed using software (SAS, version 9.3; SAS Institute Inc, Cary, NC).

RESULTS

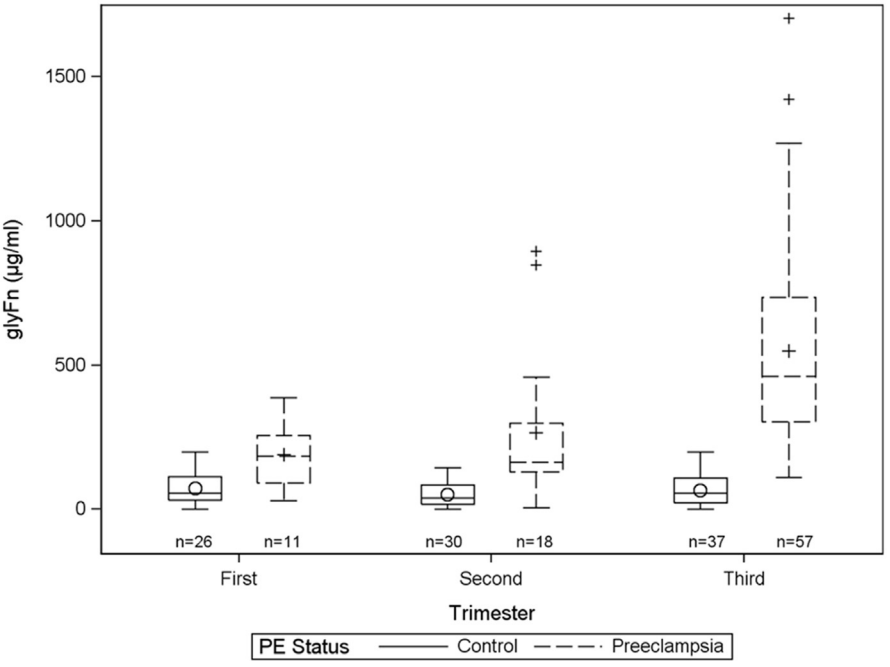
Patients in the clinical preeclampsia group were more likely to give birth earlier ($P < .01$) and have lower neonatal birthweights ($P < .01$) (Table 1). There was no difference in maternal age ($P = .14$) and nulliparity ($P = .31$) between the cohorts. Median gestational age at diagnosis of preeclampsia was significantly later in the longitudinal

preeclampsia group than in the clinical preeclampsia cohort. A comparison of the longitudinal normotensive and preeclampsia groups found that, within each trimester, GlyFn levels were significantly higher in patients with preeclampsia than in controls ($P < .01$) (Table 2 and Figure 1). To assess the change in serum biomarkers during the third trimester, levels of GlyFn, sFLt1, PlGF, and the sFLT1/PlGF ratio were compared between age-matched samples from the normotensive control and clinical preeclampsia cohorts (Table 3). There was a significant difference in all serum biomarkers between participants with and without preeclampsia ($P < .01$) (Table 3).

A repeated-measures analysis of change across all cohorts over time in biomarkers found that, in controls, there was not a significant change in GlyFn ($P = .83$) across the span of pregnancy. In patients with preeclampsia, the weekly change between 33-38 weeks was 81.7 (SE 94.1) μg/mL for participants with mild preeclampsia and 195.2 (SE 88.2) μg/mL for participants with severe preeclampsia (Table 4).

The clinical utility of these biomarkers for detection of preeclampsia was tested via ROC curves. The AUROCs for GlyFn, sFlt1, PlGF, and the sFlt1/PlGF ratio are show in Table 5 and the respective ROC curves in Figure 2. Since the sFlt1 assay requires significant serum quantities, this analysis was restricted to 15 control and 39 preeclampsia participants between 20-39 weeks of gestation with sufficient serum for sFlt1 analysis. The AUROC for GlyFn was 0.99, and trended toward being significantly different from the AUROC for sFlt1 (AUROC, 0.96; $P = .11$) and PlGF (AUROC, 0.94; $P = .10$). Upon categorization at a threshold of 176.4 μg/mL, GlyFn demonstrated a sensitivity of 0.97 (0.85-1.00) and a specificity of 0.93 (0.66-1.00). At this threshold, and with an estimated population prevalence of 5% for preeclampsia, the positive and negative predictive values for diagnosis of preeclampsia were 47% (95% CI, 23–72%) and 89% (95% CI, 80–98%), respectively (Table 6).

FIGURE 1
First-, second-, and third-trimester glycosylated fibronectin concentrations in control and preeclampsia



GlyFn levels in 45 normotensive control (*circles* and *solid lines*) and 62 PE (*pluses* and *dotted lines*) subjects across first, second, and third trimesters. PE samples include those from both longitudinal and clinical cohorts.

GlyFn, glycosylated fibronectin; *PE*, preeclampsia.

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The results of post hoc analyses of maternal and fetal outcomes vs GlyFn are presented in Table 7. GlyFn values had a significant linear relationship with gestational age at delivery, birthweight, blood pressure, uric acid, and alanine

TABLE 3
Serum biomarker concentrations in normotensive and clinical preeclampsia cohorts

	Third trimester		
Biomarker concentrations	Normotensive cohort (n = 34)	Clinical preeclampsia cohort (n = 44)	Group difference *P* value[a]
Gestational age at sample collection, wk	34.8 (27.0–39.0)	35.0 (27.0–39.0)	.21
GlyFn, μg/mL	55 (1–199)	517 (151–1703)	< .01
sFlt1	6.1 (0–15.6)	23.2 (0–71.4)	< .01
PlGF	361.7 (115.3–673.7)	105.6 (14.6–260)	< .01
sFlt1/PlGF	0.021 (0.000–0.066)	0.208 (0.061–2.781)	< .01

GlyFn, glycosylated fibronectin; *PlGF*, placental growth factor; *sFlt1*, soluble vascular endothelial growth factor receptor 1.

[a] Data are median (range). Group differences were determined using Wilcoxon nonparametric *t* test. Due to insufficient sample volume, data are missing for 7, 22, and 22 normotensive patients and for 1, 14, and 14 clinical preeclampsia patients for Flt1, PlGF, and Flt1/PlGF ratio, respectively.

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TABLE 4
Average weekly change in GlyFn concentration by week and preeclampsia status across all cohorts

Preeclampsia status	No. of subjects (no. of total measurements)	Gestational week	Weekly change GlyFn, μg/mL
Normotensive	38 (87)	7-40	0.1 ± 0.6
Mild preeclampsia	10 (5)	33-38	81.7 ± 94.1
Severe preeclampsia	8 (4)	33-38	195.2 ± 88.2

Weekly change was determined via linear regression with repeated measurements, using repeated measures for each subject. Data are average change ± SE.

GlyFn, glycosylated fibronectin.

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transaminase. For every 100-μg/mL increase in GlyFn, there was a predicted decrease in gestational age at delivery of 0.59 weeks (4 days) ($P < .01$), a decrease in birthweight of 129.4 g ($P < .01$), an increase in systolic blood pressure of 1.39 mm Hg ($P = .04$), an increase in diastolic blood pressure of 1-14 mm Hg ($P = .01$), an increase in uric acid of 13.6 μmol/L ($P < .01$), and an increase of alanine transaminase of 5.88 U/L ($P < .01$). GlyFn was not significantly related to gestational age of diagnosis of preeclampsia ($P = .27$) or proteinuria ($P = .68$).

For participants with preeclampsia, there was a significant relationship between having an infant that was small-for-gestational age and having a GlyFn level ≥500 μg/mL. Of participants with preeclampsia who had a GlyFn level <500 μg, 8% (1/32) had small-for-gestational-age infants, while for participants with preeclampsia who had GlyFn levels ≥500 μg/mL, 26% (6/24) of infants were small-for-gestational age ($P = .03$). There was not a significant relationship between having high GlyFn levels and HELLP syndrome or placental insufficiency in patients with preeclampsia ($P = .13$ and $P = .27$, respectively); however, a higher percentage of women with GlyFn levels ≥500 μg/mL developed HELLP syndrome (26% vs 8%; $P = .13$) and placental insufficiency (26% vs 12%; $P = .27$).

Results from the GlyFn plate assay were compared with the GlyFn POC assay on a subset of the samples. There was a strong correlation (r = 0.76, $P < .01$) between the plate and POC assays. ROC curves were generated for the 2 methods separately, and the AUROCs were similar between the plate (AUROC, 0.99; 95% CI, 0.99−1.00) and POC (AUROC, 0.93; 95% CI, 0.85−1.00) assays. ROC curves were generated to compare the ability to distinguish between mild and severe preeclampsia for the POC and plate assays, and the POC outperformed the plate assay (AUROC, 0.78 vs 0.68).

TABLE 5
Third-trimester preeclampsia classification performance of biomarkers within all cohorts

Biomarker	AUROC (95% CI)	*P* value for comparison to GlyFn ROC
GlyFn, μg/mL	0.99 (0.98−1.00)	NA
sFlt1, ng/mL	0.96 (0.89−1.00)	.11
PlGF, pg/mL	0.94 (0.86−1.00)	.10
Flt1/PlGF	0.98 (0.94−1.00)	.29

AUROC, area under receiver operating characteristic curve; *CI*, confidence interval; *GlyFn*, glycosylated fibronectin; *PlGF*, placental growth factor; *ROC*, receiver operating characteristic; *sFlt1*, soluble vascular endothelial growth factor receptor 1.

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Comment

Principal findings

In this paper, we describe the performance of GlyFn as a biomarker for preeclampsia and a method for assessing the progression of preeclampsia over time based on elevated levels in first-trimester maternal serum that progressively increase throughout pregnancy. In this study, increasing GlyFn levels were correlated with important clinical characteristics and outcomes, including earlier delivery, decreases in birthweight, and increases in blood pressure, uric acid, and alanine transaminase. GlyFn is a uniquely useful analyte to monitor preeclampsia, since GlyFn levels remain constant in controls throughout pregnancy. The best sensitivity and specificity for prediction of preeclampsia were found to be at a cutoff for GlyFn of 176.4 μg/mL.

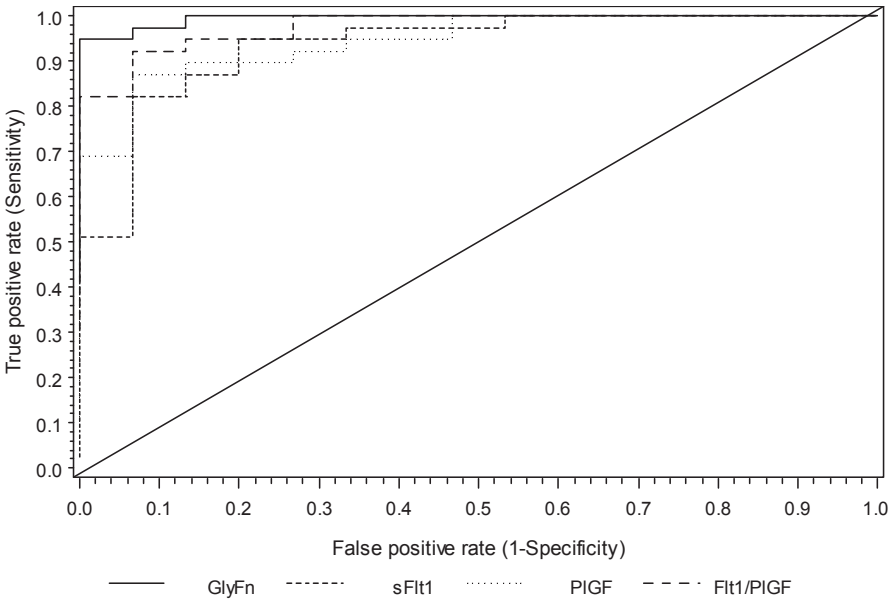
Strengths and weaknesses

GlyFn can be used as a method for the management of preeclampsia, a method of prediction of poor clinical outcomes (eg, low birthweight, HELLP syndrome), and a method to distinguish between mild and severe preeclampsia. The POC portion of this study will be performed with a larger number of participants so that these relationships may be more fully explored.

Mechanisms

The association of elevated GlyFn with preeclampsia is not entirely unexpected in light of the strong association of total serum Fn with preeclampsia, as we, and others, have previously reported.[17-23] The superior performance of this Fn fraction presumably reflects the stronger involvement of a specific fraction of Fn with the pathological processes that initiate preeclampsia.[1] This,

FIGURE 2
Receiver operating characteristic curves showing third-trimester PE classification performance of biomarkers within all cohorts



Flt1, vascular endothelial growth factor receptor 1; *GlyFn*, glycosylated fibronectin; *PE*, preeclampsia; *PlGF*, placental growth factor; *sFlt1*, soluble vascular endothelial growth factor receptor 1.

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in turn, may reflect the particular involvement in preeclampsia development of Fn splice variants or proteolytic fragments that exhibit unique glycosylation patterns. We do not know whether this specific Fn species is derived from pFn, cFn, or both, as they are both glycosylated and found in the circulation,[26,28-32] although an earlier study[20] described an association of elevated cFn with preeclampsia. It is of interest that oxygen levels have recently been reported to regulate expression of the core-1 O-glycan Galβ1-3GalNac epitope in human placenta[42]; thus, placental insufficiency may also contribute to altered glycoprotein levels in preeclampsia.

The association of GlyFn with gestational diabetes[36] as well as preeclampsia is potentially a consequence of the fact that both conditions are associated with inflammation[43-51] and endothelial dysfunction.[52-54] Thus, first-trimester inflammation and endothelial dysfunction related to disrupted spiral artery remodeling may be linked to increased levels of a specific form of GlyFn. We do not have an explanation for the distinct patterns of GlyFn abundance in these 2 related conditions (ie, consistently elevated in all trimesters in gestational diabetes[36] but a progressive increase during the course of preeclampsia), but it could suggest that the factors that trigger gestational diabetes are established early in pregnancy and remain at a constant level, while initiation and development of preeclampsia involves a continuous increase in the conditions that produce GlyFn.

The primary aim of this analysis was to assess the ability of GlyFn to monitor the severity of preeclampsia. sFlt1 and PlGF are currently used principally in investigational studies for the diagnosis, early prediction, and monitoring of disease progression.[10-16] The correlation found between GlyFn and clinical outcomes is important and unique in the literature in that it establishes a potential way to predict which patients will have poor maternal and/or fetal outcomes. This analysis showed that GlyFn is significantly different between patients with and without preeclampsia across the span of pregnancy (including before the clinical presentation of preeclampsia), which has not been shown for other preeclampsia analytes and supports the idea of an early pathogenesis of the disease. This provides preliminary evidence that GlyFn may be an early indicator of risk for preeclampsia.

The ability to use this test in a POC format provides a method for practitioners to quickly determine risk for preeclampsia in their pregnant patients and to determine risk for poor maternal and fetal outcomes among those patients with preeclampsia. ■

TABLE 6
GlyFn POC values for third-trimester diagnosis of preeclampsia at varying prevalence estimates

Threshold of GlyFn POC	Sensitivity and specificity	Predictive value	Predictive value (95% CI) with varying prevalence estimates		
			3%	5%	7%
176.4	Sensitivity: 0.97	Positive predictive value	0.41 (0.19–0.67)	0.47 (0.23–0.72)	0.50 (0.26–0.75)
	Specificity: 0.93	Negative predictive value	0.95 (0.83–0.99)	0.94 (0.80–0.98)	0.93 (0.77–0.98)

CI, confidence interval; *GlyFn*, glycosylated fibronectin; *POC*, point-of-care.

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TABLE 7
Relationship of third-trimester GlyFn levels to clinical characteristics and outcomes in all cohorts

Clinical characteristic or outcome	Pearson correlation coefficient	Change in outcome for every 100-μg change in GlyFn[a]	*P* value[a]
Gestational age at delivery[b]	−0.59	−0.49 wk	< .01
Birthweight[b]	−0.57	−129.4 g	< .01
Systolic blood pressure[b]	0.27	+1.39 mm Hg	.04
Diastolic blood pressure[b]	0.34	+1.14 mm Hg	.01
Gestational age of diagnosis of preeclampsia[b]	−0.17		.27
Uric acid[b]	0.52	+13.6 mμmol/L	< .01
Alanine transaminase[c]	0.42	+5.88 U/L	< .01
Proteinuria[c]	−0.07		.68

GlyFn, glycosylated fibronectin.

[a] Change in outcome was determined via linear regression slope and *P* value was calculated based on this slope; [b] Blood pressure, gestational age of delivery, and birthweight data were assessed across all cohorts and were unavailable for 35, 7, and 7 normotensive participants; [c] Gestational age of diagnosis of preeclampsia, uric acid, alanine transaminase, and proteinuria values were only collected from preeclampsia participants and were missing for 6, 2, and 9 clinical preeclampsia patients.

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