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Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia

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ABSTRACT

BACKGROUND

The ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF) is elevated in pregnant women before the clinical onset of preeclampsia, but its predictive value in women with suspected preeclampsia is unclear.

METHODS

We performed a prospective, multicenter, observational study to derive and validate a ratio of serum sFlt-1 to PlGF that would be predictive of the absence or presence of preeclampsia in the short term in women with singleton pregnancies in whom preeclampsia was suspected (24 weeks 0 days to 36 weeks 6 days of gestation). Primary objectives were to assess whether low sFlt-1:PlGF ratios (at or below a derived cutoff) predict the absence of preeclampsia within 1 week after the first visit and whether high ratios (above the cutoff) predict the presence of preeclampsia within 4 weeks.

RESULTS

In the development cohort (500 women), we identified an sFlt-1:PlGF ratio cutoff of 38 as having important predictive value. In a subsequent validation study among an additional 550 women, an sFlt-1:PlGF ratio of 38 or lower had a negative predictive value (i.e., no preeclampsia in the subsequent week) of 99.3% (95% confidence interval [CI], 97.9 to 99.9), with 80.0% sensitivity (95% CI, 51.9 to 95.7) and 78.3% specificity (95% CI, 74.6 to 81.7). The positive predictive value of an sFlt-1:PlGF ratio above 38 for a diagnosis of preeclampsia within 4 weeks was 36.7% (95% CI, 28.4 to 45.7), with 66.2% sensitivity (95% CI, 54.0 to 77.0) and 83.1% specificity (95% CI, 79.4 to 86.3).

CONCLUSIONS

An sFlt-1:PlGF ratio of 38 or lower can be used to predict the short-term absence of preeclampsia in women in whom the syndrome is suspected clinically. (Funded by Roche Diagnostics.)

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Preeclampsia, a heterogeneous, multisystem disorder defined by the new onset of hypertension and proteinuria after 20 weeks of gestation, affects 2 to 5% of pregnancies worldwide.[1-5] Preeclampsia is associated with high risks of iatrogenic preterm delivery, intrauterine growth restriction, placental abruption, and perinatal mortality, along with maternal morbidity and mortality.[6,7]

The cause of preeclampsia is incompletely understood, but the disorder is thought to be due to placental malperfusion resulting from abnormal remodeling of maternal spiral arteries.[8,9] In preeclampsia, circulating maternal serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1) are increased, and placental growth factor (PlGF) levels are decreased.[10,11] An antagonist of PlGF and vascular endothelial growth factor, sFlt-1 causes vasoconstriction and endothelial damage that may lead to fetal growth restriction and preeclampsia.[12-14] A high ratio of sFlt-1 to PlGF is associated with an increased risk of preeclampsia and may be a better predictor of risk than either biomarker alone.[11,15-20]

Proteinuria and elevated blood pressure are diagnostic criteria for preeclampsia, but the clinical presentation is variable. The Elecsys immunoassays for sFlt-1 and PlGF (Roche Diagnostics) have received Conformité Européenne (CE) marking for use as in vitro medical devices. The sFlt-1:PlGF ratio has been approved as a diagnostic aid for preeclampsia in conjunction with other clinical findings.[21]

There is a need for a reliable predictor of preeclampsia (particularly its absence) in the short term in women with suspected preeclampsia. Women with suggestive symptoms or signs are often hospitalized until preeclampsia and related adverse outcomes have been ruled out. Others who require hospitalization may be overlooked. Although no preventive or therapeutic strategy is yet available, with the exception of low-dose acetylsalicylic acid, which has a moderate preventive effect in high-risk pregnancies after the first trimester,[22] clinical experience suggests that early detection and monitoring are beneficial.[23]

PROGNOSIS (Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia Study) was designed to investigate the value of using the sFlt-1:PlGF ratio for the prediction of the presence or absence of preeclampsia in the short term.

Methods

Study Oversight

PROGNOSIS was a prospective observational study conducted in 14 countries (see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Full details of the methods have been published previously.[24] The protocol (available at NEJM.org) was approved by applicable national and regional independent ethics committees and institutional review boards. The study adhered to the Guidelines for Good Clinical Practice. Roche Diagnostics designed the study, with scientific and practical input from a medical adviser and clinical investigators. Data were analyzed by the sponsor's biostatistician. A medical writer funded by Roche Diagnostics provided medical writing assistance to all the authors. All the authors vouch for the fidelity of the study to the protocol and made the decision to submit the manuscript for publication. The research contract between the sponsor and the institutions participating in the study included a confidentiality agreement.

Study Participants

In the study, we included pregnant women who were 18 years of age or older (24 weeks 0 days to 36 weeks 6 days of gestation at the first visit) with suspected preeclampsia according to protocol-defined criteria (Table S2 in the Supplementary Appendix). Women who had manifest preeclampsia or a confirmed diagnosis of the HELLP syndrome (characterized by hemolysis, elevated liver-enzyme levels, and low platelet counts) and those who had received treatment with an investigational medicine within 90 days before enrollment were excluded. Participants provided written informed consent.

Study Design

We designed the study to derive and validate a cutoff point of the sFlt-1:PlGF ratio for the prediction of the short-term absence or presence of preeclampsia, in a two-phase approach (development and validation). In the development phase, we used data from 500 participants to derive the sFlt-1:PlGF ratio cutoff point for the prediction model, which was validated with the use of data from 550 additional participants (see the Supplementary Appendix). Measurements were not available until after the study; neither the investigators nor the participants were informed of the

results during the study (i.e., results could not influence clinical decisions). Assessments were made at visit 1 (baseline visit); visit 2 (7 to 9 days after visit 1); visits 3, 4, and 5 (7±2 days after the previous visit); at delivery; and at the postpartum visit. Information collected at these visits included an updated medical history, clinical assessments, laboratory testing and determination of the sFlt-1:PlGF ratio (visits 1 through 5), and documentation of maternal and neonatal outcomes.

STUDY OBJECTIVES

The primary objectives were, first, to determine whether sFlt-1:PlGF ratios that were at or below a defined cutoff point predicted the absence of preeclampsia, eclampsia, and the HELLP syndrome for 1 week after the baseline visit (rule out) and, second, to determine whether sFlt-1:PlGF ratios that were above a defined cutoff point predicted a diagnosis of preeclampsia, eclampsia, or the HELLP syndrome within 4 weeks after the baseline visit (rule in). Secondary objectives included determination of whether sFlt-1:PlGF ratios at or below a defined cutoff point were associated with the absence of preeclampsia-related maternal and fetal adverse outcomes within 1 week and whether values above the cutoff point were associated with the presence of such adverse outcomes within 4 weeks.

We performed post hoc exploratory analyses of associations between sFlt-1:PlGF ratios and combined outcomes (preeclampsia, eclampsia, or the HELLP syndrome and maternal or fetal adverse outcomes) within 1 week and 4 weeks after the baseline visit. An additional post hoc analysis compared the value of clinical data alone (the results of a dipstick test for proteinuria plus blood-pressure measurement) with the value of clinical data plus the sFlt-1:PlGF ratio for predicting preeclampsia.

DIAGNOSTIC CRITERIA

Diagnostic criteria for each preeclampsia-related disorder were based on international guidelines[25-30] (Table S3 in the Supplementary Appendix). Diagnostic criteria for preeclampsia were a new onset of both hypertension (systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or both) and proteinuria (2+ protein or greater on dipstick urinalysis, ≥300 mg of protein per 24-hour urine collection, ≥30 mg of protein per deciliter in a spot urine sample, or a ratio of protein to creatinine of ≥30 mg per millimole) after 20 weeks of gestation. Only cases that met these prespecified criteria were included in the analyses. (Cases of preeclampsia diagnosed according to local criteria that did not meet the criteria defined in the protocol were excluded.) Preeclampsia status was classified as no preeclampsia; suspected preeclampsia (defined according to the criteria for inclusion in the study but not applicable at delivery or post partum); preeclampsia; and severe preeclampsia, eclampsia, the HELLP syndrome, or a combination of these disorders. Neurologic symptoms (headache or visual disturbances), epigastric pain, severe edema, and oliguria were recorded.

Protocol-defined maternal adverse outcomes other than preeclampsia, eclampsia, or the HELLP syndrome were death, pulmonary edema, acute renal failure, cerebral hemorrhage, cerebral thrombosis, and disseminated intravascular coagula-

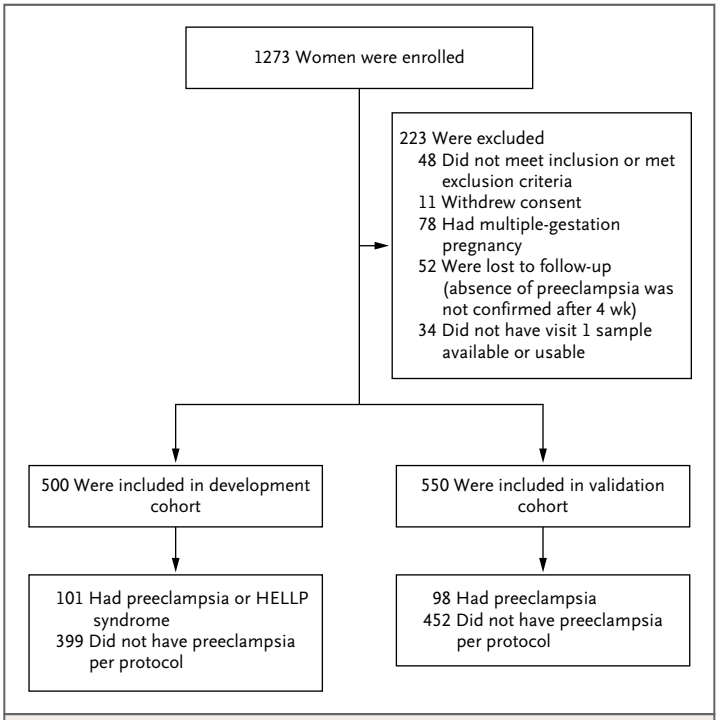

Figure 1. Numbers of Women Enrolled and Outcomes in the Development and Validation Cohorts.

A total of 52 participants who did not have protocol-defined preeclampsia as assessed during the first four visits were excluded from the analyses because they were subsequently lost to follow-up, with no data available at 28 days or later to definitively confirm the absence of preeclampsia. In the development cohort, 99 women had preeclampsia only, 1 had preeclampsia and hemolysis, elevated liver-enzyme levels, and low platelet counts (HELLP syndrome), and 1 had the HELLP syndrome only.

Table 1. Baseline Characteristics of the Study Participants and Reasons for Suspicion of Preeclampsia.*

Characteristic	Development Cohort		Validation Cohort	
	No Preeclampsia, Eclampsia, or HELLP Syndrome (N=399)	Preeclampsia, Eclampsia, or HELLP Syndrome (N=101)	No Preeclampsia, Eclampsia, or HELLP Syndrome (N=452)	Preeclampsia, Eclampsia, or HELLP Syndrome (N=98)
Median age (IQR) — yr	32 (27–36)	32 (28–36)	31 (26–36)	32 (25–36)
Median wk of gestation (IQR)	31.6 (27.3–34.7)	32.1 (27.7–34.4)	31.4 (27.6–34.3)	31.6 (28.0–34.6)
Median BMI before pregnancy (IQR)†	27.0 (22.3–32.0)	24.9 (21.5–31.2)	26.1 (22.5–30.6)	26.4 (22.8–29.4)
Median blood pressure (IQR) — mm Hg				
Systolic	128 (115–140)	137 (130–149)‡	125 (110–137)	137 (126–146)‡
Diastolic	80 (70–90)	85 (80–94)‡	78 (70–86)	90 (80–95)‡
Smoking — no. (%)				
Past	71 (17.8)	22 (21.8)	105 (23.2)	18 (18.4)
Current	60 (15.0)	13 (12.9)	70 (15.5)	9 (9.2)
Race — no. (%)§				
Asian	14 (3.5)	7 (6.9)	24 (5.3)	9 (9.2)
Black	26 (6.5)	7 (6.9)	20 (4.4)	8 (8.2)
White	355 (89.0)	87 (86.1)	345 (76.3)	73 (74.5)
Other	4 (1.0)	0	63 (13.9)	8 (8.2)
Reasons for suspected preeclampsia — no. (%)¶				
New-onset hypertension	109 (27.3)	43 (42.6)‖	103 (22.8)	55 (56.1)‡
Exacerbation of preexisting hypertension	57 (14.3)	18 (17.8)	57 (12.6)	13 (13.3)
New-onset proteinuria	144 (36.1)	50 (49.5)**	157 (34.7)	35 (35.7)
Exacerbation of preexisting proteinuria	6 (1.5)	1 (1.0)	3 (0.7)	2 (2.0)
Other	304 (76.2)	80 (79.2)	364 (80.5)	81 (82.7)
Epigastric pain	31 (7.8)	7 (6.9)	34 (7.5)	7 (7.1)
Headache	105 (26.3)	35 (34.7)	141 (31.2)	33 (33.7)
Excessive edema	33 (8.3)	12 (11.9)	64 (14.2)	17 (17.3)
Visual disturbances	38 (9.5)	9 (8.9)	56 (12.4)	15 (15.3)

Severe swelling of face, hands, feet	55 (13.8)	14 (13.9)	51 (11.3)	20 (20.4)‡
Sudden weight gain, >1 kg/wk	36 (9.0)	8 (7.9)	47 (10.4)	8 (8.2)
Low platelet count	35 (8.8)	8 (7.9)	26 (5.8)	2 (2.0)
Elevated liver-enzyme levels	12 (3.0)	4 (4.0)	15 (3.3)	8 (8.2)
Intrauterine growth restriction, an inclusion criterion	54 (13.5)	14 (13.9)	78 (17.3)	9 (9.2)**
Abnormal uterine perfusion	87 (21.8)	20 (19.8)	94 (20.8)	19 (19.4)

* Preeclampsia, eclampsia, and the HELLP syndrome (hemolysis, elevated liver-enzyme levels, and low platelet counts) were diagnosed according to protocol-specified criteria. P values were calculated with the use of the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. IQR denotes interquartile range.

† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

‡ P<0.01 for the comparison with participants in whom preeclampsia, eclampsia, and the HELLP syndrome did not develop.

§ Race was determined by the investigator.

¶ There may have been more than one reason for suspected preeclampsia.

‖ P<0.001 for the comparison with participants in whom preeclampsia, eclampsia, and the HELLP syndrome did not develop.

** P<0.05 for the comparison with participants in whom preeclampsia, eclampsia, and the HELLP syndrome did not develop.

tion. Fetal adverse outcomes were perinatal or fetal death, delivery at a gestational age of less than 34 weeks, intrauterine growth restriction, placental abruption, the respiratory distress syndrome, necrotizing enterocolitis, and intraventricular hemorrhage.

ASSESSMENT OF SERUM MARKERS

Serum samples (≥2 ml), collected according to a standard operating procedure, were analyzed retrospectively at an independent laboratory (Kreiskliniken Altoetting–Burghausen, Zentrallabor, Altoetting, Germany). Maternal serum levels of sFlt-1 and PlGF (with both levels measured in picograms per milliliter) were determined by means of the fully automated Elecsys assays for sFlt-1 and PlGF on an electrochemiluminescence immunoassay platform (cobas e analyzers, Roche Diagnostics) and were used to calculate the sFlt-1:PlGF ratio. The within-run coefficient of variation for control samples is below 4% for both assays. Between-run coefficients of variation are 2.3 to 5.6% for the Elecsys sFlt-1 assay and 2.4 to 4.6% for the Elecsys PlGF assay.

STATISTICAL ANALYSIS

We calculated that we would need to enroll approximately 1000 women (500 each for the development and validation cohorts), on the basis of previous data,[17] expert medical opinion on requirements to achieve a positive predictive value higher than 25% and a negative predictive value higher than 96%, and an assumed preeclampsia prevalence of 15% among women with signs or symptoms of preeclampsia[24] (see the Supplementary Appendix). For analysis of the validation cohort alone, the study had 90% power to show a negative predictive value greater than 96% (rule out of preeclampsia, eclampsia, and the HELLP syndrome within 1 week) and to show a positive predictive value greater than 25% (rule in of preeclampsia, eclampsia, or the HELLP syndrome within 4 weeks).

For the development phase of the study, prediction algorithms were derived for primary outcomes on the basis of sFlt-1:PlGF cutoff points and gestational age. Three models were applied for each prediction (1-week rule out and 4-week rule in): a model with one cutoff point (independent of gestational age); a model with two cutoff points, one for the earlier gestational phase (24 to <34 weeks) and one for the later gesta-

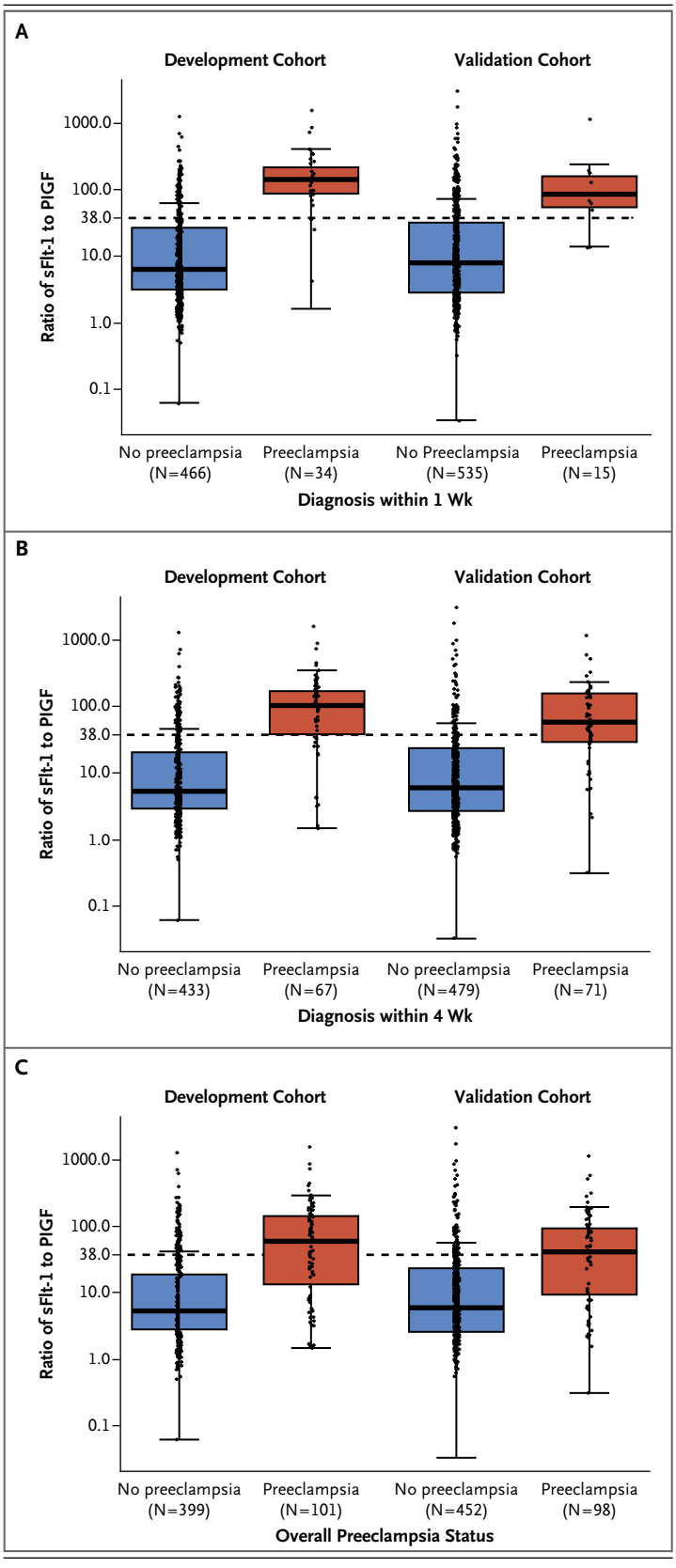

Figure 2. Ratio of sFlt-1 to PlGF for Participants with and Those without Preeclampsia in the Development and Validation Cohorts.

Preeclampsia status is shown at 1 week (Panel A), at 4 weeks (Panel B), and overall (Panel C). The bottom and top edges of each box represent the first and third quartiles, respectively, the band within the box represents the median value, the whiskers represent values that are 1.5 times the interquartile range, and the horizontal dotted line represents the cutoff point of 38 for the ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF), with both levels measured in picograms per milliliter.

tional phase (≥34 weeks); and a model with a cutoff point for each gestational week. For each model, the negative predictive value, positive predictive value, sensitivity, and specificity were estimated with the use of stratified Monte Carlo cross-validation (see the Supplementary Appendix).[31] For validation, the single-cutoff model for both rule out and rule in was selected from the development phase because the area under the curve (AUC) for this model was similar to that for each of the other models and the single cutoff point of 38 was preferred for reasons of simplicity and ease of use. Predictive performance was assessed in the validation cohort by estimating negative and positive predictive values, sensitivity and specificity, and the AUC with receiver-operating-characteristic (ROC) curves, with corresponding 95% confidence intervals. Predictive performance was also assessed in the development and validation cohorts combined.

We recruited women with either singleton or multiple pregnancies. However, only women with singleton pregnancies were included in the primary analysis.

RESULTS

BASELINE CHARACTERISTICS

Between December 2010 and January 2014, a total of 1273 women with suspected preeclampsia were enrolled (Fig. 1). The analysis included 1050 eligible participants at 30 sites who could be evaluated. Age, gestational age, body-mass index before pregnancy, and smoking status did not differ significantly between participants in whom preeclampsia developed and those in whom it did not (Table 1, and Table S4 in the Supplementary Appendix). The incidence of preeclamp-

sia, the HELLP syndrome, or both according to the protocol-defined criteria was 20.2% in the development cohort and 17.8% in the validation cohort. There were no cases of eclampsia. The frequency and duration of hospitalization for mothers and neonates are reported in Table S5 in the Supplementary Appendix.

DEVELOPMENT PHASE

The median sFlt-1:PlGF ratio was elevated among participants in whom preeclampsia or the HELLP syndrome developed within 1 week (146.4) or within 4 weeks (104.8). For participants in whom these disorders did not develop, the median ratio was 6.3 at 1 week and 5.5 at 4 weeks (Fig. 2).

For the single-cutoff model, the gestational-phase model, and the gestational-week model, respectively, the AUCs were 89.2%, 90.9%, and 90.5% for 1-week rule out and 86.4%, 86.2%, and 86.2% for 4-week rule in. For the selected single-cutoff model, the median cutoff points derived from the development cohort were 38.2 (1-week rule out) and 37.5 (4-week rule in). The application of a single cutoff point of 38 for all gestational ages and for both primary prediction claims (1-week rule out and 4-week rule in) was appropriate as a simple prediction model to be validated.

VALIDATION PHASE

In the validation cohort, the median sFlt-1:PlGF ratio was 87.8 and 59.4 for participants in whom preeclampsia or the HELLP syndrome developed within 1 week and within 4 weeks, respectively, as compared with 8.0 and 6.3 among participants in whom these disorders did not develop (Fig. 2). The negative predictive value (no diagnosis of preeclampsia, eclampsia, or the HELLP syndrome within 1 week) of 38 or lower for the sFlt-1:PlGF ratio was 99.3% (95% confidence interval [CI], 97.9 to 99.9), and the positive predictive value (a diagnosis of preeclampsia, eclampsia, or the HELLP syndrome within 4 weeks) was 36.7% (95% CI, 28.4 to 45.7) (Table 2 and Fig. 3). Results for negative and positive predictive values with the use of the full data set (development and validation cohorts) are shown in Figures S1 and S2 in the Supplementary Appendix.

A post hoc analysis used the revised criteria of the American College of Obstetricians and Gynecologists for the diagnosis of preeclampsia (new-onset hypertension in the absence of new-onset proteinuria, provided one or more predefined other new-onset clinical signs or features of the syndrome were present).[23] The results were similar to those obtained with the protocol-defined criteria for preeclampsia (Table S6 in Supplementary Appendix).

ROC curves for the individual biomarkers in the development and validation cohorts are shown in Figure S3 in the Supplementary Appendix; cutoff points were not derived. The predictive performance of sFlt-1 and PlGF, used separately, was not superior to the predictive performance of the sFlt-1:PlGF ratio. A post hoc analysis suggested that the addition of the sFlt-1:PlGF ratio to proteinuria and blood-pressure assessments improved the prediction of preeclampsia (both rule out within 1 week and rule in within 4 weeks) (Fig. S4 in the Supplementary Appendix).

MATERNAL AND FETAL ADVERSE OUTCOMES

Two maternal adverse outcomes occurred. One participant (with an sFlt-1:PlGF ratio of 143.7) had severe preeclampsia and cerebral hemorrhage within 1 week. Another participant (with an sFlt-1:PlGF ratio of 64.4) had cerebral thrombosis within 4 weeks, despite the apparent absence of a clinical risk factor for thrombosis, but

Table 2. Validation of a Cutoff Point of 38 for the sFlt-1:PlGF Ratio in Predicting Preeclampsia.*

Preeclampsia	Development Cohort	Validation Cohort
	percent (95% CI)	
Within 1 wk		
Negative predictive value: rule out	98.9 (97.3–99.7)	99.3 (97.9–99.9)
Sensitivity	88.2 (72.5–96.7)	80.0 (51.9–95.7)
Specificity	80.0 (76.1–83.6)	78.3 (74.6–81.7)
Within 4 wk		
Positive predictive value: rule in	40.7 (31.9–49.9)	36.7 (28.4–45.7)
Sensitivity	74.6 (62.5–84.5)	66.2 (54.0–77.0)
Specificity	83.1 (79.3–86.5)	83.1 (79.4–86.3)

* Sensitivity was calculated on the basis of the number of participants in whom preeclampsia developed within 1 week or 4 weeks. Specificity was calculated on the basis of the number of participants in whom preeclampsia did not develop within 1 week or 4 weeks. Maternal serum levels of sFlt-1 and PlGF were both measured in picograms per milliliter.

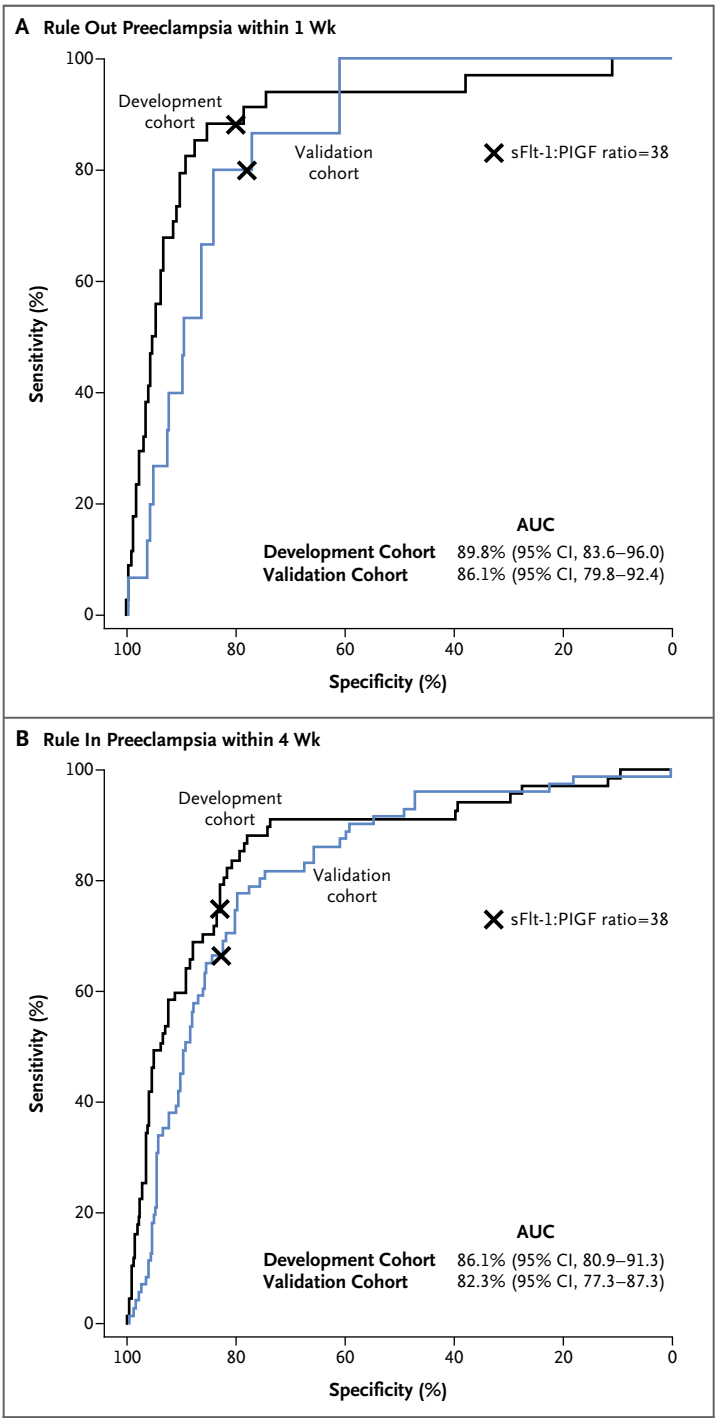

Figure 3. Predictive Performance of the sFlt-1:PlGF Ratio for Protocol-Defined Preeclampsia in the Development and Validation Cohorts.

The predictive performance of a cutoff point of 38 for the sFlt-1:PlGF ratio is shown for ruling out preeclampsia within 1 week (Panel A) and ruling in preeclampsia within 4 weeks (Panel B). AUC denotes area under the curve.

preeclampsia, eclampsia, and the HELLP syndrome did not develop in this participant.

An sFlt-1:PlGF ratio of 38 or lower was predictive of the absence of fetal adverse outcomes within 1 week (negative predictive value in the development cohort, 99.5% [95% CI, 98.1 to 99.9]; negative predictive value in the validation cohort, 99.3% [95% CI, 97.9 to 99.9]); a ratio greater than 38 was predictive of the presence of these outcomes at 4 weeks (positive predictive value in the development cohort, 37.2% [95% CI, 28.6 to 46.4]; positive predictive value in the validation cohort, 47.5% [95% CI, 38.4 to 56.8]) (Fig. S5 and S6 in the Supplementary Appendix). An sFlt-1:PlGF ratio of more than 38 was also associated with a shorter time to delivery (Fig. S7 in the Supplementary Appendix). The results of post hoc analyses using a combined end point of preeclampsia, eclampsia, or the HELLP syndrome or adverse maternal or fetal outcomes are shown in Figures S8 and S9 and Table S7 in the Supplementary Appendix. Outcomes for participants with high sFlt-1:PlGF ratios in whom preeclampsia did not develop are reported in Table S8 in the Supplementary Appendix.

DISCUSSION

The present study identified and validated a cutoff point of 38 for the sFlt-1:PlGF ratio, assessed with the use of the Elecsys sFlt-1 and PlGF immunoassays, as a useful predictor of the short-term absence of preeclampsia in women with singleton pregnancies and clinical signs that are suggestive of the disorder. In the validation cohort, the negative predictive value of a ratio at or below this cutoff point (i.e., for ruling out preeclampsia within 1 week) was 99.3% (95% CI, 97.9 to 99.9).

Preeclampsia is a major contributor to pregnancy-associated morbidity and mortality, and the management of this complex syndrome needs to be improved.[8,20,32] High blood pressure and proteinuria have low predictive value for preeclampsia and its associated adverse outcomes. Angiogenic and antiangiogenic factors have been implicated in the pathophysiology of preeclampsia.[8,9] In PROGNOSIS, a single cutoff point for the sFlt-1:PlGF ratio, independent of

the weeks of gestation, was validated for ruling out preeclampsia, eclampsia, and the HELLP syndrome within 1 week after assessment of the ratio. The ability to accurately rule out preeclampsia, eclampsia, and the HELLP syndrome within 1 week on the basis of the sFlt-1:PlGF ratio is likely to improve clinical decisions with regard to hospitalization versus outpatient monitoring and the intensity of outpatient monitoring. In clinical practice, a very high negative predictive value is crucial in the evaluation of a patient with suspected preeclampsia, since failure to detect imminent disease could have devastating consequences for the fetus or the mother.

The observed positive predictive value of the sFlt-1:PlGF ratio was 36.7%, which appears to represent an improvement in prediction, as compared with clinical variables in post hoc analyses. Assessment for proteinuria and measurement of blood pressure have a reported positive predictive value of only 20% in detecting preeclampsia-related adverse outcomes.[32]

Generally, the sFlt-1:PlGF ratio has shown better diagnostic performance than have the single biomarkers.[17,18,21,33] A recent study suggested that PlGF alone predicted delivery within 14 days for women with confirmed preeclampsia before 35 weeks' gestation.[34] In the present study, the predictive performance of sFlt-1 and PlGF, evaluated individually, was not superior to the predictive performance of the sFlt-1:PlGF ratio.

An sFlt-1:PlGF ratio cutoff point of 38 or lower also had value in predicting the absence of fetal adverse outcomes within 1 week, as well as the absence of the combined end point of preeclampsia or adverse maternal or fetal outcomes within 1 week. In the two cohorts combined, comprising 1050 participants, only two maternal adverse outcomes occurred, both in women who had high sFlt-1:PlGF ratios. It was not possible to evaluate the predictive performance of the sFlt-1:PlGF ratio separately for maternal adverse outcomes, since there were only two such outcomes.

Previous studies have investigated the sFlt-1:PlGF ratio for the prediction of preeclampsia, but these studies were not prospective, included fewer participants than ours, or had different inclusion and exclusion criteria.[19,20,35-38] The current study extends these previous studies by prospectively validating an sFlt-1:PlGF ratio cutoff point of 38, calculated with the use of commercially available and fully automated immunoassays, for the prediction of preeclampsia in the short term.

Our study has limitations. The data were validated with the use of the Elecsys immunoassays, and the optimal cutoff point for the ratio may differ when other assays are used. In addition, PROGNOSIS was an observational study. Data from randomized trials are needed to establish whether use of this ratio in clinical practice, as compared with the current standard of care, could reduce unnecessary hospitalizations and costs, with improved or similar results with respect to fetal and maternal adverse outcomes. In conclusion, this study shows that a cutoff point of 38 for the sFlt-1:PlGF ratio is useful for predicting the short-term absence of preeclampsia in women in whom the disorder is suspected clinically.

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REFERENCES

1. Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980-2010: age-period-cohort analysis. BMJ 2013;347:f6564.
2. Hernández-Díaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. BMJ 2009;338:b2255.
3. Skjaerven R, Wilcox AJ, Lie RT. The interval between pregnancies and the risk of preeclampsia. N Engl J Med 2002;346:33-8.
4. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol 2009;33:130-7.
5. World Health Organization. The World Health Report 2005: make every mother and child count. November 1, 2014 (http://www.who.int/whr/2005/en/).
6. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
7. McClure JH, Cooper GM, Clutton-Brock TH. Saving mothers' lives: reviewing maternal deaths to make motherhood safer: 2006-8: a review. Br J Anaesth 2011;107:127-32.
8. Staff AC, Benton SJ, von Dadelszen P, et al. Redefining preeclampsia using placenta-derived biomarkers. Hypertension 2013;61:932-42.
9. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia. 1. Current understanding of its pathophysiology. Nat Rev Nephrol 2014;10:466-80.
10. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649-58.
11. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992-1005.
12. Karumanchi SA, Epstein FH. Placental ischemia and soluble fms-like tyrosine kinase 1: cause or consequence of preeclampsia? Kidney Int 2007;71:959-61.
13. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993;90:10705-9.
14. Lu FX, Longo M, Tamayo E, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol 2007;196(4):396.e1-7.
15. Vatten LJ, Eskild A, Nilsen TIL, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. Am J Obstet Gynecol 2007;196(3):e1-e6.
16. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.
17. Verlohren S, Galindo A, Schlembach D, et al. An automated method for the determination of the sFlt-1/PlGF ratio in the assessment of preeclampsia. Am J Obstet Gynecol 2010;202(2):e1, e11.
18. Verlohren S, Herraiz I, Lapaire O, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. Am J Obstet Gynecol 2012;206(1):e1-8.
19. Villa PM, Hämäläinen E, Mäki A, et al. Vasoactive agents for the prediction of early- and late-onset preeclampsia in a high-risk cohort. BMC Pregnancy Childbirth 2013;13:110.
20. Rana S, Powe CE, Salahuddin S, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation 2012;125:911-9.
21. Verlohren S, Herraiz I, Lapaire O, et al. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. Hypertension 2014;63:346-52.
22. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson JH, Rowland MG. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med 2014;160:695-703.
23. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Obstet Gynecol 2013;122:1122-31.
24. Hund M, Allegranza D, Schoedl M, Dilba P, Verhagen-Kamerbeek W, Stepan H. Multicenter prospective clinical study to evaluate the prediction of short-term outcome in pregnant women with suspected preeclampsia (PROGNOSIS): study protocol. BMC Pregnancy Childbirth 2014;14:324.
25. Magann EF, Martin JN Jr. Twelve steps to optimal management of HELLP syndrome. Clin Obstet Gynecol 1999;42:532-50.
26. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 2001;20:IX-XIV.
27. ACOG Committee on Obstetric Practice, American College of Obstetricians and Gynecologists. ACOG practice bulletin: diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Int J Gynaecol Obstet 2002;77:67-75.
28. Simhan HN, Caritis SN. Prevention of preterm delivery. N Engl J Med 2007;357:477-87.
29. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy 2003;22:143-8.
30. Stepan H. Intrauterine Wachstumsretardierung. In: Wacker J, Bastert G, Sillem M, Beckmann MW, eds. Therapiehandbuch Gynäkologie und Geburtshilfe. Heidelberg, Germany: Springer Medizin, 2007:45-50.
31. Xu QS, Liang YZ, Du YP. Monte Carlo cross-validation for selecting a model and estimating the prediction error in multivariate calibration. J Chemometrics 2004;18:112-20.
32. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gynecol 2001;97:261-7.
33. Álvarez-Fernández I, Prieto B, Rodríguez V, Ruano Y, Escudero AI, Álvarez FV. New biomarkers in diagnosis of early onset preeclampsia and imminent delivery prognosis. Clin Chem Lab Med 2014;52:1159-68.
34. Chappell LC, Duckworth S, Seed PT, et al. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. Circulation 2013;128:2121-31.
35. Holmes VA, Young IS, Patterson CC, et al. The role of angiogenic and anti-angiogenic factors in the second trimester in the prediction of preeclampsia in pregnant women with type 1 diabetes. Diabetes Care 2013;36:3671-7.
36. Chaiworapongsa T, Romero R, Korzeniewski SJ, et al. Plasma concentrations of angiogenic/anti-angiogenic factors have prognostic value in women presenting with suspected preeclampsia to the obstetrical triage area: a prospective study. J Matern Fetal Neonatal Med 2014;27:132-44.
37. Moore AG, Young H, Keller JM, et al. Angiogenic biomarkers for prediction of maternal and neonatal complications in suspected preeclampsia. J Matern Fetal Neonatal Med 2012;25:2651-7.
38. Lai J, Garcia-Tizon Larroca S, Peeva G, Poon LC, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by serum placental growth factor and soluble fms-like tyrosine kinase-1 at 30-33 weeks' gestation. Fetal Diagn Ther 2014;35:240-8.