The development of an accurate first trimester screening test for early onset preeclampsia

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Objective To develop a biochemical prenatal assay protocol to support accurate identification of pregnancies at high risk of developing early onset preeclampsia (EOPE) prior to onset of symptoms.

Methods Using an immunoassay protocol, 1,079 first trimester serum samples were tested for levels of three biomarkers: placental growth factor (PlGF), pregnancy associated plasma protein-A (PAPP-A) and alpha-fetoprotein (AFP). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined in a manner similar to the model used for Down syndrome screening.

Results When combined with patient history and demographics, the three biomarkers together demonstrated 60% sensitivity and 95% specificity, with a 95% confidence interval (CI). With the addition of mean arterial pressure (MAP) and uterine artery Doppler pulsatility index (UtAD-PI) measurements, sensitivity increased to 91%.

Conclusion We have developed a screening test that for the first time enables effective identification of pregnancies at high risk of EOPE with a low false positive rate (5%) during the first trimester. Preeclampsia**Screen**™ | T1 shows the highest sensitivity available in a biochemical screen and may be used as early as 10 weeks of pregnancy, with MAP and UtAD-PI measurements available starting at 11 weeks. This opens the door to potential treatments that prevent or delay onset of symptoms for this early and often more serious form of preeclampsia.

INTRODUCTION

On August 1, 2013, PerkinElmer Labs/NTD began offering the Preeclampsia**Screen**™|T1 testing service for identification of pregnancies at high risk of developing early onset preeclampsia (EOPE) prior to onset of symptoms. The screening test modifies the patient's risk based on demographics and clinical history using the first trimester biochemical markers placental growth factor (PlGF), pregnancy associated plasma protein-A (PAPP-A) and alpha-fetoprotein (AFP). The screening test can be expanded to include optional biophysical results of mean arterial pressure (MAP) measurement or uterine artery Doppler pulsatility index (UtAD-PI), or both. The screening test was approved by the New York State Department of Health (NYSDOH) and is available for use by all qualified healthcare providers.

Preeclampsia is a frequent medical complication during pregnancy, characterized by development of hypertension (systolic blood pressure (BP) ≥140 and/or diastolic BP ≥90 mmHg, measured at two occasions with at least 4 hours in between measurements) and proteinuria (≥300 mg/day) after 20 weeks' gestation,[1] and it is one of the leading causes worldwide of maternal and perinatal mortality.[2] It is estimated that preeclampsia develops in 5%-7.5% of all pregnancies,[3,4] leading to more than 4 million cases worldwide every year.[5] Current estimates suggest that more than 72,000 maternal deaths per year are attributable to preeclampsia,[5] and up to 40% of all fetal mortality is due to preeclampsia.[6]

The distinction between early and late onset preeclampsia is a more modern concept and is becoming widely accepted as a better indicator of disease significance than the classic "mild" versus "severe" terminology. Although it is less frequent than the more commonly occurring late onset form of the condition (early onset preeclampsia occurs in approximately 0.5% of pregnancies),[7,8] there is evidence that more severe disease is associated with the early onset form, variously defined but generally recognized as symptom onset prior to 34 weeks' gestation[9] coupled with an increased risk of delivery prior to 34 weeks.[10,11]

Early onset preeclampsia can be associated with abnormal uterine and umbilical artery Doppler waveforms, intrauterine growth restriction (IUGR) and gestational diabetes mellitus (GDM), as well as other adverse maternal and neonatal outcomes, while late onset preeclampsia exhibits milder maternal disease and lower rates of fetal involvement.[12] The identification of asymptomatic patients at risk for early onset preeclampsia provides opportunities for intervention and treatment that were not available prior to the Preeclampsia**Screen**™|T1.

Previous work by our lab and others has identified PAPP-A and PlGF as being decreased in the first trimester in asymptomatic patients when the pregnancy is destined to develop preeclampsia, relative to unaffected cases,[6,12] whereas AFP has been shown to be increased in the second trimester when accompanied by preeclampsia.[13,14]

First trimester Down syndrome screening in a general population using a combination of demographic information, biochemical markers and ultrasound markers has been used for over 10 years at PerkinElmer Labs/NTD and can achieve a detection rate of 91% at a 5% false positive rate.[15,16] This type of multivariate analysis is also utilized in the Preeclampsia**Screen**™|T1. Below we present an evaluation of the performance of first trimester PlGF, PAPP-A and AFP in the identification of at-risk pregnancies for early onset preeclampsia.

METHODS

Samples

First trimester de-identified serum samples collected between 10 weeks 0 days' and 13 weeks 6 days' gestation and containing relevant outcome data were obtained by PerkinElmer Labs/NTD. Table 1 provides the breakdown of this data set by ethnicity.

Table 1: Patient Demographics and Outcome

Ethnicity	Unaffected		EOPE		Total
	n	%	n	%	n
Caucasian	817	78	14	45	831
African American/ Caribbean	55	5	7	23	62
Asian	71	7	6	19	77
Hispanic	83	8	3	10	86
Other	22	2	1	3	23
Total	1048	100	31	100	1079

Samples were delivered frozen on dry ice and upon receipt stored at -20°C until processed. Samples were bar coded and matched to the original identification code prior to evaluation for biomarker levels using laboratory-developed tests (LDTs) for placental growth factor (PlGF), pregnancy associated plasma protein-A (PAPP-A) and alpha-fetoprotein (AFP).

Time-Resolved Immunoassay

Assays for PlGF, PAPP-A and AFP were developed at PerkinElmer Labs/NTD on PerkinElmer AutoDELFIA systems. Monoclonal antibodies directed against specific antigenic sites of PlGF, PAPP-A or AFP were coated in wells of 96 well plates. Each assay plate contained calibrators in duplicate, controls in singlicate and samples in singlicate. After specimen transfer, a second monoclonal antibody directed to a different antigenic site on PlGF, PAPP-A or AFP and labeled with the lanthanide europium was added to complete the immunoassay sandwich. A fluorescent signal proportional to the antigen was produced by the formation of highly fluorescent chelates created by the dissociation of the europium ions mediated by enhancement solution. The fluorescent signal for each specimen was then compared with the signal from calibrators with known amounts of antigen to determine the analyte level.

Assay precision was evaluated by running 1 low and 1 high specimen in duplicate for 2 assays per day for 10 days. A total of 40 low and high specimens was run. Analysis of variance was performed to determine within-run, between-run and between-day variance of each of the assays.

Statistical Analysis

Analyte levels were converted to multiples of the median (MoM) by dividing the analyte levels by the regressed gestational day-specific median determined from regression of observed medians versus gestational age and weighted by the number of patients at each gestational week. Regression analysis included only the 817 unaffected Caucasian patients. MoMs were then adjusted for maternal weight, ethnicity and smoking.

Assessment of effectiveness of the biochemical markers was determined by logistic regression analysis including each of the markers and the interactions between each pair of markers, with continuous log MoM values used as the independent variable. Variables were considered significant if the P value was less than 0.05.

Calculation of Risks

Risk calculations were determined using a protocol similar to that used in aneuploidy screening. Risk of early onset preeclampsia = likelihood ratio X prior risk.

Likelihood Ratios

Likelihood ratios are determined by comparing the relative frequency of the natural log MoM values in early onset preeclampsia with the relative frequency in unaffected pregnancy. The relative frequencies are based on multivariate Gaussian distributions. Parameters for the distributions were determined as follows: 1) median of the unaffected distribution was set equal to 1.0; 2) standard deviations were determined from the difference between the 90th and 10th percentiles dividing by 2.563; and 3) correlation coefficients were determined based on the Spearman correlation coefficient. The distribution parameters for MAP and UtAD-PI were based on published meta-analysis data.[17] The correlation between biochemical and biophysical markers was assumed to be 0.0.

Prior Risk

The prior risk of early onset preeclampsia is dependent on a number of demographic factors.[17-26] BMI (body mass index) is a known risk factor for preeclampsia – its impact on early onset preeclampsia is not as strong, and its impact is different based on ethnicity. Patients of African American/Caribbean descent are at significantly greater risk than those of other ethnic groups. Other risk factors include parity and prior history of preeclampsia for the patient or her mother. Table 2 provides a breakdown of the prior risk factors based on demographic factors.

Table 2: Prior Risk of EOPE (1 in X)

African American/ Caribbean	BMI	Parous	Parous	Nulliparous	Nulliparous
		Patient & Mother No PE	Patient or Mother PE	Mother No PE	Mother PE
No	<25	587	67	230	51
No	25-34	612	70	240	53
No	35+	466	53	183	40
Yes	<25	130	16	51	12
Yes	25-34	220	26	87	20
Yes	35+	379	44	149	33

Chronic Hypertension

In addition to the factors shown in Table 2, a history of chronic hypertension increases the risk thirteenfold. When MAP measurement is included in the risk assessment, the final risk is adjusted to account for the interaction between MAP and chronic hypertension.

Determination of Sensitivity, Specificity, PPV and NPV

As with aneuploidy screening, specificity and sensitivity will vary depending on prior risk. Therefore, overall specificity and sensitivity are dependent on the incidence of the various demographic factors in the general population. The estimated incidence of the 5 demographic factors – ethnicity,[27] BMI,[28] parity,[27] patient's and patient's mother's history of preeclampsia,[22] and chronic hypertension[27] – in a general screening population in the U.S. was based on published rates. Combinations of factors were determined by multiplying the rates for each factor.

Sensitivity, specificity, PPV and NPV were based on modeling in a manner similar to that used in Down syndrome screening. A simulation of 100,000 sets of MoM values in the unaffected and early onset preeclampsia distributions was generated to determine likelihood ratios. For each combination of demographic factors, risk values were estimated by multiplying the likelihood ratio by prior risk. Based on these risk values, specificity, sensitivity, PPV and NPV were determined. Overall rates were determined from a weighted average of the demographic-specific rates where the weights were based on the incidence of factors in the population.

RESULTS

Assay performance for the laboratory-developed tests approved for use by the New York State Department of Health in the identification of at-risk patients for early onset preeclampsia is presented in Table 3.

Table 3: Assay Performance

Analyte	Level	Target	% Coefficient of Variation		
			Within Run	Between Run	Between Day
PlGF (pg/mL)	LOW	21.43	7.8	4.8	4.7
	HIGH	319.76	1.5	4.5	4.7
PAPP-A (mU/L)	LOW	29.39	4.0	10.4	4.9
	HIGH	966.29	1.9	5.4	2.5
AFP (u/mL)	LOW	7.53	1.5	1.8	1.6
	HIGH	204.58	1.6	1.9	2.8

Analyte levels increase by approximately 20%, 50% and 40% per gestational week for PlGF, PAPP-A and AFP, respectively, as shown in Figure 1. The data is used to determine the GA (gestational age)-specific MoM for each analyte.

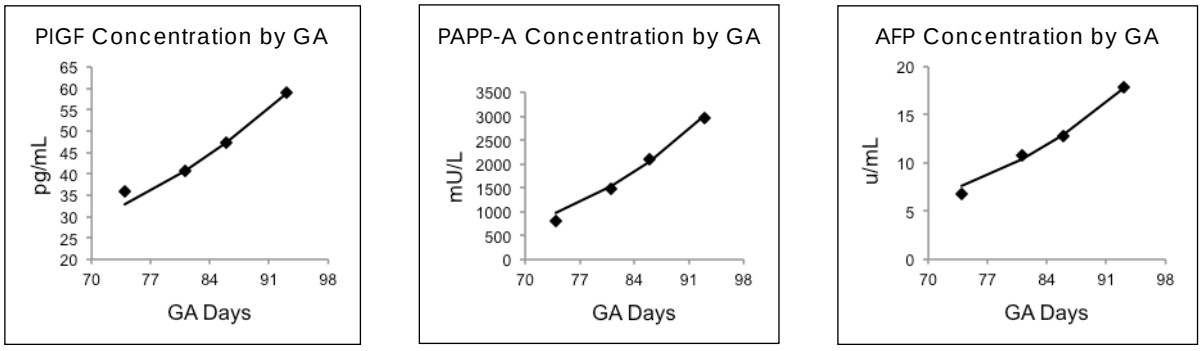

Figure 1: Squares are observed medians. Solid lines show regressed values by GA.

Logistic regression (Table 4) for each of the biochemical marker MoM values and their interactions demonstrated that all the biomarkers and their interactions were significant except for the interaction between PlGF and PAPP-A. Data is shown after the PlGF/PAPP-A interaction term was excluded from the model.

Table 4: Logistic Regression Analysis in the Prediction of EOPE Using Log MoM Values Derived from 1048 Unaffected and 31 EOPE Patients

Variable(s)	Odds Ratio, 95% CI	P Value
PlGF	0.339 (0.145-0.791)	0.012
PAPP-A	0.263 (0.152-0.453)	<0.001
AFP	5.527 (1.947-15.685)	0.001
PlGF x AFP	5.384 (1.382-20.980)	0.015
PAPP-A x AFP	2.875 (1.063-7.775)	0.037

The distribution parameters for the multivariate distribution of MoM values for the biochemical and biophysical markers are shown in Table 5. Results are based on 1048 unaffected and 31 early onset preeclampsia pregnancies. These distribution parameters are used to determine the likelihood ratios in the risk calculation.

Table 5: Parameters for Multivariate Gaussian Distributions in Unaffected and EOPE Patients

Unaffected					
Parameter	**PIGF**	**PAPP-A**	**AFP**	**MAP**	**UtAD-PI**
Median MoM	1.00	1.00	1.00	1.00	1.00
SD ln (MoM)**	0.3704	0.6154	0.4187	0.0889	0.2901
Unaffected Correlation Matrix					
PIGF	1.0000	0.1879	0.0100	0.0000	0.0000
PAPP-A	0.1879	1.0000	0.0547	0.0000	0.0000
AFP	0.0100	0.0547	1.0000	0.0000	0.0000
MAP	0.0000	0.0000	0.0000	1.0000	0.0000
UtAD-PI	0.0000	0.0000	0.0000	0.0000	1.0000
EOPE					
Median MoM	0.83	0.60	1.39	1.14	1.60
SD ln (MoM)**	0.3924	0.7724	0.6057	0.0857	0.2229
EOPE Correlation Matrix					
PIGF	1.0000	0.1056	0.4496	0.0000	0.0000
PAPP-A	0.1056	1.0000	0.2988	0.0000	0.0000
AFP	0.4496	0.2988	1.0000	0.0000	0.0000
MAP	0.0000	0.0000	0.0000	1.0000	0.0000
UtAD-PI	0.0000	0.0000	0.0000	0.0000	1.0000

**ln (MoM) = natural log (MoM)

Table 6 shows the specificity, sensitivity, PPV and NPV for various combinations of the biochemical and biophysical markers. Utilizing the full protocol, including MAP and UtAD-PI, the sensitivity is 91%, the specificity is 95%, the PPV is 1 in 9.5 and the NPV is 99.94%. These results are similar to those seen with Down syndrome screening.[15,16]

Table 6: Screening Performance for Various EOPE Screening Protocols (History and Demographics Included for All Protocols)

Protocol	Specificity (95% CI)	Sensitivity (95% CI)	PPV	NPV
PIGF, PAPP-A, AFP	95% (93.5%-96.3%)	60% (48.2%-78.2%)	1 in 14	99.73%
PIGF, PAPP-A, AFP, MAP	95% (93.5%-96.3%)	77% (58.9%-90.4%)	1 in 11	99.84%
PIGF, PAPP-A, AFP, UtAD-PI	95% (93.5%-96.3%)	82% (62.5%-92.5%)	1 in 10.5	99.88%
PIGF, PAPP-A, AFP, MAP, UtAD-PI	95% (93.5%-96.3%)	91% (74.2%-98.0%)	1 in 9.5	99.94%

Confidence intervals are based on a binomial distribution with sample size equal to 31 for sensitivity and 1048 for specificity.

DISCUSSION

Timing of the Test

Preeclampsia is a serious maternal and fetal condition that presents after 20 weeks' gestation. Common evaluation for preeclampsia includes both uterine and umbilical artery Doppler assessment starting at 20 weeks as well as assessment of blood pressure and proteinuria. In addition, symptoms such as sudden weight gain; swelling of hands, feet or face; headache that won't go away; changes in vision; upper abdominal pain or chest pain; breathing with difficulty; gasping; and panting are often evaluated as signs of possible preeclampsia. These assessments usually take place at or near the time of onset of the disease, and although essential in treating and caring for the pregnancy, they are not useful in preventing or delaying onset of disease except by potentially marginal amounts.

The Preeclampsia**Screen**™ | T1 test is performed during the first trimester of pregnancy, with biomarker testing available as early as 10 weeks of pregnancy and MAP and UtAD-PI measurements available starting at 11 weeks, opening the door to potential treatments that prevent or delay onset of symptoms.

Treatment of High-Risk Asymptomatic Patients

Most preeclampsia interventions currently employed are performed later in pregnancy, often during escalation into eclampsia. These interventions are designed to control seizures and enable maturity of the fetal lungs prior to delivery. The ability to provide treatment at an earlier time in pregnancy before the onset of symptoms would obviously provide significant advantages.

Placental damage caused by preeclampsia is thought to lead to activation of platelets and the clotting system. Women with preeclampsia produce excess thromboxane, a vasoconstrictor and stimulant of platelet aggregation. Aspirin potentially inhibits platelet production of thromboxane. As a result, aspirin has been tried for both prevention and treatment.

Two large-scale meta-analyses by Bujold[29] and Roberge[30] showed that use of aspirin prior to 16 weeks of pregnancy significantly reduced the risk of preeclampsia. They evaluated a total of 13 studies with initial treatment prior to 16 weeks and 20 studies with treatment at 16 weeks, or later. In the group with initial treatment before 16 weeks, the risk of preeclampsia was reduced by 53%, while in the latter group the risk was reduced by only 22%. There was a statistically significant (P<0.01) difference between the rates for the two groups. Due to the evaluation of these and other studies, the New York State Department of Health, as part of its Maternal Mortality Review Initiative,[31] issued an executive summary that indicated low-dose aspirin as a treatment option for high-risk individuals.

Blood Pressure

High blood pressure is a well-known indicator for preeclampsia. With early onset preeclampsia risk assessment, the binary assessment of hypertension is no longer acceptable. A measured blood pressure reading that is incorporated into a risk algorithm is of great importance. For such an approach to be successful, highly accurate blood pressure measurement is essential.

Recent studies have shown that conventional office blood pressure measurement is no longer the best method for evaluating a patient's blood pressure status. Typical manual blood pressure readings are subject to significant inter-operator variability and digit preference in which numbers are rounded to the nearest 5 or 0 digit. As such, automated office blood pressure (AOBP) in which multiple blood pressure readings are taken with a fully automated device, provides better accuracy and higher correlation with ambulatory blood pressure.[32]

Uterine Artery Doppler

Since the development of preeclampsia is thought to include abnormal development of the placenta and its vascular supply, it logically follows that evaluation of the uterine artery blood flow resistance in pregnancy may be helpful in establishing this risk. Some success in predicting preeclampsia was achieved by measuring uterine artery blood flow in the second trimester. Studies on screening using this marker at 20-24 weeks of gestation have reported that the detection rate of pregnancies that subsequently develop early onset preeclampsia is 80%-90%, with a false positive rate of 30%-35%. Data from the Fetal Medicine Foundation (FMF) and others has now shown that uterine artery Doppler assessment is effective in the first trimester as well.[23,33] Our study presented here shows that the incorporation of UtAD-PI with PlGF, PAPP-A, AFP and MAP can achieve a detection rate of 91% for early onset preeclampsia. To ensure that this detection rate can be maintained in routine clinical practice, a standard protocol must be followed for obtaining UtAD-PI measurements. The FMF has established such a protocol, and an accreditation procedure has been developed for UtAD-PI.

CONCLUSION

The data show that the combination of biochemical and biophysical markers used in this study can be employed as an effective first trimester screening test for early onset preeclampsia, with detection rates ranging from 60% when utilizing biochemistry only to up to 91% when utilizing the combination of biochemistry, MAP and UtAD-PI. Positive predictive values range from 1 in 14 with biochemistry only to 1 in 9.5 when utilizing all markers. Instead of applying medical monitoring and management resources to those patients with incidence rates of 1 in 200 for early onset preeclampsia, the Preeclampsia**Screen**™ | T1 test can be used to focus care on those patients with an incidence rate in the range of 1 in 9.5 to 1 in 14, thus better allocating such resources to those at the highest risk of developing early onset preeclampsia.

DISCLAIMER: This test is a part of a lab service offering provided by PerkinElmer Labs/NTD. This test was developed and its performance characteristics determined by PerkinElmer Labs/NTD. Consistent with laboratory-developed tests, it has not been cleared or approved by the U.S. Food and Drug Administration. The methods and performance characteristics have been reviewed and approved by the New York State Department of Health.

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