SIEMENS Healthineers

A-351

Gestational Phase–Specific Cutoff Values for the Use of the sFlt-1/ PlGF Ratio as an Aid in Diagnosis for Preeclampsia

S A Love, A Wright, N Carmosino, S K Shah, S Lange, M Pagano, J E Wilson, A Palladino
Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.

Background

Preeclampsia (PE) is estimated to occur in 2–8% of all pregnancies worldwide and is one of the leading causes of maternal morbidity.[1] PE and the other hypertensive disorders of pregnancy (HPD) also remain a leading cause of maternal, neonatal, and fetal mortality worldwide. In the US, according to the CDC, preeclampsia disproportionally affects Black women 60% more of the time compared to White women.[2] Similar disparities exist in the US for maternal mortality.

As defined by the American College of Obstetricians and Gynecologists (ACOG), PE is characterized by high blood pressure, defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and end-organ dysfunction with or without proteinuria after 20 weeks gestation.[3] Some of the known risk factors of PE include nulliparity, PE with previous pregnancy, and chronic hypertension, yet in many cases of PE patients do not have identifiable risks.[3] Though the underlying pathophysiology has not been fully elucidated for PE, the literature currently supports a spectrum of disease with differences with timing of PE onset.[3,4] This multi-factor process involves placental dysfunction and angiogenic imbalance. Biomarkers characterizing this imbalance are used in combination with measurements of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF).

The purpose of this multicenter case-control study was to derive the sFlt-1/ PlGF ratio, or PE ratio, cutoff values in terms of sensitivity (SE) and specificity (SP) to rule-in and rule-out PE for an early gestation phase (GP) spanning 20+0 to 33+6 weeks and a late GP (34 weeks to delivery).

Methods

This multicenter US case-control study enrolled consenting, adult women (18+ years) with singleton pregnancy between 20+0 and 42+6 weeks who had not had heparin within the prior 24 hours. Enrolled PE subjects were adjudicated by pairs of experienced clinicians. The control cohort consisted of twice as many healthy pregnant females, where healthy pregnancy outcome was normotensive patients not diagnosed with any form of PE, eclampsia, HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, or intrauterine growth restriction at any point for the duration of pregnancy. Serum specimens were stored frozen (-70°C) and subsequently tested with the Atellica IM sFlt-1 and PlGF assays on the Atellica IM analyzer to determine the sFlt-1/PlGF ratio.

Results

Participants. A total of 695 participants were enrolled in the study, with a mean age of 30 years (range: 18–49 years). The cohort was demographically diverse, including 23% (159/695) Black and 27% (190/695) Hispanic individuals. See Table 1. Adjudication identified 452 patients with uneventful pregnancy outcomes and 243 patients diagnosed with PE. Univariate comparisons between patients with PE and their gestationally age-matched controls showed that PE patients were significantly more likely to be older, of greater weight, have a history of diabetes, chronic hypertentsion and PE in a prior pregnancy, and have higher systolic and diastolic blood pressure (Table 1). In the early GP, a greater proportion of Black subjects had PE (34.8%) as compared to controls (13%, P<0.001). However, when these characteristics were modeled in a logistic regression for each GP, PE patients were only distinguished from their gestationally age-matched controls by significantly higher systolic and diastolic blood pressure (P<0.05 or P<0.001).

Table 1. Demographic and Clinical Characteristics at Enrollment for Participants (n=695).

Demographics & Clinical Characteristics		Early Gestational Phase (weeks 20+0–33+6)				Late Gestational Phase (weeks 34+0–42+6)			
		PE, N=135 (100%)		Control, N=253 (100%)		PE, N=108 (100%)		Control, N=199 (100%)	
		N	%	N	%	N	%	N	%
GA (days)	Mean (SD)	135	214.70 (18.52)	253	193.77 (24.54)	108	253.56 (10.38)	199	261.99 (13.19)
MA (years)	Mean (SD)	135	31.16 (6.42)**	253	29.57 (5.33)	108	30.85 (6.59)**	199	28.15 (5.95)
Height (inches)	Mean (SD)	135	63.79 (2.79)	253	63.62 (2.57)	108	63.98 (2.79)	199	63.89 (2.88)
Weight (lbs)	Mean (SD)	135	205.00 (47.02)***	253	183.52 (42.14)	108	206.80 (47.29)***	199	186.17 (41.07)
BMI[a]	Underweight	1	0.7%	2	0.8%	0	0.0%	0	0.0%
	Normal	9	6.7%	42	16.6%	6	5.6%	18	9.0%
	Overweight	30	22.2%	64	25.3%	21	19.4%	68	34.2%
	Obesity	95	70.4%*	145	57.3%	81	75%*	113	56.8%
Race	White	54	40.0%	209	82.6%	61	56.5%	133	66.8%
	Black	47	34.8%***	33	13.0%	27	25.0%	52	26.1%
	Asian	7	5.2%	7	2.8%	4	3.7%	4	2.0%
	Native Am	0	0.0%	0	0.0%	1	0.9%	0	0.0%
	Other	11	8.1%	3	1.2%	3	2.8%	5	2.5%
	Not specified	16	11.9%	0	0.0%	12	11.1%	0	0.0%
Ethnicity	Hispanic	25	18.5%	90	35.6%	26	24.1%	49	24.6%
Gravidity	Mean (SD)	135	2.97 (2.22)	253	2.68 (1.70)	108	2.17 (1.54)	199	2.66 (1.76)
Positive for history of	PE	38	28.1%***	0	0.0%	16	14.8%***	0	0.0%
	Diabetes	23	17%***	8	3.2%	13	12%*	9	4.5%
	Smoking	18	13.3%	18	7.1%	12	11.1%	29	14.6%
	Renal disease	4	3.0%	0	0.0%	0	0.0%	0	0.0%
	Chronic HTN	46	34.1%***	2	0.8%	16	14.8%***	0	0.0%
BP systolic	Mean (SD)	135	164.28 (18.04)***	253	115.35 (10.30)	108	159.09 (16.67)***	199	116.98 (9.99)
BP diastolic	Mean (SD)	135	98.24 (11.03)***	253	69.91 (7.52)	108	97.58 (11.64)***	199	72.12 (8.29)

*Notes: PE=Preeclampsia; control=subject with uneventful pregnancy outcome; BP=blood pressure; GA=gestational age; MA=maternal age; HTN=hypertension; Am=American; Statistical significance was denoted for P-values of <0.001 as ***, <0.01 as **, and <0.05 as * when compared to gestationally age-matched controls; [a]Underweight (BMI<18.5), Normal (BMI =18.5–24.9), Overweight (BMI=25–29.9), Obese (BMI=30+)*

Gestational Age–Dependent Ratio Values

Controls versus Patients with PE. The PE ratio (i.e. sFlt-1 result/PlGF result) was calculated for each participant. The distribution of the PE ratio among PE patients versus controls was examined for each GP (see Figure 1, panel A). In the early GP, the median PE ratio was 313.73 for PE patients (n=135) versus 2.68 for controls (n=253; P<0.001), respectively. In the late GP, the median PE ratio was 162.92 for PE patients (n=108) versus 9.98 for controls (n=199; P<0.001), respectively.

Distribution of the PE ratio for PE versus controls over time, as displayed in Figure 1, panel B, shows that among controls, PE ratio values were lowest in gestational weeks 20–23 weeks (mean=4.27); the ratio remained low until a slight increase at 37–42 weeks (mean=27.47). In contrast, ratio distribution resembled a bell-shaped curve among the population with PE that was consistent with published reports[5]; highest PE ratio results were observed in gestational weeks 29–33 (mean=444.20). Ratio results among PE patients were significantly higher than controls across all gestational windows shown in Figure 1, panel B (P<0.001).

Diagnostic Accuracy of the PE Ratio in Early- and Late-Onset Preeclampsia. An optimized cutoff for each GP was evaluated based on the ROC analysis using Youden's J-Statistic.[6] For the early GP (Figure 2), the optimized PE ratio cutoff was 34.5, resulting in a SE of 86.7% and a SP of 98.0%; the ROC area under curve was 0.94. For the late GP (Figure 3), the optimized cutoff was 41.4, which resulted in an SE of 79.6% and an SP of 91.5%; the ROC area under curve was 0.91.

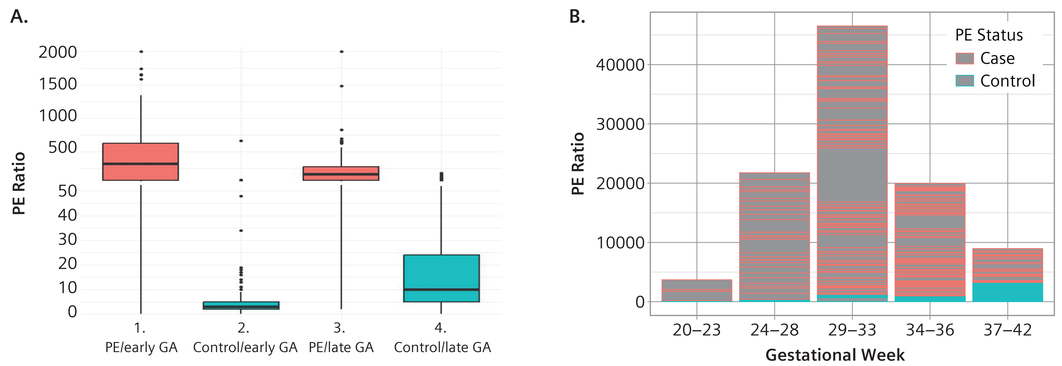

Figure 1. A, Box plot of PE ratio for PE vs. controls by gestational age and B, PE ratio by weeks of gestation, from 20+0 to 42+6 weeks, for patients with PE (red) vs. controls (blue), respectively.
(PE=Preeclampsia; Control=healthy pregnancy; GA=gestational age; Boxes = interquartile range; whiskers = range; error bars = median)

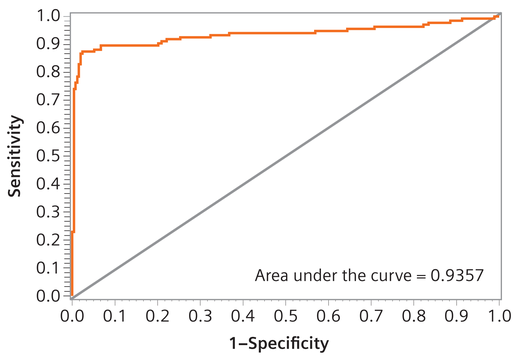

Figure 2. ROC Curve for PE Status, Early Gestational Phase

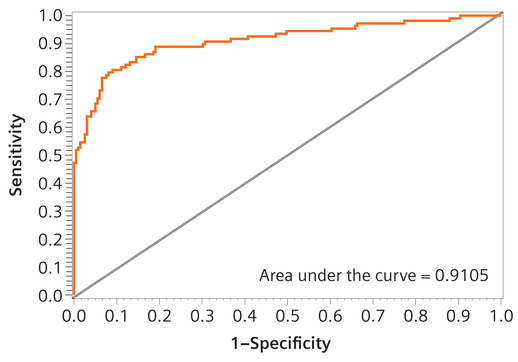

Figure 3. ROC Curve for PE Status. Late Gestational Phase

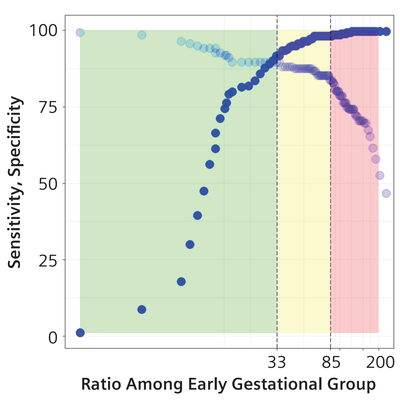

Figure 4. Early GP PE ratio performance

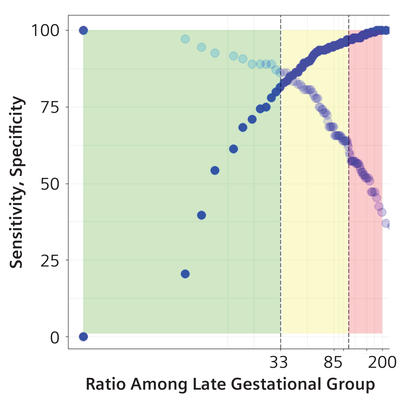

Figure 5. Late GP PE ratio performance

To enhance the diagnostic accuracy of the PE ratio, 2 cutoffs (i.e. rather than 1 cutoff) were determined for each GP. The PE ratio ranged from 0–9092 (N=695). Most non-disease patients may not be captured by a very low ratio cutoff (i.e. PE Ratio < 5), which translates into suboptimal SP. However, all patients with PE are likely to have results above a very low PE ratio cutoff, which translates into high SE. Therefore, the lower cutoff (i.e. rule-in level) focused on selecting a low PE ratio cutoff that maximized SP while maintaining sufficiently high SE. In the healthy population, most non-disease patients may have PE ratio results that fall below a very high ratio cutoff, which translates into optimal SP. However, all patients with PE may not have results above a very high cutoff, which translates into suboptimal SE. Therefore, the decision for the high PE ratio cutoff (i.e. rule-out level) focused on selecting a high PE ratio while maximizing SE and maintaining sufficiently high SP. Acceptance criteria for rule-in/rule-out PE ratio cutoffs for each GP are shown in Table 2.

Table 2. Clinical Performance of the PE Ratio by Gestational Phase

Early Gestational Phase (Wk 20+0–33+6), N=388				
SE/SP	Estimate		95% CI	Acceptance Criteria
cutoff 33 (lower cutoff)				
SE	**121/135**	**89.6%**	**83.3%–93.7%**	SE≥89.0%
SP	232/253	91.7%	87.6%–94.5%	SP≥90.0%
cutoff 85 (higher cutoff)				
SE	113/135	83.7%	76.6%–89.0%	SE≥81.0%
SP	**248/253**	**98.0%**	**95.5%–99.2%**	SP≥97.0%
Late Gestational Phase (Wk 34+0–Delivery), N=307				
SE/SP	Estimate		95% CI	Acceptance Criteria
cutoff 33 (lower cutoff)				
SE	**93/108**	**86.1%**	**78.3%–91.4%**	SE≥84.0%
SP	162/199	81.4%	75.4%–86.2%	SP≥68.0%
cutoff 110 (higher cutoff)				
SE	67/108	62.0%	52.6%–70.6%	SE≥50.0%
SP	**193/199**	**97.0%**	**93.6%–98.6%**	SP≥92.0%

Notes: SE=Sensitivity; SP=Specificity; CI=Confidence Intervals; Bold values indicate the outer borders of the cut-off.

Gestational Phase–Specific Cutoff Values

Optimized rule-out and rule-in PE ratio cutoffs were identified for each gestational phase that generated sensitivities and specificities, as seen in Table 2. In the early GP, a cutoff of 33 resulted in a SE of 89.6% and a SP of 91.7%, and a PE ratio cutoff of 85 resulted in a SE of 83.7% and a SP of 98.0%. Therefore, when combining the PE ratio cutoffs of ≤33 & ≥85, a SE of 89.6% and a specificity of 98.0% were reached (see bolded estimates in Table 2). For the late GP, a PE ratio cutoff of 33 resulted in a SE of 86.1% and a SP of 81.4%, and a cutoff of 110 resulted in a SE of 62.0% and a SP of 97.0%. Therefore, when combining the cutoffs of ≤33 & ≥110, a SE of 86.1% and a specificity of 97.0% were reached (see bolded estimates in Table 2). Clinical performance results (i.e. SE, SP) with GP-specific PE ratio cutoffs exceeded acceptance criteria thresholds.

Gerhard plots in Figures 4 and 5 display the zones framed by rule-in and rule-out cutoffs for each GP. Values were taken from receiver operating characteristics analysis: the dotted blue line represents SE, behind which is a faded blue line that represents SP. The color fills represent interpretations of the test result: below the (lower) cutoff, green indicates low risk; above the (upper) cutoff, red stands for high risk; yellow indicates intermediate risk. Figure 4 shows the Gerhard plot for the early GP population. The zones framed by these 2 PE ratio cutoffs correspond to a LR+ of 160.5 (95% CI, 22.6–1140.5) and a LR– of 0.30 (95% CI, 0.20–0.30). Figure 5 shows the Gerhard plot for the late GP population. The zones framed by these 2 cutoffs corresponded to a LR+ of 13.5 (95% CI, 5.90–30.6) and a LR– of 0.30 (95% CI, 0.20–0.50). Less than 5% of the early GP population and 20% of the late GP population fell into the intermediate zone, which is consistent with prior research.[3] A precise diagnosis at the outer borders, combined with a necessity of timely retesting in patients inside the intermediate zone allows for maximum diagnostic safety.

Conclusions

This study provides gestational-specific rule-in and rule-out PE ratio cutoffs in a large multicenter study with a large proportion of Black and Hispanic pregnant females. The approach to use multiple PE ratio cutoffs for the early and late gestational phase enhances the diagnostic accuracy of the sFlt-1/PlGF ratio as a diagnostic tool for preeclampsia.

References

1. World Health Organization. (2025) Preeclampsia. Retrieved from: www.who.int/news-room/fact-sheets/detail/pre-eclampsia
2. Centers for Disease Control (2022) Preeclampsia, Genomics and Public Health. Retrieved from: blogs.cdc.gov/genomics/2022/10/25/preeclampsia/
3. American College of Obstetrics and Gynecology (ACOG) Practice Bulletin No. 222. *Obstet Gynecol.* 2020;135: e237-e260.
4. Rana S, Lemoine E, Granger JP, et. al.(2019) Preeclampsia: Pathophysiology, Challenges, and Perspectives. *Circulation*,124(7):1094-1112.
5. 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: 161.e1-11.
6. Fluss, R., Faraggi, D., & Reiser, B. (2005) Estimation of the Youden Index and its associated cutoff point. *Biometrical Journal*, 47 (4), 458-472.

The products/features mentioned here are in development; future availability cannot be guaranteed.

Scan QR code for downloadable copy of this poster or visit www.medicalaffairs.siemens-healthineers.com

Published by Siemens Healthcare Diagnostics Inc.