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Population based biomarker screening and the development of severe preeclampsia in California

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Abstract

Objective—To examine the relationship between second trimester maternal serum biomarkers and the development of early- and late-onset severe preeclampsia in euploid pregnancies.

Study Design—Included were 136,139 pregnancies participating in second trimester prenatal screening through the California Prenatal Screening Program with live births in 2006 through 2008. We identified severe preeclampsia diagnoses from hospital discharge records. We used log binomial regression to examine the association between abnormal second trimester maternal serum biomarkers and the development of severe preeclampsia.

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Results—Approximately 0.9% of all women (n= 1,208) in our sample developed severe preeclampsia; 329 before 34 weeks gestation and 879 at or after 34 weeks. High levels of alpha fetoprotein (AFP), human gonadotropin (hCG) and inhibin (INH) (multiple of the median (MoM) ≥ 95th percentile), and low estriol (uE3) (MoM ≤ 5th percentile), were associated with severe preeclampsia (RRs 2.5 – 11.7). Biomarkers were more predictive of early-onset severe preeclampsia (RRs 3.8 – 11.7). One in 9.5 pregnancies with combined high AFP, INH and low uE3 developed severe early-onset preeclampsia compared to one in 680.5 pregnancies without any abnormal biomarkers.

Conclusions—The risk of developing severe preeclampsia increases for women with high second trimester AFP, hCG, INH and/or low uE3; this is especially true for early-onset severe preeclampsia. When abnormal biomarkers co-occur, risk dramatically increases. Although the screening value of second trimester biomarkers is low, abnormal biomarkers, especially when occurring in combination, appear to indicate placental dysfunction associated with the development of severe preeclampsia.

Keywords

early-onset severe preeclampsia; second trimester biomarker screening; serum analytes

INTRODUCTION

Abnormal maternal serum analytes obtained for the purpose of prenatal screening for fetal anomalies are associated with adverse pregnancy outcomes; this is particularly true when their values are at extreme levels. [1–5] Preeclampsia (PE), a placental-based disease, is one such adverse pregnancy outcome. [1,2,6] PE occurs in about 3–5% of births, with the majority of cases occurring at term.[7] Approximately 10% of PE disorders have early-onset disease, defined as occurring prior to 34 weeks gestation.[8] Although early-onset PE represents the minority of cases, it is more closely associated with significant maternal and neonatal morbidity and mortality.[9]

While routine markers may be useful in identifying pregnancies at increased risk for severe PE,[1,2,6] identifying those that develop early-onset severe preeclampsia could potentially impact maternal and fetal outcomes. A few studies have used routinely collected maternal serum analytes to identify pregnancies at increased risk for severe PE while also differentiating between early and late onset disease.[6,10,11] However these studies have tended to be limited by small sample size (n<460).

We examined the association between routinely collected second trimester maternal serum analytes (alpha fetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE3), inhibin (INH)) and the development of early- and late-onset severe PE in a population-based sample.

MATERIALS AND METHODS

We included women with singleton pregnancies that underwent second trimester prenatal screening through the California Prenatal Screening Program within the Genetic Disease

Screening Program (GDSP) at the California Department of Public Health (CDPH) with live births in 2006 through 2008 for whom there was linked maternal and baby outcome data available from the Office of Statewide Health Planning and Development (OSHPD) hospital discharge records.[12,13] We excluded pregnancies with GDSP records (prenatal screening records, newborn screening records, chromosomal, and neural tube defect registries) that indicated a chromosomal or neural tube defect. Severe PE diagnosis was based on International Classification of Diseases, 9th Revision, Clinical Modifications (ICD-9-CM) code 642.5 which defines severe PE as hypertension in pregnancy, childbirth or puerperium, not specified as preexisting, with albuminuria, edema (or both) characterized as severe.[14] Controls had no severe PE or any other PE disorder (ICD-9-CM code 642.4 (mild PE) or 642.6 (eclampsia).[14] Early-onset was defined as severe PE and delivery before 34 weeks gestation or delivery in the 34th week of gestation with hospitalization prior to 34 weeks. Late-onset was defined as severe PE and delivery in the 34th week without continuous hospitalization prior to 34 weeks or delivery after 34 weeks gestation.

Second trimester maternal blood samples were collected between 15 and 20 completed weeks of gestation and sent to California state-designated regional laboratories for serum testing of AFP, hCG, uE3, and INH. Regional laboratories all adhered to the same protocols for measuring these analytes using fully automated equipment (Auto DELFIA; Perkin Elmer Life Sciences, Waltham, MA). Analyte levels were reported directly into the state database along with patient information. Information provided by the regional laboratories was used to convert the analyte values into a multiple of the median (MoM) used for interpretation of the final result. All women in our sample had AFP, hCG, uE3, and INH MoMs adjusted for gestational age, maternal weight, smoking status, preexisting diabetes and race/ethnicity.

We obtained hospital discharge records for cases with severe PE diagnoses and controls. We obtained race/ethnicity, age, weight and smoking variables from prenatal screening records and diabetic status from hospital discharge diagnoses (ICD-9-CM code 648.0 for pre-existing diabetes, 648.8 for gestational diabetes). We did not have date of diagnosis of PE in the hospital discharge records. Because the standard of care is to deliver patients who develop severe PE, we used the gestation of delivery as indicator of early- and late- onset.

Analyses utilized logistic binomial regression methods to estimate relative risks (RRs) of developing early- and late-onset severe PE in pregnancies with abnormal levels of second trimester AFP, hCG, INH, and/or uE3 relative to pregnancies without any marker abnormalities. A biomarker was considered abnormally high if the MoM was ≥ the 95th percentile and abnormally low if the MoM was ≤ the 5th percentile. Pregnancies with normal biomarkers were considered to be those who had all associated MoMs between the 5th and 95th percentiles. Biomarker analyses controlled for maternal characteristics found to be significantly different in those who developed severe PE versus those who did not. The performance of biomarkers found to be significantly predictive of early- or late-onset severe PE (considered in isolation and in combination) was tested using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) statistics.

All analyses were performed using Statistical Analysis Software (SAS) version 9.3 (Cary, NC). Methods and protocols for the study were approved by the Committee for the

Protection of Human Subjects within the Health and Human Services Agency of the State of California and the Institutional Review Board of the University of California, Davis.

RESULTS

A total of 136,139 pregnancies met entry criteria for evaluation of which 1,208 (0.9%) were classifiable as cases having severe PE or controls (n=134,931). Early onset and late-onset PE developed in 329 (0.2%) and 879 (0.7%) of all women. Maternal demographics associated with an increased risk for early- and late-onset severe PE included black race/ethnicity and diabetes (any, preexisting, and gestational) (RRs 1.5 – 6.9). Hispanic race/ethnicity, maternal age ≤ 17 or ≥ 35 years and weight at testing > the 95th percentile (by race/ethnicity at gestational age at testing) were associated with an increased risk for late-onset PE only (RR 1.2 – 2.1) (Table 1).

Single factor biomarker models for severe PE indicated an increased risk for early- and late-onset severe PE among pregnancies with AFP, hCG and INH MoMs ≥ the 95th percentile or a uE3 MoM ≤ the 5th percentile (RRs 2.5 to 11.7) (Table 2). Pregnancies with any of the at-risk biomarkers (elevated AFP, hCG, INH, and/or low uE3) had a 5-fold increased risk of developing early-onset severe PE compared with pregnancies without any of these biomarker patterns (RR 5.0, 95% CI 3.4 – 7.4, sensitivity 49.5%, specificity 84.4%, PPV 0.8%, NPV 99.9%). This same direction of risk was observed for late-onset severe PE wherein pregnancies with any at-risk biomarker had more than a two-fold increased risk compared to those without any of these marker patterns (RR 2.3, 95% CI 1.6 – 3.3, sensitivity 25.9%, specificity 84.4%, PPV 1.1%, NPV, 99.4%).

When at-risk biomarker patterns co-occurred, risks were higher for both early- and late-onset severe PE. For pregnancies with early-onset severe preeclampsia, high AFP and INH with low uE3 had the highest risk for development of the disease, with a one in 9.5 chance of this diagnosis compared to a one in 680.5 chance among pregnancies without any at-risk biomarker pattern (RR 36.9, 95% CI 5.6 – 244.3) (Table 3). For pregnancies with late-onset severe preeclampsia, the highest risk biomarker pattern was high AFP, hCG and INH with low uE3, with a one in 20.0 chance of having late-onset severe PE compared to a one in 176.2 chance among pregnancies without any at-risk biomarker pattern (RR 36.9, 95% CI 5.6 – 244.3) (Table 4). Overall, pregnancies with any at-risk biomarker pattern were nearly three times as likely to be diagnosed with severe PE compared to those without any risk pattern (RR 2.7, 95% CI 2.0 – 3.6). The highest risks for severe PE were also observed when three or more biomarker abnormalities were observed (RRs 13.0 to 34.2) (Table 5).

COMMENTS

California, with the highest number of births per year in the United States, provides a rich source of data on a heterogeneous population of pregnant women undergoing prenatal screening. Our study sought to determine the associations between second trimester maternal serum biomarkers and the development of early- and late-onset severe PE.

We have established that women with elevated second trimester AFP, hCG, INH and/or lowered uE3 are at increased risk of developing early and late-onset severe PE. Our study to

date provides the largest sample of women who underwent prenatal screening and had biomarkers used in the context of the development of preeclampsia. This is consistent with others who have examined these same relationships,[6,15] and that of Dugoff et al who, while observing crude biomarker-PE associations, did not observe an association between PE and AFP, hCG or uE3 when considered in isolation but observed an association between increased INH and PE and noted increased risk when biomarkers occurred in combination.[1]

While biomarker risk patterns were predictive of early-onset and late-onset severe PE, the magnitude of observed risks was especially high for early-onset severe PE. For instance, with the specific biomarker combination of elevated AFP, hCG and INH, 1 in 14.4 developed early-onset PE. This corresponds to a 37.6-fold increased risk over those without any abnormal at-risk markers (rate of 1 in 680.5). In contrast, 1 in 57.6 women with the same biomarker pattern (elevated AFP, hCG and INH) developed late-onset severe PE, a 9.3-fold increased risk compared to pregnancies without any abnormal at-risk markers (rate of 1 in 176.2). The predictive difference of biomarkers in early and late-onset severe disease can be explained by the varying pathogenesis of these diseases along the preeclampsia spectrum. Early-onset disease is thought to be due to abnormal placental implantation whereas late-onset disease is thought to result as a consequence of certain maternal medical co-morbidities.[16] This pathogenesis of preeclampsia explanation is supported by the lack of association we found between maternal age >35 years and maternal weight > 95th percentile with the development of early-onset PE.

A strength of this study is that our occurrence rates and demographic associations with PE were similar to reported findings. The incidence of early-onset severe PE among women participating in the California prenatal screening program between 2005–2008 was 0.2%, similar to reported rates in prior studies (range 0.1 to 0.38%).[17–20] Additionally, the associations we found between black race/ethnicity and diabetes with the development of early- and late-onset severe PE are well supported in the literature, as is the association between maternal age ≥ 35 years or maternal weight > the 95th percentile (by race/ethnicity and gestational age at testing) and late-onset severe PE.[21–23] The null finding of maternal age ≥ 35 years with the development of early-onset severe PE is not surprising and is supported in the literature, particularly when controlling for co-morbid medical conditions that are more common in this age group (chronic hypertension, diabetes).[24,25] The lack of association we found between maternal weight > 95th percentile and early-onset severe PE has been inconsistently shown in the literature, likely due to lack of specific preeclampsia subtype classification. Some support the lack of association,[26–27] while others find increased maternal weight associated with mild PE but not severe PE,[27–28] or PE in general.[29]

The only noticeable finding that did not match other reports was our rate of late-onset severe PE, 0.7%, which is lower than recently reported rates of 2.72 and 1.8%.[18,30,] Our rate difference could be attributed to our selecting for severe preeclampsia occurring at >34 weeks and did not include mild preeclampsia.

While considerable strengths of the present study include its size and diversity and as such, observed risks that are more likely to generalize broadly, these strengths should be considered together with the limitations of the study. PE diagnoses were derived from

hospital discharge data; we did not personally review the records to ensure accurate diagnosis. It is certainly possible that the hospital discharge data could have been miscoded. In addition, clinicians may differ in their interpretations of 'mild' versus 'severe' PE diagnoses. Given that the study included more than 130,000 pregnancies and that our findings for early-onset preeclampsia are consistent with studies with more clinical definitions of PE,[1,6,15] we believe that any errors were minimal and likely would not have changed the overall findings.

Although the performance analyses did not demonstrate this test to be sensitive enough to be used as a screening tool for early- or late-onset severe PE, observed risks can be utilized to identify at-risk pregnancies. Such information may be especially useful for nulliparous women for whom no prior pregnancy history is available. The information could also be used to further target an at-risk population and assist in risk stratification. Importantly, since we know that aspirin when started in the early second trimester in a higher risk population reduces the risk of developing severe preeclampsia,[31–32] the information from our study could be used to identify other potential candidates for aspirin therapy. To date, no study has specifically addressed the clinical management of the pregnant patient with abnormal serum biomarker findings.

Our results provide a framework for further investigations. Biomarkers are made and released by the fetal-placental unit. AFP is secreted by the fetus, hCG and Inhibin by the placenta, and uE3 a combination of the fetal-placental unit.[33] Further investigation of biomarker patterns for severe PE, particularly those associated with early-onset, could aid in the identification of underlying disease mechanisms. From a clinical perspective, our findings provide data for future evaluations of the potential use of screening marker data to further enrich an "at-risk" population who may benefit from preventative treatment.

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Table 1

Maternal characteristics associated with early- and late-onset preeclampsia (PE).

	No Preeclampsia or Eclampsia[a]	Severe preeclampsia	
		Early onset	Late onset
	n (%)	n (%) RR (95% CI)	n (%) RR (95% CI)
Sample	134,931 (100.0)	319 (100.0)	889 (100.0)
Race/Ethnicity			
White not Hispanic	36,738 (27.2) Reference	79 (24.8)	219 (24.6)
Hispanic	77,476 (57.4)	198 (62.1) 1.2 (0.9, 1.5)	554 (62.3) 1.2 (1.0, 1.4)[b]
Black	6,806 (5.0)	30 (9.4) 2.0 (1.3, 3.1)[c]	69 (7.8) 1.7 (1.3, 2.2)[c]
Asian	9,605 (7.1)	4 (1.3) 0.2 (0.1, 0.5)[d]	31 (3.5) 0.5 (0.4, 0.8)[d]
Other[e]	4,306 (3.2)	8 (2.5) 0.9 (0.4, 1.8)	16 (1.8) 0.6 (0.4, 1.0)
Maternal Age			
≤ 17 years	2,272 (1.7)	2 (0.6) 0.4 (0.1, 1.5)	29 (3.3) 2.0 (1.4, 2.9)[c]
18 – 34 years	109,225 (81.0) Reference	256 (80.3)	681 (76.6)
≥ 35 years	23,434 (17.4)	61 (19.1) 1.1 (0.8, 1.5)	179 (20.1) 1.2 (1.0, 1.4)[b]
Maternal Weight[f]			
< 5th percentile	6,259 (4.6)	13 (4.1) 0.9 (0.5, 1.6)	46 (5.2) 1.2 (0.9, 1.6)
5th – 95th percentile	121,699 (90.2) Reference	284 (89.0)	767 (86.3)
> 95th percentile	6,973 (5.2)	22 (6.9) 1.4 (0.9, 2.1)	76 (8.6) 1.7 (1.4, 2.2)[c]
Diabetes			
No	124,617 (92.4) Reference	274 (85.9)	757 (85.2)
Yes	10,314 (7.6)	45 (14.1) 2.0 (1.4, 2.7)[c]	132 (14.9) 2.1 (1.7, 2.5)[c]
Pre-gestational	1,049 (0.8)	12 (3.8) 5.2 (2.9, 9.2)[c]	45 (5.1) 6.8 (5.1, 9.1)[c]
Gestational	9,265 (6.9)	33 (10.3) 1.7 (1.1, 2.3)[d]	87 (9.8) 1.5 (1.2, 1.9)[c]
Mother smoked			
No	132,847 (98.5) Reference	318 (99.7)	876 (98.5)
Yes	2,084 (1.5)	1 (0.3) 0.2 (0.0, 1.4)	13 (1.5) 10.0 (0.5, 1.6)

[a] No mild or severe preeclampsia or eclampsia.

[b] $p < 0.05$

[c] $p < 0.001$

[d] $p < 0.01$

[e] Includes: Asian East Indian, Pacific Islander, Native American, Middle Eastern, Other race/ethnicity, and Unknown race/ethnicity

[f] Percentile by race/ethnicity at gestational age at testing.

Table 2

Log binomial regression analyses examining the association between second trimester maternal serum biomarkers and severe preeclampsia.

	No Preeclampsia or Eclampsia[a]	Severe preeclampsia	
		Early onset[b]	Late onset[c]
	n (%)	n (%) RR (95% CI)	n (%) RR (95% CI
No abnormal biomarkers[d] (n = 93,228)	92,562 (99.3) Referent	133 (0.1)	533 (0.6)
"High" Biomarker (MoM ≥ 95th)			
AFP (n = 6,833)	6,687 (97.9)	74 (1.1) 7.1 (4.3, 11.7)[e]	72 (1.1) 2.5 (1.5, 4.3)[e]
hCG (n = 6,863)	6,709 (97.8)	68 (1.0) 6.9 (4.3, 11.0)[e]	86 (1.3) 3.5 (2.3, 5.4)[e]
uE3 (n = 7,179)	7,112 (99.1)	13 (0.2) 1.3 (0.5, 3.2)	54 (0.8) 1.0 (0.5, 2.1)
INH (n = 6,719)	6,494 (96.7)	106 (1.6) 11.4 (7.5, 17.4)[e]	119 (1.8) 3.5 (2.2, 5.5)[e]
"Low" Biomarker (MoM ≤ 5th)			
AFP (n = 6,789)	6,739 (99.3)	9 (0.1) 0.6 (0.2, 2.6)	41 (0.6) 0.5 (0.2, 1.6)
hCG (n = 6,321)	6,273 (99.2)	11 (0.2) 0.3 (0.0, 2.5)	37 (0.6) 0.8 (0.3, 2.0)
uE3 (n = 5,450)	5,360 (98.4)	34 (0.6) 3.8 (2.0, 7.3)[e]	56 (1.0) 2.8 (1.6, 4.9)[e]
INH (n = 7,155)	7,111 (99.4)	8 (0.1) 0.7 (0.2, 2.3)	36 (0.5) 0.6 (0.4, 1.6)

RR, Relative Risk; 95% CI, Confidence Interval; AFP, alpha-fetoprotein; MoM, Multiple of the Median; hCG, human choronic gonatotropin; uE3, unconjugated estriol; INH, inhibin-A

[a] No mild or severe preeclampsia or eclampsia.

[b] Binomial analyses included black race/ethnicity and any diabetes (all dichotomized as yes versus no).

[c] Binomial analyses included Hispanic and black race/ethnicity, maternal age ≤ 17 years, maternal age ≥ 35 years, weight at testing > 95th percentile and any diabetes (all dichotomized as yes versus no).

[d] AFP, hCG, uE3 and INH MoMs all between the 5th and 95th percentile (AFP > 0.60, < 1.74; hCG > 0.42, < 2.35; uE3 >0.61, < 1.49; INH > 0.48, < 1.95)

[e] $p < 0.001$

Table 3

Results of log binomial regression and associated performance analyses examining the association between second trimester serum biomarker pattern and severe early onset preeclampsia

	Severe Early Onset Preeclampsia						
	n (%)	Rate (1/x)	RR (95% CI)[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Sample (n = 136,139)	319 (0.2)	426.8	--				
No abnormal biomarkers[b] (n = 93,228)	133 (0.1)	701.0	Reference				
Any Early Onset Preeclampsia 'at risk' Quad Biomarker[c] (n = 21,290)	160 (0.8)	133.1	4.9 (3.3, 7.3)[d]	50.2	84.4	0.8	99.9
One 'at risk' biomarker							
High AFP (n = 5,270)	27 (0.5)	195.2	1.7 (0.6, 4.7)	8.5	96.1	0.5	99.8
High hCG (n = 3,902)	10 (0.3)	390.2	2.3 (1.0, 5.5)	3.1	97.1	0.3	99.8
High INH (n = 3,845)	29 (0.8)	132.6	4.9 (2.5, 9.7)[d]	9.1	97.2	0.8	99.8
Low uE3 (n = 4,471)	14 (0.3)	319.4	0.4 (0.1, 3.2)	4.4	96.7	0.3	99.8
Two 'at risk' biomarkers							
High AFP and hCG (n = 470)	2 (0.4)	235.0	6.5 (1.6, 26.9)[e]	0.6	99.7	0.4	99.8
High AFP and INH (n = 424)	10 (2.4)	42.4	25.0 (10.8, 57.9)[d]	3.1	99.7	2.4	99.8
High hCG and INH (n = 1,526)	20 (1.3)	76.3	10.0 (4.9, 20.3)[d]	6.3	98.9	1.3	99.8
High AFP, Low uE3 (n = 144)	1 (0.7)	144.0	12.8 (1.8, 90.5)[e]	0.3	99.9	0.7	99.8
High hCG, Low uE3 (n = 290)	0 (0.0)	--	--	--	--	--	--
High INH, Low uE3 (n = 235)	7 (3.0)	33.6	21.9 (7.0, 68.8)[d]	2.2	99.8	3.0	99.8
Three or more 'at risk' biomarkers							
High AFP, hCG, and INH (n = 403)	28 (7.0)	14.4	37.6 (17.4, 81.3)[d]	8.8	99.7	6.9	99.8
High AFP, hCG and Low uE3 (n = 24)	0 (0.0)	--	--	--	--	--	--
High AFP, INH and Low uE3 (n = 38)	4 (10.5)	9.5	36.9 (5.6, 244.3)[d]	1.3	100.0	10.5	99.8
High hCG, INH and Low uE3 (n = 188)	6 (3.2)	31.3	27.4 (8.8, 85.5)[d]	1.9	99.9	3.2	99.8
High AFP, hCG, INH and Low uE3 (n = 60)	2 (3.3)	30.0	79.0 (21.3, 293.4)[d]	0.6	100.0	3.3	99.8

PPV, positive predictive value; NPV, negative predictive value; AFP, alpha-fetoprotein; hCG, human choronic gonatotropin; uE3, unconjugated estriol; INH, inhibin-A; "High" biomarker = Multiple of the Median (MoM) ≥ 95th percentile; "Isolated" biomarker = all 'at risk'other biomarker MoMs > 5th and < 95th percentile.

[a]Binomial analyses included black race/ethnicity and any diabetes (all dichotomized as yes versus no).

[b] AFP, hCG, uE3 and INH MoMs all between the 5th and 95th percentile (AFP > 0.60, < 1.74; hCG > 0.42, < 2.35; uE3 >0.61, < 1.49; INH > 0.48, < 1.95). 21,621 pregnancies were not included who had neither 'at risk' biomarkers nor 'no abnormal' biomarkers.

[c] Any high biomarker and/or low uE3 (all biomarkers found to be predictive in Table 2)

[d] $p < 0.001$

[e] $p < 0.01$

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Table 4

Results of log binomial regression and associated performance analyses examining the association between second trimester serum biomarker pattern and severe late onset preeclampsia

	Severe Late Onset Preeclampsia						
	n (%)	Rate (1/x)	RR (95% CI)[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Sample (n = 136,139)	889 (0.7)	153.1	--				
No abnormal biomarkers[b] (n = 93,228)	533 (0.6)	174.9	Reference				
Any Early Onset Preeclampsia 'at risk' Quad Biomarker[c] (n = 21,290)	231 (1.1)	92.2	2.3 (1.6, 3.3)[d]	26.0	84.4	1.1	99.4
One 'at risk' biomarker							
High AFP (n = 5,270)	340 (0.8)	135.1	1.3 (0.6, 2.9)	4.5	96.1	0.8	99.4
High hCG (n = 3,902)	25 (0.6)	156.1	1.6 (0.8, 3.5)	2.8	97.1	0.6	99.3
High INH (n = 3,845)	52 (1.4)	73.9	2.0 (1.0, 4.2)	5.8	97.2	1.4	99.4
Low uE3 (n = 4,471)	33 (0.7)	135.5	1.5 (0.7, 3.5)	3.7	96.7	0.7	99.3
Two 'at risk' biomarkers							
High AFP and hCG (n = 470)	8 (1.7)	58.8	7.2 (2.3, 22.3)[d]	0.9	99.7	1.7	99.4
High AFP and INH (n = 424)	11 (2.6)	38.5	2.9 (0.4, 20.2)	1.2	99.7	2.6	99.4
High hCG and INH (n = 1,526)	32 (2.1)	50.9	3.3 (1.4, 8.1)[e]	3.6	98.9	2.1	99.4
High AFP, Low uE3 (n = 144)	1 (0.7)	144.0	1.2 (0.2, 8.6)[f]	0.1	99.9	0.7	99.3
High hCG, Low uE3 (n = 290)	4 (1.4)	72.5	5.6 (1.4, 22.4)[g]	0.4	99.8	1.4	99.3
High INH, Low uE3 (n = 235)	7 (3.0)	33.6	4.5 (0.6, 31.7)	0.8	99.8	3.0	99.4
Three or more 'at risk' biomarkers							
High AFP, hCG, and INH (n = 403)	7 (1.7)	57.6	9.3 (3.0, 28.7)[d]	0.8	99.7	1.7	99.4
High AFP, hCG and Low uE3 (n = 24)	1 (4.2)	24.0	7.3 (1.1, 50.0)[f,g]	0.1	100.0	4.2	99.3
High AFP, INH and Low uE3 (n = 38)	1 (2.6)	38.0	5.2 (0.7, 35.5)[f]	0.1	100.0	2.6	99.3
High hCG, INH and Low uE3 (n = 188)	6 (3.2)	31.3	13.0 (4.3, 39.3)[d]	0.7	99.9	3.2	99.4
High AFP, hCG, INH and Low uE3 (n = 60)	3 (5.0)	20.0	44.8 (12.9, 155.1)[d]	0.3	100.0	5.0	99.3

PPV, positive predictive value; NPV, negative predictive value; AFP, alpha-fetoprotein; hCG, human choronic gonatotropin; uE3, unconjugated estriol; INH, inhibin-A; "High" biomarker = Multiple of the Median (MoM) ≥ 95th percentile; "Isolated" biomarker = all 'at risk'other biomarker MoMs > 5th and < 95th percentile.

[a] Unless otherwise indicated, binomial analyses included Hispanic or black race/ethnicity, maternal age ≤ 17 years, maternal age ≥ 35 years, weight at testing > 95th percentile and any diabetes (all dichotomized as yes versus no).

[b] AFP, hCG, uE3 and INH MoMs all between the 5th and 95th percentile (AFP > 0.60, < 1.74; hCG > 0.42, < 2.35; uE3 >0.61, < 1.49; INH > 0.48, < 1.95). 21,621 pregnancies were not included who had neither ‘at risk’ biomarkers nor ‘no abnormal’ biomarkers.

[c] Any high biomarker and/or low uE3 (all biomarkers found to be predictive in Table 2)

[d] $p < 0.001$

[e] $p < 0.01$

[f] Crude model (insufficient power to adjust for other factors listed in a).

[g] $p < 0.05$

Table 5

Results of log binomial regression and associated performance analyses examining the association between second trimester serum biomarker pattern and severe preeclampsia

	Severe Preeclampsia						
	n (%)	Rate (1/x)	RR (95% CI)[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Sample (n = 136,139)	1,208 (0.9)	112.7	--				
No abnormal biomarkers[b] (n = 93,228)	666 (0.7)	140.0	Reference				
Any Early Onset Preeclampsia 'at risk' Quad Biomarker[c] (n = 21,290)	391 (1.8)	54.5	2.7 (2.0, 3.6)[d]	32.4	84.5	1.8	99.3
One 'at risk' biomarker							
High AFP (n = 5,270)	67 (1.3)	78.7	1.5 (0.7, 2.9)	5.5	96.1	1.3	99.1
High hCG (n = 3,902)	35 (0.9)	111.5	1.6 (0.8, 3.2)	2.9	97.1	0.9	99.1
High INH (n = 3,845)	81 (2.1)	47.5	2.7 (1.6, 4.7)[d]	6.7	97.2	2.1	99.1
Low uE3 (n = 4,471)	47 (1.1)	95.1	1.2 (0.5, 2.7)	3.9	96.7	1.1	99.1
Two 'at risk' biomarkers							
High AFP and hCG (n = 470)	10 (2.1)	47.0	7.2 (2.7, 19.1)[d]	0.8	99.7	2.1	99.1
High AFP and INH (n = 424)	21 (5.0)	20.2	10.4 (4.4, 24.7)[d]	1.7	99.7	5.0	99.1
High hCG and INH (n = 1,526)	52 (3.4)	29.3	5.0 (2.7, 9.4)[d]	4.3	98.9	3.4	99.1
High AFP, Low uE3 (n = 144)	2 (1.4)	72.0	1.9 (0.5, 7.7)[e]	0.2	99.9	1.4	99.1
High hCG, Low uE3 (n = 290)	4 (1.4)	72.5	4.3 (1.1, 17.1)[f]	0.3	99.8	1.4	99.1
High INH, Low uE3 (n = 235)	14 (6.0)	16.8	3.5 (0.5, 23.2)	1.2	99.8	6.0	99.1
Three or more 'at risk' biomarkers							
High AFP, hCG, and INH (n = 403)	35 (8.7)	11.5	15.8 (7.7, 32.5)[d]	2.9	99.7	8.7	99.1
High AFP, hCG and Low uE3 (n = 24)	1 (4.2)	24.0	5.8 (0.9, 39.8)[e]	0.1	100.0	4.2	99.1
High AFP, INH and Low uE3 (n = 38)	5 (13.2)	7.6	18.4 (8.1, 41.8)[d,e]	0.4	100.0	13.2	99.1
High hCG, INH and Low uE3 (n = 188)	12 (6.4)	15.7	13.0 (5.0, 33.7)[d]	1.0	99.9	6.4	99.1
High AFP, hCG, INH and Low uE3 (n = 60)	5 (8.3)	12.0	34.2 (9.9, 117.9)[d]	0.4	100.0	8.3	99.1

PPV, positive predictive value; NPV, negative predictive value; AFP, alpha-fetoprotein; hCG, human choronic gonatotropin; uE3, unconjugated estriol; INH, inhibin-A; "High" biomarker = Multiple of the Median (MoM) ≥ 95th percentile; "Isolated" biomarker = all 'at risk'other biomarker MoMs > 5th and < 95th percentile.

[a] Unless otherwise indicated, binomial analyses included Hispanic and black race/ethnicity, maternal age ≤ 17 years, maternal age ≥ 35 years, weight at testing > 95th percentile and any diabetes (all dichotomized as yes versus no).

[b] AFP, hCG, uE3 and INH MoMs all between the 5th and 95th percentile (AFP > 0.60, < 1.74; hCG > 0.42, < 2.35; uE3 >0.61, < 1.49; INH > 0.48, < 1.95). 21,621 pregnancies were not included who had neither ‘at risk’ biomarkers nor ‘no abnormal’ biomarkers.

[c] Any high biomarker and/or low uE3 (all biomarkers found to be predictive in Table 2)

[d] $p < 0.001$

[e] Crude model (insufficient power to adjust for other factors listed in a).

[f] $p < 0.05$