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ORIGINAL RESEARCH

Decision Threshold for Kryptor sFlt-1/PlGF Ratio in Women With Suspected Preeclampsia: Retrospective Study in a Routine Clinical Setting

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BACKGROUND: The objective was to evaluate predictive performance and optimal decision threshold of the Kryptor soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio when implemented for routine management of women presenting with symptoms of preeclampsia.

METHODS AND RESULTS: Observational retrospective study of a cohort of 501 women with suspected preeclampsia after 20 weeks of gestation. Women referred to maternity ward for observation of preeclampsia had an sFlt-1/PlGF ratio test included in routine diagnostic workup. Maternal and offspring characteristic data included maternal risk factors, outcomes, delivery mode, and indication for suspected preeclampsia. Biochemical measurements to determine sFlt-1/PlGF ratio were performed using the BRAHMS/Kryptor sFlt-1/PlGF ratio immunoassays. Results were analyzed by area under receiver-operating characteristic curve. Preeclampsia occurred in 150 of 501 (30%) of symptomatic women with an sFlt-1/PlGF ratio determined before the time of diagnosis. Area under receiver-operating characteristic curve for diagnosis of early-onset preeclampsia within 1 and 4 weeks was 0.98 (95% CI, 0.96–1.00) and 0.95 (95% CI, 0.92–0.98), respectively. For late-onset preeclampsia, predictive performance within 1 and 4 weeks was lower: 0.90 (95% CI, 0.85–0.94) and 0.85 (95% CI, 0.80–0.90), respectively. The optimal single sFlt-1/PlGF ratio threshold for all preeclampsia and late-onset preeclampsia within 1 and 4 weeks was 66. The negative and positive predictive values for ruling out and ruling in developing preeclampsia within 1 week were 96% and 70%, respectively.

CONCLUSIONS: The Kryptor sFlt-1/PlGF ratio is a useful clinical tool ruling out and in preeclampsia within 1 week. Prediction within 4 weeks is superior for early-onset preeclampsia. A single decision threshold of 66 is indicated for use in clinical routine.

Key Words: clinical routine ■ Kryptor ■ preeclampsia ■ soluble fms-like tyrosine kinase-1/placental growth factor ratio ■ threshold value

Preeclampsia is a multiorgan pregnancy disorder associated with significant maternal and neonatal morbidity and mortality, affecting 3% to 8% of pregnancies worldwide.[1-4] Preeclampsia is characterized by new-onset hypertension after 20 weeks of gestation, often along with proteinuria in the mother. The condition can progress to multiorgan dysfunction, including hepatic, renal, and cerebral disease, if the fetus and placenta are not delivered.[5]

The pathophysiological characteristics of preeclampsia have yet to be fully established, but increasing evidence supports the view that the condition can be subclassified into early- and late-onset preeclampsia.[2,6,7] Early-onset preeclampsia, defined as onset of symptoms before gestational week (GW) 34, is characterized by impaired implantation of the placenta caused by insufficient trophoblast invasion and spiral artery remodeling. Abnormal placentation,

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CLINICAL PERSPECTIVE

What Is New?

- The soluble fms-like tyrosine kinase-1/placental growth factor ratio test was implemented and evaluated retrospectively in a clinical routine setting.
- We provide Kryptor soluble fms-like tyrosine kinase-1/placental growth factor thresholds for both early- and late-onset preeclampsia and time to diagnosis.
- An optimal Kryptor soluble fms-like tyrosine kinase-1/placental growth factor threshold of 66 may be used for clinical decision making.

What Are the Clinical Implications?

- Identifying pregnant women at risk of developing preeclampsia reduces severe maternal and neonatal morbidity and mortality.
- Accurate diagnosis of preeclampsia is challenging because signs and symptoms often are nonspecific.
- Implementation of additional diagnostic tools may improve maternal and neonatal outcome, and a single Kryptor soluble fms-like tyrosine kinase-1/placental growth factor decision threshold of 66 provides clinicians with a simpler alternative to gestation-specific dual threshold values.

Nonstandard Abbreviations and Acronyms

GA	gestational age
GW	gestational week
PlGF	placental growth factor
sFlt-1	soluble fms-like tyrosine kinase-1

in turn, leads to placental hypoperfusion that eventually results in fetal growth restriction.[3] Late-onset preeclampsia, defined as preeclampsia that developed at or after 34 weeks of gestation, is associated with a shift from placental endothelial dysfunction to more widespread maternal endothelial dysfunction.[8] The 2 conditions have different implications for both the mother and the fetus/neonate, with a 10-fold higher risk of perinatal mortality in the early-onset preeclampsia group and 1.5-fold increased risk among mothers with late-onset disease, compared with mothers without preeclampsia.[6]

One key factor in development of endothelial dysfunction is soluble fms-like tyrosine kinase-1 (sFlt-1), a circulating splice variant of the membrane-bound receptor vascular endothelial growth factor receptor 1 with significant antiangiogenic function.[9] Excess levels of sFlt-1, produced in the placenta, bind and antagonize placental growth factor (PlGF) and vascular endothelial growth factor, leading to reduced ligand-mediated angiogenic signaling. Consequently, an imbalance in circulating angiogenic factors induces maternal endothelial dysfunction associated with preeclamptic signs and symptoms.[10]

The sFlt-1/PlGF ratio has been established as a strong predictor of preeclampsia.[11-13] Altered levels of sFlt-1 and PlGF are detectable weeks before the onset of preeclampsia symptoms and complications. Monitoring the sFlt-1/PlGF ratio can support the diagnosis and eventually the time of delivery to facilitate better maternal and fetal outcome.[12] Identification of women at risk, and especially not at risk, is essential in the daily clinic to optimize perinatal care, reduce complications, and ensure optimal allocation of available resources.

Increasing evidence has supported the clinical benefit of using the sFlt-1/PlGF ratio in women with suspected preeclampsia after 20 weeks of gestation.[14,15] However, the optimal clinical and biochemical setup for implementation has been a matter of debate. Initially, it was proposed to use multiple gestational phase adapted cutoffs focusing on high sensitivity for ruling out preeclampsia (33) and a second focusing on high specificity for ruling in early-onset preeclampsia (85) and late-onset preeclampsia (110).[14] In the prospective, multicenter Predictive Value of the sFlt-1:PlGF Ratio in women with suspected preeclampsi study, a uniform cutoff of ≤38 was shown to predict the short-term absence of preeclampsia in women in whom the syndrome was clinically suspected.[15] Although most studies were performed using the automated Roche Elecsys immunoassays and thus with comparable ratios, method comparison studies have shown that the BRAHMS Kryptor automated sFlt-1 and PlGF immunoassays result in an elevated ratio compared with Roche Elecsys.[16,17] Hence, there is a need to establish vendor-specific predictive cutoff value(s) for clinical implementation.

The objective of this retrospective study was to evaluate the predictive performance and optimal Kryptor-specific threshold value(s) for the sFlt-1/PlGF ratio when implemented in routine clinical management of women presenting with symptoms of preeclampsia after 20 weeks of gestation. Herein, we provide evidence for the use of a single gestation-independent threshold value for ruling out and ruling in developing preeclampsia.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design, Population, and Clinical Implementation

This is an observational retrospective study from a Danish University Hospital, a tertiary referral center with ≈4000 deliveries per year receiving high-risk pregnancies from southern region of Denmark. We included women with suspected preeclampsia from the first 18 months after implementation of the sFlt-1/PlGF ratio in clinical routine (Figure 1). Women were referred to the outpatient clinic or admitted at the maternity ward in suspicion of or observation for preeclampsia after GW 20. The women not developing preeclampsia were defined as controls.

The symptoms raising suspicion were proteinuria as only finding, marginally increased blood pressure (suspected hypertension), white coat hypertension, adverse biochemistry (eg, solitary thrombocytopenia), or fetal growth restriction. Other indications for having the sFlt-1/PlGF ratio taken (noted as adverse symptoms in) were headache, nausea, epigastric pain, right upper quadrant abdominal pain, vomiting, dizziness, blurred vision or eye fluttering, indeterminable malaise, molimina, unspecific symptoms, or suspicion of preeclampsia. All patients had ≥1 blood pressure measurements at the visit, urine dipstick, sFlt-1/PlGF ratio, and preeclampsia routine blood test taken (thrombocytes, serum-creatinine, liver function test, serum-sodium, serum-potassium, and hemoglobin).

Implementation of the sFlt-1/PlGF ratio in clinical routine was according to a local guideline in which healthcare professionals (primary physicians and midwives on call) were educated by a senior obstetrician. Published data for the Kryptor sFlt-1/PlGF ratio suggested a cutoff value of 33 for rule out and 85 for rule in before GW 34 and 33 for rule out and 99 to 110 for rule in after GW 34.[16,18,19]

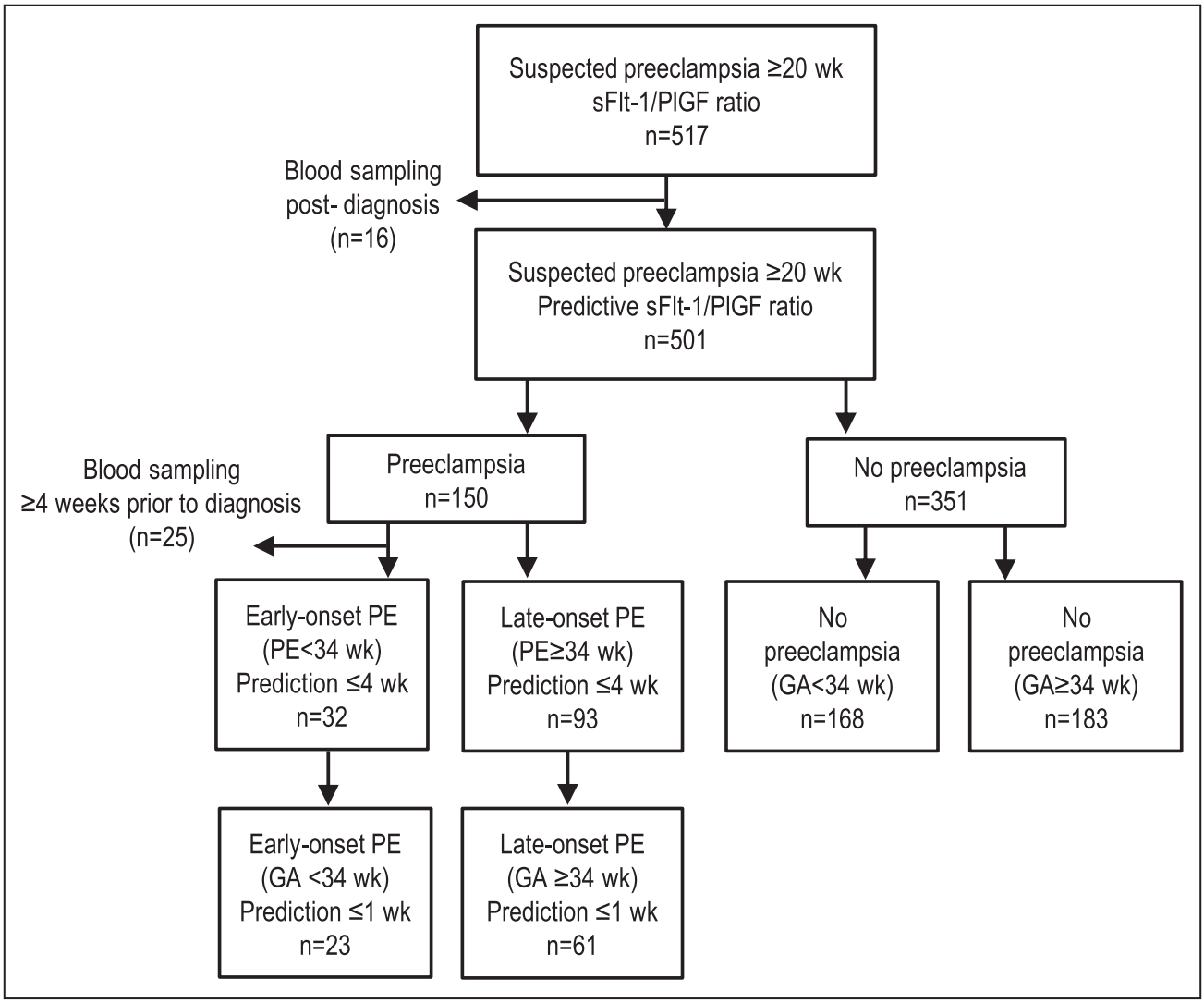

Figure 1. Flowchart of the study population, prediction windows, and outcomes.
GA indicates gestational age; PE, preeclampsia; PlGF, placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1.

The study protocol was performed in accordance with the Declaration of Helsinki II and approved by the institutional review board (project identifier 8/49439), according to the Danish Health Care Act (§42 d, pcs. 2).

Outcome Definition and Medication

Preeclampsia was defined according to the Danish 2018 national clinical guideline for hypertensive disorders in pregnancy and preeclampsia, which relates to the international definitions of preeclampsia.[20,21] The definition was onset of hypertension (≥140/90 mm Hg) on 2 separate occasions, ≥4 hours apart, and proteinuria (≥300 mg/24 h or ≥1+ on dipstick) after GW 20, superimposed proteinuria in women with chronic hypertension before pregnancy or in pregnancy diagnosed before GW 20 or gestational hypertension (hypertension diagnosed after GW 20), and/or signs of organ dysfunction (thrombocytopenia/hematological complications, renal and/or hepatic involvement, pulmonary edema, neurological complications, and/or signs of uteroplacental dysfunction).

Gestational age (GA) was based on estimated day of delivery, which was determined at the nuchal translucency scan at GA 11+5–13+5 as part of the Danish first-trimester screening program for trisomy 21.

Small for GA at birth is defined as birth weight <–22% compared with expected birth weight for a given gestation. Fetal growth restriction is defined as estimated fetal weight in pregnancy <–33% compared with expected weight for a given gestation or –15% but with abnormal Doppler waveform or oligohydramnios each observed at least twice and 12 hours apart.[22]

Chronic hypertension was defined as hypertension before pregnancy or blood pressure >140/90 mm Hg before GW 20. The antihypertensive treatment in Denmark is methyldopa, labetalol, calcium antagonist, or a combination of the 3. Pregestational angiotensin-converting enzyme inhibitor treatment is always changed to 1 of the 3 mentioned drugs before or in early pregnancy. Aspirin treatment in Denmark is according to the national guideline: 150 mg from early pregnancy or latest GW 16. The guideline was implemented during the study period.

Biochemical Analyses

Serum sFlt-1 and PlGF concentrations were measured in clinical routine using BRAHMS Kryptor sFlt-1 and PlGF plus immunofluorescent homogeneous assays (Thermo Scientific). Automated analysis was performed on a Kryptor compact PLUS analyzer at the Clinical Biochemistry Department, Odense University Hospital (Odense, Denmark). Blood samples were drawn in BD Vacutainer SST tubes (Becton Dickinson) and centrifuged within 8 hours, according to standard procedures. Samples were analyzed according to the manufacturer either at the same day or after storage at 4°C for up to 3 days.

The limit of detection and limit of quantitation for Kryptor sFlt-1 and PlGF assays were 6.9 and 34 pg/mL, and 3.6 and 22 pg/mL, respectively (measuring range: sFlt-1, 22–90 000 pg/mL; PlGF, 3.6–7000 pg/mL). The assays are calibrated against internal reference standards prepared from recombinant human sFlt-1 or PlGF. The Kryptor sFlt-1 and PlGF assays has a turnaround time of 9 and 29 minutes, respectively.

Internal quality control (QC), interassay coefficients of variation for sFlt-1 in routine were <17.3% (QC level 1; 32.9 pg/mL, Seronorm Immunoassay Liq-1 [Sero]) and <4.7% (QC level 2; 4127 pg/mL, L2 SERO Maternal Health Control [Sero]). Interassay coefficients of variation for PlGF were <16.7% (QC level 1; 12.1 pg/mL, Seronorm Immunoassay Liq-1 [Sero]) and <8.2% (QC level 2; 123 pg/mL, L2 SERO Maternal Health Control [Sero]). External QC was monitored using the Preeclampsia Marker (625) survey from Instand e.V. (Germany).

The sFlt-1/PlGF ratio was provided in the range of 1 to 13 040.

Statistical Analysis

Data were extracted from electronic medical records and stored in a secure SharePoint web application. Data handling and statistical analysis were performed using Microsoft Excel 2010 (Microsoft) and Stata version 16.0. Data were presented as median and interquartile range or number (percentage). For univariate statistical analysis of continuous variables, we applied Mann-Whitney *U* test; and for categorical variables, we used the Pearson χ^2 test or the Fisher exact test. Receiver-operating characteristic (ROC) analysis was performed using nonparametric estimation of the ROC curve with Bamber and Hanley CIs for the area under the ROC curve (AUC). The optimal threshold value was calculated using the Stata module cutpt by the Liu method. A significance level of $P<0.05$ was applied for all statistical tests in this study.

RESULTS

The study cohort consisted of pregnant women (n=501) who presented with symptoms in the clinic and had at least a single measurement of the sFlt-1/PlGF ratio because of suspicion of developing preeclampsia (Figure 1). We excluded

16 women having preeclampsia diagnosed before blood sampling for the ratio determination. A total of 150 women developed preeclampsia during pregnancy and 351 did not (controls). For calculation of diagnostic accuracy measures, we further excluded those women who had a ratio measurement >4 weeks before preeclampsia diagnosis (n=25). Finally, the cohort was divided into early- and late-onset preeclampsia groups (<34+0 and ≥34+0 weeks, respectively), according to GA at diagnosis, and these were further subdivided into groups who developed preeclampsia within 1 and 4 weeks (Figure 1). Women, who did not develop preeclampsia (controls), were divided into 2 groups, according to GA at the first ratio measurement.

Baseline characteristics of the women in the study with and without preeclampsia are given in Table 1.

Table 1. Baseline Maternal and Pregnancy Characteristics of Women Suspected of Having Preeclampsia

Characteristic	All (n=501)	Preeclampsia (n=150)	No Preeclampsia (n=351)	*P* Value
Maternal characteristics				
Maternal age, y	29 (27–33)	29 (26–33)	30 (27–34)	0.11
BMI, kg/m^2	26.3 (22.6–31.5)	26.0 (22.3–31.2)	26.9 (23.5–32.1)	0.31
Race, n (%)				
Asian	25 (5.1)	17 (5.1)	8 (5.0)	0.82
Black	5 (1.0)	2 (1.3)	3 (0.9)	0.64
White	453 (90.4)	137 (91.3)	316 (90.0)	0.74
Smoking, n (%)	46 (9.2)	16 (10.7)	30 (8.5)	0.78
Multiple gestations,† n (%)	22 (4.4)	12 (8.0)	10 (2.8)	0.012
Gestational age at delivery, wk	38.7 (37.4–40.0)	37.5 (35.7–38.4)	39.2 (38.1–40.6)	<0.001
Eclampsia, n (%)	1 (0.2)	1 (0.67)	0 (0.0)	0.30
HELLP, n (%)	20 (4.0)	19 (12.7)	1 (0.28)	<0.001
Medication, n (%)				
Antihypertensive, n (%)	158 (31.5)	103 (68.7)	55 (15.7)	<0.001
Aspirin <16 wk, n (%)	57 (11.4)	17 (11.3)	40 (11.4)	0.98
Maternal risk factors, n (%)				
Previous preeclampsia	42 (8.4)	14 (9.3)	28 (7.1)	0.60
GDM	35 (7.0)	12 (8.0)	23 (6.6)	0.57
Aged >40 y	24 (4.8)	7 (4.7)	17 (4.9)	1.00
BMI >35 kg/m^2	69 (13.8)	23 (15.3)	46 (13.1)	0.58
Pregestational diabetes mellitus	21 (4.2)	8 (5.3)	13 (3.7)	0.47
Gestational hypertension	66 (13.2)	24 (16.0)	42 (12.0)	0.25
Chronic hypertension	46 (9.2)	20 (13.3)	26 (7.4)	0.043
Mode of delivery, n (%)				
Vaginal (spontaneous)	150 (29.9)	11 (7.3)	139 (39.6)	<0.001
Vaginal (induced)	144 (28.7)	57 (38.0)	87 (24.8)	0.003
Cesarean section (induced)	49 (9.8)	27 (18.0)	22 (6.3)	<0.001
Cesarean section (elective)	67 (13.4)	14 (9.3)	53 (15.1)	0.08
Cesarean section (emergency)	85 (17.0)	38 (25.3)	47 (13.4)	0.001
Offspring characteristics				
Sex, n (%)				
Female	261 (52.1)	70 (46.7)	191 (54.1)	0.14
Male	240 (48.1)	80 (53.3)	160 (45.8)	0.11
Birth weight, g	3215 (2830–3650)	2905 (2341–3283)	3320 (3005–3750)	<0.001
SGA, n (%)	107 (21.4)	47 (31.5)	60 (17.1)	0.001
Placental weight, g	650 (520–780)	580 (460–740)	670 (560–800)	<0.001

†Data are presented as median (interquartile range) or number (percentage). *P* values for differences between preeclampsia and no preeclampsia were calculated using the Mann-Whitney *U* test for continuous variables and the Pearson χ^2 test/Fisher exact test for categorical variables. BMI indicates body mass index (before pregnancy); GDM, gestational diabetes mellitus; HELLP, hemolysis, elevated liver enzymes, and low platelet count; and SGA, small for gestational age (<10th percentile).

There was no difference in maternal age, body mass index, or race. The percentage of women with multiple gestations was higher in the preeclampsia group (8.0% versus 2.8%; *P*=0.012), and the preeclampsia group delivered significantly earlier (37.6 versus 39.3 weeks; *P*<0.001) and had a higher risk of developing HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome (12.7% versus 0.28%; *P*<0.001). More women in the preeclampsia group received antihypertensive medication (68.7% versus 15.5%; *P*<0.001). There was no difference in aspirin intake. The percentage of women with chronic hypertension was marginally higher in the preeclampsia group (13.3% versus 7.4%; *P*=0.043), but there was no difference in other preeclampsia risk factors. Women in the preeclampsia group delivered less spontaneously, had more frequent induced delivery, and had higher rates of induced and emergency cesarean section (Table 1). Offspring characteristics showed no difference in offspring sex but a significantly lower birth weight, higher frequency of small for GA, and lower weight of the placenta in the preeclampsia group.

Among indications for taking an sFlt-1/PlGF ratio (Table 2), proteinuria was more common in the preeclampsia group (54.7% versus 27.4%; *P*<0.001), and more women presented with suspected preeclampsia (7.3% versus 2.3%; *P*<0.01). There were significantly fewer adverse symptoms compared with controls among women in the preeclampsia group (23.3% versus 43.1%; *P*<0.001). The indications of fetal growth restriction, borderline hypertension, and abnormal blood test results did not differ between the groups.

GA at blood sampling did not differ between groups (Table 2). Median sFlt-1 and sFlt-1/PlGF ratio were significantly higher in the preeclampsia group (5792 versus 1872 pg/mL [*P*<0.001] and 105 versus 7 [*P*<0.001]), whereas median PlGF was significantly lower (62 versus 225 pg/mL; *P*<0.001) (Table 2 and Table S1). AUC-ROC for preeclampsia within 4 weeks was 0.90 (95% CI, 0.87–0.93) (Table 3 and Figure 2A). The diagnostic performance was increased for the 4-week prediction of early-onset preeclampsia (AUC, 0.95; 95% CI, 0.92–0.98) and reduced for late-onset preeclampsia (AUC, 0.85; 95% CI, 0.80–0.90) (Table 3 and Figure 2A and 2B). Similar results were obtained, albeit with higher overall performance, for prediction of preeclampsia within 1 week (Table 3 and Figure 2A and 2B). The optimal ratio threshold for preeclampsia within 1 and 4 weeks was 66. The diagnostic performance was calculated for this threshold as well as previous suggested threshold values for rule out (33) and rule in (85) (Table 3). For prediction of preeclampsia within 1 week, a ratio of 66 yielded similar negative predictive value compared with a threshold value of 33 (96% versus 97%), but with a higher positive predictive value (70% versus 49%). Similar results are evident in the early- and late-onset preeclampsia groups (Table 3), suggesting a benefit of using a single cutoff as a clinical decision threshold. When comparing threshold values of 66 and 85, the sensitivities were equal (0.87) in the early-onset group (preeclampsia ≤1 week) but not in the late-onset group (preeclampsia ≤1 week) (0.80 versus 0.72).

DISCUSSION

This study confirms the usefulness of the sFlt-1/PlGF ratio as a prognostic factor for developing preeclampsia in clinical routine. Our data were obtained

Table 2. Indication for sFlt-1/PlGF Ratio and Biochemical Measures

Characteristic	All (n=501)	Preeclampsia (n=150)	No Preeclampsia (n=351)	*P* Value
Indication, n (%)				
Proteinuria	178 (35.5)	82 (54.7)	96 (27.4)	<0.001
FGR	24 (4.8)	7 (4.7)	17 (4.8)	1.00
Suspected hypertension	25 (5.0)	7 (4.7)	18 (5.1)	1.00
Biochemistry*	16 (3.2)	6 (4.0)	10 (4.6)	0.58
Adverse symptoms†	189 (37.7)	35 (23.3)	154 (43.9)	<0.001
Biochemistry				
GA at blood sampling, wk	34.1 (31.1–36.0)	34.4 (30.7–36.3)	34.1 (31.1–35.9)	0.27
sFlt-1, pg/mL	2461 (1363–4936)	5792 (2966–9522)	1872 (1168–3118)	<0.001
PlGF, pg/mL	148 (71–350)	62 (32–113)	225 (111–429)	<0.001
sFlt-1/PlGF ratio	15 (4–65)	105 (30–276)	7 (3–26)	<0.001

Data are presented as median (interquartile range) or number (percentage). *P* values for differences between preeclampsia and no preeclampsia were calculated using the Mann-Whitney *U* test for continuous variables and the Pearson χ^2 test/Fisher exact test for categorical variables. Indications are defined in the Methods section. FGR indicate fetal growth restriction; GA, gestational age; PlGF, placental growth factor; and sFlt-1, soluble fms-like tyrosine kinase-1.

*Pregnant women with abnormal blood test results.

†Adverse symptoms (eg, headache, nausea, and epigastric pain) (defined in Methods).

Table 3. Diagnostic Performance and Predictive Values of sFlt-1/PlGF Ratio at Different Threshold Values

sFlt-1/PlGF Ratio	AUC (95% CI)	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Preeclampsia ≤4 wk (n=125)	0.90 (0.87–0.93)				
33		0.82	0.78	57	93
66*		0.72	0.92	75	90
85		0.64	0.92	75	88
Eo PE ≤4 wk (n=32)	0.95 (0.92–0.98)				
33		0.88	0.88	57	97
66		0.75	0.94	73	95
85		0.75	0.95	72	95
Lo PE ≤4 wk (n=93)	0.85 (0.80–0.90)				
33		0.83	0.68	57	88
66*		0.71	0.88	76	86
85		0.62	0.90	76	82
Preeclampsia ≤1 wk (n=84)	0.93 (0.90–0.96)				
33		0.92	0.77	49	97
66*		0.82	0.91	70	96
85		0.76	0.92	70	94
Eo PE ≤1 wk (n=23)	0.98 (0.96–1.00)				
33		0.96	0.88	51	99
66		0.87	0.94	69	98
85		0.87	0.94	69	98
Lo PE ≤1 wk (n=61)	0.90 (0.85–0.94)				
33		0.92	0.66	50	95
66*		0.80	0.88	70	93
85		0.72	0.90	71	90

Diagnostic performance at specific thresholds was calculated on the basis of the exact or nearest higher threshold value to those indicated. AUC indicates area under the receiver-operating characteristic curve; Eo PE, early-onset preeclampsia; Lo PE, late-onset preeclampsia; NPV, negative predictive value; PlGF, placental growth factor; PPV, positive predictive value; and sFlt-1, soluble fms-like tyrosine kinase-1.

*Optimal determined threshold.

retrospectively in a cohort where women presented with a variety of specific and/or unspecific signs and symptoms of preeclampsia in the outpatient clinic or at the maternity ward. Almost one third of the women, who had indications for an sFlt-1/PlGF ratio, developed preeclampsia later in pregnancy. The overall performance of the marker in our clinical routine was similar to AUC-ROC values for early- and late-onset preeclampsia reported previously.[15,17] Notably, the predictive performance was markedly higher for early-onset preeclampsia compared with late-onset preeclampsia within 1 week (0.98 versus 0.90) or 4 weeks (0.95 versus 0.85). We calculated an optimal Kryptor threshold ratio of 66 for predicting all preeclampsia and late-onset preeclampsia both within 1 and 4 weeks from blood sampling (Table 3). Zeisler and coworkers identified and validated a single ratio threshold value of 38 based on the Elecsys immunoassays.[15] Our finding of an optimal Kryptor threshold value of 66 is in line with previous method comparisons and data from external QC (Instand) that indicated that calibration of PlGF is different for Kryptor and Elecsys assays, resulting in lower Kryptor PlGF concentrations and higher ratios in a concentration-dependent manner.[16,17] For clinical routine, there have been data to support the use of gestation-specific threshold values focusing on high sensitivity and a high negative predictive value for ruling out preeclampsia (33) and ruling in preeclampsia (85/110), focusing on high specificity and high positive predictive value for early- and late-onset preeclampsia, respectively.[14] However, because this setup leaves a gray zone for women with ratios in between, it may be argued that single-threshold value may be easier to use for clinical decision making. When considering most preeclampsia pregnancies, our sFlt-1/PlGF ratio data suggest that not much performance will be gained in terms of positive predictive and negative predictive values if dual rule-in and rule-out values are used, compared with a single threshold value of 66 (Table 3). Conversely, for the women with early-onset preeclampsia, some degree of sensitivity will be lost; however, the negative predictive value is still high for ruling out preeclampsia within 1 week (98%) and fair within 4 weeks (95%).

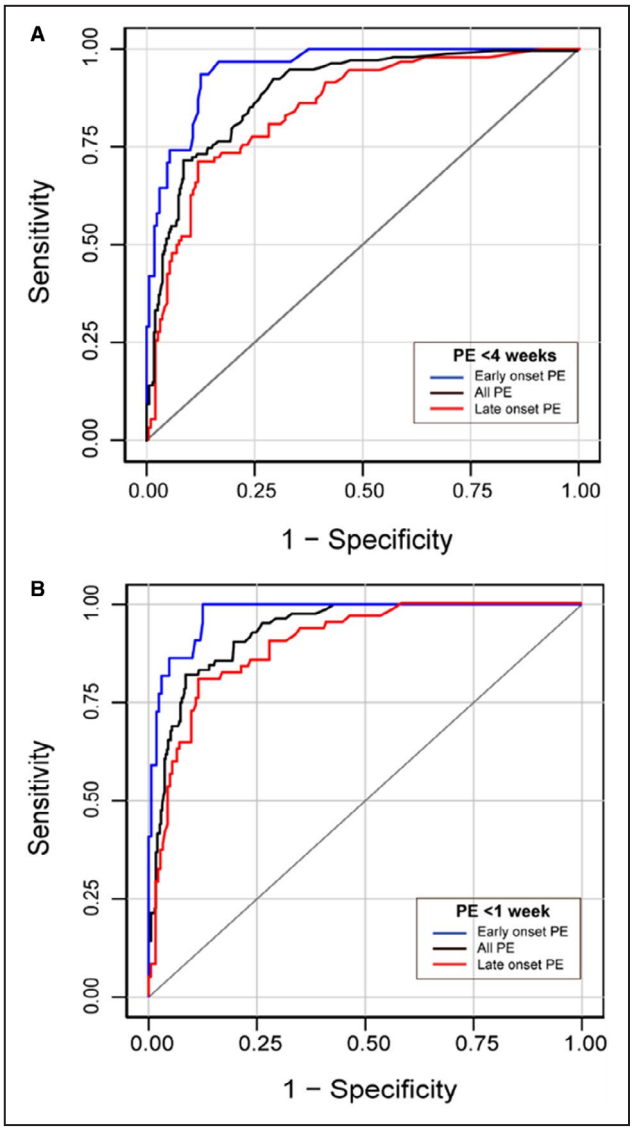

Figure 2. Receiver-operating characteristic curves for soluble fms-like tyrosine kinase-1/placental growth factor ratio at different gestational ages.
A, Four-week prediction of all preeclampsia (PE) (black line), early-onset PE (blue line), and late-onset PE (red line). **B**, One-week prediction of all PE (black line), early-onset PE (blue line), and late-onset PE (red line).

To address the added diagnostic value of using the sFlt-1/PlGF ratio instead of any of the 2 single markers, we calculated AUC-ROC for all the relevant outcome variables (Table S2). Although sFlt-1 and PlGF performed similarly for early- and late-onset preeclampsia, the ratio outperformed them in all outcome and prediction windows. Thus, the ratio rather than any single marker is needed for optimal discrimination between women developing preeclampsia and those not developing preeclampsia.

In our study, the controls were from an inhomogeneous group of women referred to the ratio test because of a variety of specific and/or unspecific signs and symptoms of preeclampsia. Thus, the control group was neither normotensive nor healthy. Nevertheless, the sFlt-1/PlGF ratio performed well in discriminating developing preeclampsia in the clinical setting. Notably, it was of high value to distinguish between those women who were not to be closely followed up by an obstetrician or midwife and those women having a high risk of developing preeclampsia and requiring close monitoring or hospitalization. From an economic perspective, previous cost-benefit analyses showed that introducing the sFlt-1/PlGF ratio for guiding the management of preeclampsia in clinical routine leads to a substantial reduction in costs to unnecessary hospital admittances.[23,24]

Some strengths and limitations may apply to this study. First, the diagnostic performance and optimal Kryptor threshold values were derived from retrospective data from the first 18 months of routine ratio testing in the clinic. This should be regarded as a strength as opposed to case-control studies using stored samples from biobanks. Second, we used gestation-specific outcome data for short (1-week) and intermediate (4-week) prediction of preeclampsia. The number of women developing early-onset preeclampsia was limited, leading to less accurate calculations of diagnostic performance. During implementation, a proportion of women were already diagnosed with preeclampsia before the day of blood sampling and were thus excluded from the study. This number decreased significantly when more hospital personnel became properly trained in the clinical guideline.

CONCLUSIONS

There is strong evidence for implementing the sFlt-1/PlGF ratio in routine management of pregnant women presenting with unspecific signs and symptoms after 20 weeks of gestation. Herein, we report implementation of Kryptor sFlt-1/PlGF ratio in clinical routine, where almost one third of symptomatic women developed preeclampsia. Our clinical validation showed that the sFlt-1/PlGF ratio is a useful clinical tool for ruling out and ruling in preeclampsia within 1 week. Prediction within 4 weeks is superior for early-onset preeclampsia. Furthermore, our data suggest that a single decision threshold of 66 could be used in clinical routine as a simpler alternative to gestation-specific dual thresholds. Future retrospective studies with a larger number of women developing early-onset preeclampsia may further improve decision thresholds for clinical management. Finally, future prospective, longitudinal, and randomized studies are needed to access the added clinical and economical value of implementing sFlt-1/PlGF ratio in clinical decision making in obstetric care units.

ARTICLE INFORMATION

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None.

Supplementary Material

Tables S1–S2

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Supplemental Material

Table S1. sFlt-1, PlGF and sFlt-1/PlGF ratio for early onset and late onset preeclampsia at ≤ 4 weeks and ≤ 1 week prediction windows.

	Eo PE ≤ 4 weeks (n = 32)	Eo PE ≤ 1 week (n = 23)	No PE (GA < 34 wk) (n = 168)	Lo PE ≤ 4 weeks (n = 93)	Lo PE ≤ 1 week (n = 61)	No PE (GA ≥ 34 wk) (n = 183)
GA at blood sampling, weeks	29.5 (24.1–33.4)	29.7 (24.1–33.4)	29.7 (18.9–33.9)	35.6 (30.4–39.0)	35.9 (33.1–39.0)	35.9 (34.0–40.0)
sFlt-1, ng/L	9,436 (1,445–31,300)	11,395 (2,486–31,300)	1,969 (365–18,670)	7,422 (273–24,230)	7,960 (1,930–24,230)	3,348 (495–15,620)
PlGF, ng/L	53.3 (7.0–243)	29.9 (7.0–139)	441 (7.0–4,074)	84.4 (12.0–656)	59.7 (12.0–191)	252 (4.0–1,759)
sFlt-1/PlGF ratio	763 (6–4471)	1,026 (25–4,471)	26 (1–869)	169 (3–1,006)	203 (11–1,006)	41 (1–705)

Data are presented as mean and range
Eo PE, early-onset preeclampsia; Lo PE, late-onset preeclampsia

Table S2. Diagnostic performances of sFlt-1, PlGF and sFlt-1/PlGF ratio.

Outcome variable/ prediction window	sFlt-1	PlGF	sFlt-1/PlGF ratio
	AUC-ROC (95% CI)	AUC-ROC (95% CI)	AUC-ROC (95% CI)
PE ≤ 4 weeks (n = 125)	0.86 (0.83–0.90)	0.86 (0.82 – 0.90)	0.90 (0.87–0.93)
Lo PE ≤ 4 weeks (n = 93)	0.80 (0.75–0.86)	0.81 (0.76–0.86)	0.85 (0.80–0.90)
Eo PE ≤ 4 weeks (n = 32)	0.93 (0.89–0.97)	0.93 (0.89–0.97)	0.95 (0.92–0.98)
PE ≤ 1 week (n = 84)	0.90 (0.86–0.93)	0.91 (0.88–0.94)	0.93 (0.91–0.96)
Eo PE ≤ 1 week (n = 23)	0.96 (0.94–0.99)	0.96 (0.93 – 0.99)	0.98 (0.96–1.00)
Lo PE ≤ 1 week (n = 61)	0.83 (0.78–0.89)	0.87 (0.82–0.92)	0.90 (0.85–0.94)

Eo PE, early-onset preeclampsia; Lo PE, late-onset preeclampsia