Performance of a novel multi-biomarker rule-out preeclampsia test: a prospective verification study

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Introduction

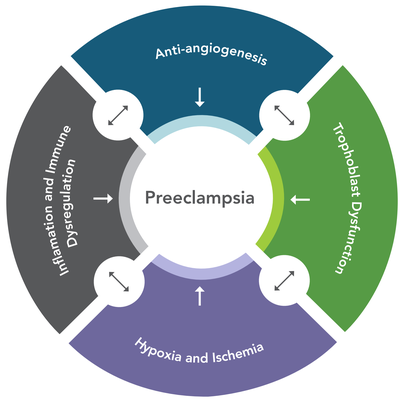
Preeclampsia complicates 2 – 8% of all pregnancies worldwide and is one of the leading causes of maternal and neonatal mortality.[1] Suspected preeclampsia is a frequent clinical presentation in obstetrical care, with 20 – 30% of pregnant women exhibiting signs or symptoms consistent with possible preeclampsia. Additionally, the rate of preeclampsia in the United States has increased 25% over the past 20 years.[1] The pathophysiology of preeclampsia is complex and includes multiple pathways **(Figure 1)**. Recent observations suggest imbalances of angiogenic factors play a role in the pathogenesis of preeclampsia.[1] However, to date, biomarker tests are limited to one angiogenic pathway, have not been widely studied in a US patient population, nor are currently available in the United States.

There is no definitive, diagnostic test for preeclampsia; rather, a multitude of non-specific, surrogate maternal and fetal evaluations are used to diagnose preeclampsia. Current tools such as blood pressure readings and urine protein tests measure the maternal clinical response of preeclampsia but are not directly linked to the underlying pathophysiology of the syndrome. Signs and symptoms of preeclampsia do not accurately predict the development of preeclampsia, adverse outcomes, or need for delivery.[2] As a result, clinical diagnoses may be rendered with tenuous accuracy, resulting in iatrogenic delivery that can add both morbidity and mortality to the fetus and the mother. Accurately assessing a low risk of developing preterm preeclampsia within 14 days, utilizing a test that evaluates biomarkers associated with multiple pathways involved in the underlying pathophysiology of preeclampsia, could potentially avoid unnecessary interventions and premature delivery of the fetus.

Aim

The goal of this multi-center sample acquisition observational clinical study was to determine the performance of the Preecludia™ test, a novel, proprietary, multi-biomarker, rule-out test, to assess the risk for preeclampsia within 14 days of sample collection in a cohort of women presenting with signs or symptoms of possible preeclampsia. Laboratory associates were kept blinded to the subjects' clinical metadata during the sample testing.

Figure 1. Molecular Pathways Involved in the Pathophysiology of Preeclampsia



Multiple pathways may be involved in the pathophysiology of preeclampsia. The Preecludia test combines eight biomarkers across these pathways.

Methods

Study Design

- The PRO-129 study **Collection of Whole Blood Samples for the Evaluation of Preeclampsia Biomarkers from Pregnant Women** was a multicenter, non-interventional, minimal-risk study conducted at 24 sites (17 OB/GYN and 7 MFM) across the United States from September 2018 to January 2020.
- 455 subjects were enrolled. The study population outlined below consisted of 376 singleton pregnant women 18 to 45 years of age and 28 0/7 to 36 6/7 weeks' gestational age (GA) who presented with one or more of the following signs and symptoms associated with the risk of developing preeclampsia:
  - New onset increased blood pressure in otherwise normotensive patient
  - Worsening hypertension in a patient with pre-existing hypertension
  - New onset proteinuria or worsening of pre-existing proteinuria
  - Any other clinical finding typically associated with suspicion of preeclampsia
- Baseline samples were collected at enrollment along with medical history and the signs and symptoms.
- Women who were previously diagnosed with preeclampsia were not included in the study.
- Women who did not exhibit signs and symptoms were not included in the study.
- The subjects were then followed through delivery and the date of diagnosis of preeclampsia was recorded.
- Preeclampsia was diagnosed according to the 2013 ACOG guidelines.

Study Population

- The study subject demographics are shown in **Table 1**. The clinical flow for assessing performance is shown in **Figure 2**. In addition to the site diagnosis, all subjects were independently adjudicated by 2 MFM specialists. Subjects with disagreement of preeclampsia diagnosis between site clinician and independent adjudicators (n = 9) were excluded.

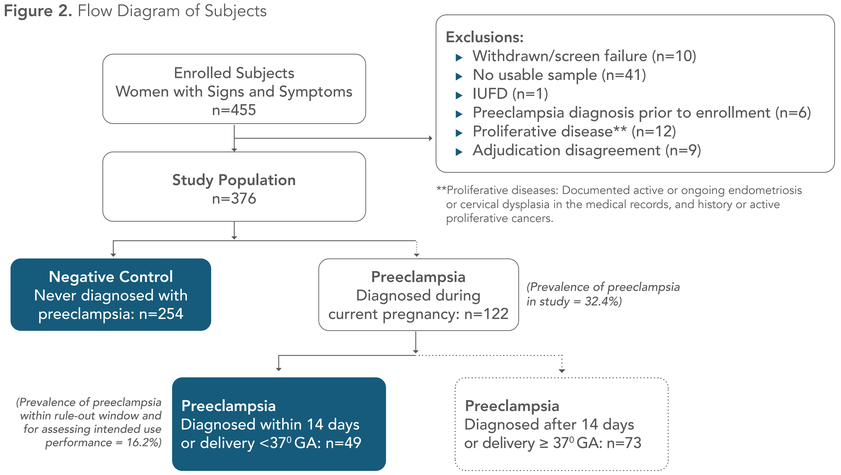
Preecludia™ Test Result

- Baseline samples from enrolled subjects were tested on the Preecludia laboratory-developed test.
- Results were expressed as a binary categorical variable for efficacy analyses with continuous values dichotomized based on a pre-specified threshold. Subjects with values below the threshold were considered at reduced risk for preeclampsia events and reported as "Negative: at reduced risk for preeclampsia within 14 days." by the test. Conversely, subjects with values at or greater than the threshold were considered at increased risk for preeclampsia events and reported as "At risk" by the test.

Efficacy Analyses

- Survival analysis (SA) was performed on the entire study population (n = 376). Subjects were stratified into two groups: "At risk" (test positive) group versus "Negative: at reduced risk for preeclampsia within 14 days" (test negative) group based on Preecludia test results (**Figure 3**). For the SA, diagnosis of preeclampsia was considered the event and subjects that were not diagnosed with preeclampsia were censored at delivery.
- In order to evaluate the Preecludia test's ability to rule out preeclampsia within the 14-day rule-out window, "Negative: at reduced risk for preeclampsia within 14 days" Preecludia test results were evaluated against negative control subjects who were never diagnosed with preeclampsia. "At risk" Preecludia test results were evaluated against preeclampsia subjects diagnosed within a 14-day window and delivery < 37 0/7 weeks' GA.
- Subjects diagnosed with preeclampsia after the 14-day window or delivery ≥37 0/7 weeks' GA were evaluated separately.
- Performance metrics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated along with their associated two-sided Wilson score 95% confidence intervals (95% CIs) on negative control subjects (n = 254) and preeclampsia subjects diagnosed within a 14-day window and delivery < 37 0/7 weeks' GA (n = 49).

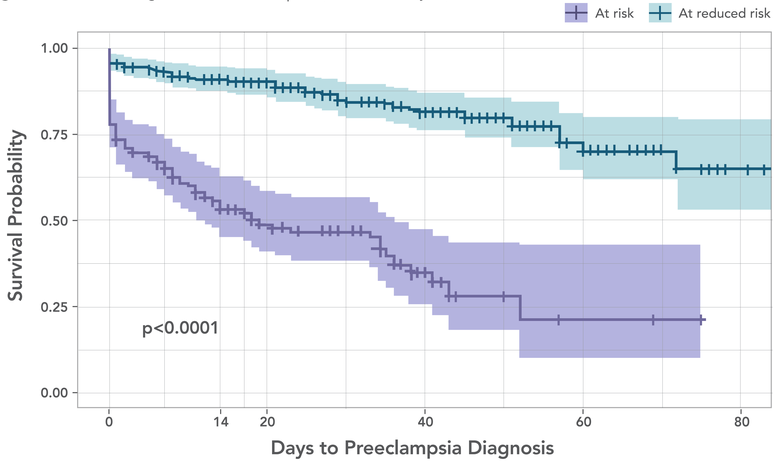
Figure 2. Flow Diagram of Subjects



**Proliferative diseases: Documented active or ongoing endometriosis or cervical dysplasia in the medical records, and history or active proliferative cancers.

Results

Figure 3. Time to Diagnosis of Preeclampsia (Survival Analysis)



Survival analysis suggests that in general, subjects that resulted "At risk" by the Preecludia test had a shorter time to diagnosis of preeclampsia (P < 0.0001) than those who were "At reduced risk." The Kaplan-Meier plot depicts the survival curves and their 95% confidence intervals.

Table 1. Subject Demographics

Demographics	Study Population (n = 376)
Baseline Blood Pressure	
Systolic (median, IQR)	136 (22)
Diastolic (median, IQR)	83 (14)
Maternal Age (median, IQR)	30 (9)
Race	
White	266 (71%)
Black or African American	53 (14%)
Asian	14 (3.7%)
Other	9 (2.4%)
Unknown/Refused	34 (9.0%)
Ethnicity	
Hispanic or Latino	85 (22%)
Not Hispanic or Latino	272 (72%)
Unknown/Not Provided	19 (5.1%)
BMI (median, IQR)	34.86 (11%)
Normal (18.5 through 24 BMI)	16 (4.3%)
Overweight (25 through 29 BMI)	76 (20%)
Obese - class 1 (30 through 34 BMI)	98 (26%)
Obese - class 2 (35 through 39 BMI)	85 (23%)
Obese - class 3 (40 or greater BMI)	100 (27%)
Missing	1 (0.27%)
Pregnancy History	
Gestational Age at Enrollment (median, IQR)	33.71 (4.3)
Gravidity (median, IQR)	2 (3)
1 (first pregnancy)	104 (28%)
> 1	272 (72%)
# of births (median, IQR)	1 (2)
Nulliparous (0)	143 (38%)
> 0	233 (62%)
History of Previous Preeclampsia	61 (16%)

Demographics	Study Population (n = 376)
Signs and Symptoms at Enrollment	
New onset increased blood pressure in otherwise normotensive patients	199 (53%)
Worsening hypertension in a patient with pre-existing hypertension	62 (16%)
New onset proteinuria or worsening proteinuria of pre-existing proteinuria	181 (48%)
Any other clinical findings typically associated with suspicion of Preeclampsia and requiring workup to rule-out Preeclampsia	148 (39%)
Medical History and Ongoing Conditions	
Chronic Hypertension (CHTN)	65 (17%)
Gestational Hypertension (GHTN)	142 (38%)
Diabetes	
Type I	3 (0.8%)
Type II	26 (6.9%)
Gestational Diabetes	
Type A1	23 (6.1%)
Type A2	39 (10%)
Smoker	
Current	13 (3.5%)
Past	52 (14%)
Autoimmune Disease	9 (2.4%)
Nephropathy/Kidney Disease	5 (1.3%)
Antiphospholipid Syndrome	0 (0.00%)
Pregnancy Complications	
Preeclampsia	122 (32%)
Eclampsia	1 (0.27%)
HELLP Syndrome	2 (0.53%)

Table 2. Preecludia Test Performance

	Sensitivity	Specificity	PPV	NPV	PLR	NLR
Test Performance	**87.8%** [75.8% – 94.3%]	**76.8%** [71.2% – 81.5%]	**42.1%** [33.0% – 51.9%]	**97.0%** [93.6% – 98.6%]	**3.78** [2.95 – 4.84]	**0.16** [0.08 – 0.34]

Test performance on 303 subjects (254 negative controls and 49 preeclampsia within rule-out window) showed high sensitivity and NPV.

Conclusion

In women with suspected preeclampsia at 28 to 36 6/7 weeks', this study concludes that the Preecludia test has high sensitivity and high negative predictive value to rule out the risk of developing preeclampsia within 14 days, up to 37 weeks' gestation.

Patients with "At Risk" results can be managed in accordance with ACOG guidelines. Notably, the study population in this study is representative of the US population.

The ability to rule out the risk for developing preterm preeclampsia may provide physician reassurance while reducing maternal anxiety, unnecessary testing, hospitalizations, interventions, and preterm delivery.

Progenity is thankful to the study participants and their families and the study principal investigators and their teams.

References

1. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin: Gestational hypertension and preeclampsia. Number 222. *Obstet Gynecol.* 2020; 135(6):e237-e260.
2. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet.* 2010; 376(9741):631-44.