OBSTETRICS

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

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OBJECTIVE: The purpose of this study was 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 that obtained second-trimester prenatal screening through the California Prenatal Screening Program with live births in 2006-2008. 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.

RESULTS: Approximately 0.9% of all women (n = 1208) in our sample experienced severe preeclampsia; 329 women at <34 weeks' gestation and 879 women ≥34 weeks' gestation. High levels of alpha fetoprotein (AFP), human chorionic gonadotropin, inhibin (multiple of the median, ≥95th percentile), and low unconjugated estriol (multiple of the median, ≤5th percentile), were associated with severe preeclampsia (relative risk, 2.5-11.7). Biomarkers were more predictive of early-onset severe preeclampsia (relative risk, 3.8-11.7). One in 9.5 pregnancies with combined high AFP, inhibin, and low unconjugated estriol levels experienced severe early-onset preeclampsia compared with 1 in 680.5 pregnancies without any abnormal biomarkers.

CONCLUSION: The risk of the development of severe preeclampsia increases for women with high second-trimester AFP, human chorionic gonadotropin, inhibin, and/or low unconjugated estriol; 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 that is associated with the development of severe preeclampsia.

Key words: biomarker, early-onset severe preeclampsia, screening, serum analyte

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Abnormal maternal serum analytes that were 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, a placental-based disease, is one such adverse pregnancy outcome.[1,2,6] Preeclampsia occurs in approximately 3-5% of births; most cases occur at term.[7] Approximately 10% of preeclampsia disorders have early-onset disease, which is defined as occurring at <34 weeks' gestation.[8] Although early-onset preeclampsia represents the minority of cases, it is associated more closely with significant maternal and neonatal morbidity and mortality rates.[9]

EDITORS' ★ CHOICE

Although routine markers may be useful in the identification of women whose pregnancies are at increased risk for severe preeclampsia,[1,2,6] the

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identification of those who experience early-onset severe preeclampsia potentially could impact maternal and fetal outcomes. A few studies routinely have used collected maternal serum analytes to identify pregnancies at increased risk for severe preeclampsia 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 pregnancies).

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

Materials and Methods

We included women with singleton pregnancies who underwent second-trimester prenatal screening through the California Prenatal Screening Program within the Genetic Disease Screening Program at the California Department of Public Health with live births in 2006 through 2008 for whom there were linked maternal and baby outcome data available from the Office of Statewide Health Planning and Development hospital discharge records.[12,13] We excluded pregnancies with Genetic Disease Screening Program records (prenatal screening records, newborn infant screening records, and chromosomal and neural tube defect registries) that indicated a chromosomal or neural tube defect. Severe preeclampsia diagnosis was based on *International Classification of Diseases, 9th Revision, Clinical Modifications* (ICD-9-CM) code 642.5, which defines severe preeclampsia as hypertension in pregnancy, childbirth, or puerperium, not specified as preexisting, with albuminuria, edema (or both) characterized as severe.[14] Control subjects had no severe preeclampsia or any other preeclampsia disorder (ICD-9-CM code 642.4 [mild preeclampsia] or 642.6 [eclampsia]).[14] *Early-onset* was defined as severe preeclampsia and delivery at <34 weeks' gestation or delivery in gestational week 34 with hospitalization at <34 weeks. *Late-onset* was defined as severe preeclampsia and delivery in gestational week 34 without continuous hospitalization at <34 weeks' gestation or delivery at >34 weeks' gestation.

Second-trimester maternal blood samples were collected from 15-20 completed weeks' gestation and were sent to California state-designated regional laboratories for serum testing of AFP, hCG, uE3, and inhibin levels. Regional laboratories all adhered to the same protocols for measuring these analytes with 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) that was used for interpretation of the final result. All women in our sample had AFP, hCG, uE3, and inhibin level MoMs adjusted for gestational age, maternal weight, smoking status, preexisting diabetes mellitus, and race/ethnicity.

We obtained hospital discharge records for cases with severe preeclampsia diagnoses and control subjects. 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 preexisting diabetes mellitus, 648.8 for gestational diabetes mellitus). We did not have the date of diagnosis of preeclampsia in the hospital discharge records. Because the standard of care is to deliver patients who experience severe preeclampsia, we used the gestation of delivery as indicator of early and late onset.

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

All analyses were performed with Statistical Analysis Software (version 9.3; SAS Institute Inc, 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 1208 pregnancies (0.9%) were classifiable as cases having severe preeclampsia or control subjects (n = 134,931). Early-onset and late-onset preeclampsia developed in 329 (0.2%) and 879 (0.7%) of all women. Maternal demographics that were associated with an increased risk for early- and late-onset severe preeclampsia included black race/ethnicity and diabetes mellitus (any, preexisting, and gestational; RR, 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 preeclampsia only (RR, 1.2-2.1; Table 1).

Single factor biomarker models for severe preeclampsia indicated an increased risk for early- and late-onset severe preeclampsia among pregnancies with AFP, hCG, and inhibin MoMs ≥95th percentile or a uE3 MoM ≤5th percentile (RR, 2.5-11.7; Table 2). Pregnancies with any of the at-risk biomarkers (elevated AFP, hCG, inhibin, and/or low uE3 levels) had a 5-fold increased risk of experiencing early-onset severe preeclampsia compared

TABLE 1
Maternal characteristics associated with early- and late-onset preeclampsia

Maternal characteristic	No preeclampsia or eclampsia[a]	Severe preeclampsia			
		Early onset		Late onset	
	n (%)	n (%)	Relative risk (95% CI)	n (%)	Relative risk (95% CI)
Sample	134,931 (100.0)	319 (100.0)		889 (100.0)	
Race/ethnicity					
White, not Hispanic	36,738 (27.2)	79 (24.8)		219 (24.6)	
	Reference				
Hispanic	77,476 (57.4)	198 (62.1)		554 (62.3)	
		1.2 (0.9–1.5)		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)
Age, y					
≤17	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	109,225 (81.0)	256 (80.3)		681 (76.6)	
	Reference				
≥35	23,434 (17.4)	61 (19.1)	1.1 (0.8–1.5)	179 (20.1)	1.2 (1.0–1.4)[b]
Weight[f]					
<5th percentile	6259 (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)	284 (89.0)		767 (86.3)	
	Reference				
>95th percentile	6973 (5.2)	22 (6.9)	1.4 (0.9–2.1)	76 (8.6)	1.7 (1.4–2.2)[c]
Diabetes mellitus					
No	124,617 (92.4)	274 (85.9)		757 (85.2)	
	Reference				
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]
Pregestational	1049 (0.8)	12 (3.8)	5.2 (2.9–9.2)[c]	45 (5.1)	6.8 (5.1–9.1)[c]
Gestational	9265 (6.9)	33 (10.3)	1.7 (1.1–2.3)[d]	87 (9.8)	1.5 (1.2–1.9)[c]
Smoked					
No	132,847 (98.5)	318 (99.7)		876 (98.5)	
	Reference				
Yes	2084 (1.5)	1 (0.3)	0.2 (0.0–1.4)	13 (1.5)	10.0 (0.5–1.6)

CI, confidence interval.

[a] No mild or severe preeclampsia or eclampsia; [b] $P < .05$; [c] $P < .001$; [d] $P < .01$; [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.

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with pregnancies without any of these biomarker patterns (RR, 5.0; 95% confidence interval [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 preeclampsia, wherein pregnancies with any at-risk biomarker had a >2-fold increased risk compared with 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

TABLE 2
Log binomial regression analyses that examined the association between second-trimester maternal serum biomarkers and severe preeclampsia

Variable	No preeclampsia or eclampsia[a]	Severe preeclampsia			
		Early onset[b]		Late onset[c]	
	n (%)	n (%)	Relative risk (95% CI)	n (%)	Relative risk (95% CI)
No abnormal biomarkers[d] (n = 93,228)	92,562 (99.3)	133 (0.1)		533 (0.6)	
	Referent				
High biomarker (MoM ≥ 95th percentile)					
Alpha-fetoprotein (n = 6833)	6,687 (97.9)	74 (1.1)	7.1 (4.3–11.7)[e]	72 (1.1)	2.5 (1.5–4.3)[e]
Human chorionic gonadotropin (n = 6863)	6,709 (97.8)	68 (1.0)	6.9 (4.3–11.0)[e]	86 (1.3)	3.5 (2.3–5.4)[e]
Unconjugated estriol (n = 7179)	7,112 (99.1)	13 (0.2)	1.3 (0.5–3.2)	54 (0.8)	1.0 (0.5–2.1)
Inhibin-A (n = 6719)	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 percentile)					
Alpha-fetoprotein (n = 6789)	6,739 (99.3)	9 (0.1)	0.6 (0.2–2.6)	41 (0.6)	0.5 (0.2–1.6)
Human chorionic gonadotropin (n = 6321)	6,273 (99.2)	11 (0.2)	0.3 (0.0–2.5)	37 (0.6)	0.8 (0.3–2.0)
Unconjugated estriol (n = 5450)	5,360 (98.4)	34 (0.6)	3.8 (2.0–7.3)[e]	56 (1.0)	2.8 (1.6–4.9)[e]
Inhibin-A (n = 7155)	7,111 (99.4)	8 (0.1)	0.7 (0.2–2.3)	36 (0.5)	0.6 (0.4–1.6)

CI, confidence interval; *MoM*, multiple of the median.

[a] No mild or severe preeclampsia or eclampsia; [b] Binomial analyses included black race/ethnicity and any diabetes mellitus (all dichotomized as yes vs 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 mellitus (all dichotomized as yes vs no); [d] Alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol, and inhibin-A multiples of the median all between the 5th and 95th percentile (alpha-fetoprotein, >0.60, <1.74; human chorionic gonadotropin, >0.42, <2.35; unconjugated estriol, >0.61, < 1.49; inhibin-A, >0.48, <1.95); [e] $P < .001$.

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early- and late-onset severe preeclampsia. For pregnancies with early-onset severe preeclampsia, high AFP and inhibin with low uE3 levels had the highest risk for development of the disease, with a 1 in 9.5 chance of this diagnosis compared with a 1 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 inhibin levels with low uE3 levels, with a 1 in 20.0 chance of having late-onset severe preeclampsia compared with a 1 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 3 times as likely to be diagnosed with severe preeclampsia compared with those without any risk pattern (RR, 2.7; 95% CI, 2.0–3.6). The highest risks for severe preeclampsia were also observed when ≥3 biomarker abnormalities were observed (RR, 13.0–34.2; 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 who undergo prenatal screening. Our study sought to determine the associations between second-trimester maternal serum biomarkers and the development of early- and late-onset severe preeclampsia.

We have established that women with elevated second-trimester AFP, hCG, inhibin, and/or lowered uE3 levels are at increased risk of the development of early- and late-onset severe preeclampsia. Our study to date provides the largest sample of women who underwent prenatal screening and who had biomarkers used in the context of the development of preeclampsia. This is consistent with others who have examined these same relationships[6,15]; Dugoff et al,[1] while observing crude biomarker-preeclampsia associations, did not observe an association between preeclampsia and AFP, hCG or uE3 levels when considered in isolation but observed an association between increased inhibin level and preeclampsia and noted increased risk when biomarkers occurred in combination.

Although biomarker risk patterns were predictive of early-onset and late-onset severe preeclampsia, the magnitude of observed risks was especially high for early-onset severe preeclampsia. For instance, with the specific biomarker combination of elevated AFP, hCG and inhibin levels, 1 in 14.4 pregnancies experienced early-onset preeclampsia. 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 inhibin levels) experienced late-onset severe preeclampsia, a 9.3-fold increased risk compared with pregnancies without any abnormal at-risk markers (rate of 1 in

TABLE 3
Associations between second-trimester biomarker patterns and severe early-onset preeclampsia

	Severe early onset preeclampsia						
Variable	n (%)	Rate (1/x)	Relative risk (95% CI)[a]	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
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" 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 = 5270)	27 (0.5)	195.2	1.7 (0.6–4.7)	8.5	96.1	0.5	99.8
High hCG (n = 3902)	10 (0.3)	390.2	2.3 (1.0–5.5)	3.1	97.1	0.3	99.8
High INH (n = 3845)	29 (0.8)	132.6	4.9 (2.5–9.7)[d]	9.1	97.2	0.8	99.8
Low uE3 (n = 4471)	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 = 1526)	20 (1.3)	76.3	10.0 (4.9–20.3)[d]	6.3	98.9	1.3	99.8
High AFP and 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 and low uE3 (n = 290)	0	—	—	—	—	—	—
High INH and 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 and hCG; low uE3 (n = 24)	0	—	—	—	—	—	—
High AFP and INH; 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 and INH; 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, and INH; low uE3 (n = 60)	2 (3.3)	30.0	79.0 (21.3–293.4)[d]	0.6	100.0	3.3	99.8

"High" biomarker: multiple of the median ≥95th percentile; "at risk" biomarker: any "at risk" biomarkers, multiples of the median, >5th and <95th percentile.

AFP, alpha-fetoprotein; *CI,* confidence interval; *hCG,* human chorionic gonadotropin; *INH,* inhibin-A; *uE3,* unconjugated estriol.

[a] Binomial analyses included black race/ethnicity and any diabetes mellitus (all dichotomized as yes vs no); [b] AFP, hCG, uE3, and INH multiples of the median 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 who had neither "at risk" biomarkers nor "no abnormal" biomarkers were not included; [c] Any high biomarker and/or low uE3 (all biomarkers found to be predictive in Table 2); [d] $P < .001$; [e] $P < .01$.

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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 comorbidities.[16] This pathogenesis of preeclampsia explanation is supported by the lack of association we found between maternal age >35 years and maternal weight at >95th percentile with the development of early-onset preeclampsia.

A strength of this study is that our occurrence rates and demographic associations with preeclampsia were similar to reported findings. The incidence of early-onset severe preeclampsia among women who participated in the California prenatal screening program from 2005-2008 was 0.2%, which is similar to reported rates in previous studies (range, 0.1–0.38%).[17-20] Additionally, the associations that we found between black race/ethnicity and diabetes mellitus with the development of early- and late-onset severe preeclampsia are well supported in the literature, as is the association between maternal age ≥35 years or maternal weight at >95th percentile (by race/ethnicity and gestational age at testing) and late-onset severe preeclampsia.[21-23] The null finding of maternal age ≥35 years with the development of early-onset severe preeclampsia is not surprising and is

TABLE 4
Association between second-trimester biomarker patterns and severe late-onset preeclampsia

Variable	Severe late-onset preeclampsia						
	n (%)	Rate (1/x)	Relative risk (95% CI)[a]	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
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" 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 = 5270)	340 (0.8)	135.1	1.3 (0.6–2.9)	4.5	96.1	0.8	99.4
High hCG (n = 3902)	25 (0.6)	156.1	1.6 (0.8–3.5)	2.8	97.1	0.6	99.3
High INH (n = 3845)	52 (1.4)	73.9	2.0 (1.0–4.2)	5.8	97.2	1.4	99.4
Low uE3 (n = 4471)	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 = 1526)	32 (2.1)	50.9	3.3 (1.4–8.1)[e]	3.6	98.9	2.1	99.4
High AFP and 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 and 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 and 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 and hCG; 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 and INH; 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 and INH; 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, and INH; low uE3 (n = 60)	3 (5.0)	20.0	44.8 (12.9–155.1)[d]	0.3	100.0	5.0	99.3

"High" biomarker: multiple of the median ≥95th percentile; "at risk" biomarker: any "at risk" biomarker multiples of the median >5th and <95th percentile.

AFP, alpha-fetoprotein; *CI,* confidence interval; *hCG,* human chorionic gonadotropin; *INH,* inhibin-A; *uE3,* unconjugated estriol.

[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 vs no); [b] AFP, hCG, uE3, and INH multiples of the median 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] $P < .001$; [e] $P < .01$; [f] Crude model (insufficient power to adjust for other factors listed in a); [g] $P < .05$.

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supported in the literature, particularly when we controlled for comorbid medical conditions that are more common in this age group (chronic hypertension, diabetes mellitus).[24,25] The lack of association that we found between maternal weight >95th percentile and early-onset severe preeclampsia has been shown inconsistently in the literature, likely because of lack of specific preeclampsia subtype classification. Some support the lack of association[26,27]; others find increased maternal weight to be associated with mild preeclampsia but not severe preeclampsia[27,28] or preeclampsia in general.[29]

The only noticeable finding that did not match other reports was our rate of late-onset severe preeclampsia, 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 that occurred at >34 weeks' gestation and did not include mild preeclampsia.

Although 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. Preeclampsia diagnoses were derived from hospital discharge data; we did not review personally 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

TABLE 5
Association between second-trimester biomarker patterns and severe preeclampsia

Variable	Severe preeclampsia						
	n (%)	Rate (1/x)	Relative risk (95% CI)[a]	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
Sample (n = 136,139)	1208 (0.9)	112.7	—				
No abnormal biomarkers[b] (n = 93,228)	666 (0.7)	140.0	Reference				
Any early onset preeclampsia "at risk" 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 = 5270)	67 (1.3)	78.7	1.5 (0.7–2.9)	5.5	96.1	1.3	99.1
High hCG (n = 3902)	35 (0.9)	111.5	1.6 (0.8–3.2)	2.9	97.1	0.9	99.1
High INH (n = 3845)	81 (2.1)	47.5	2.7 (1.6–4.7)[d]	6.7	97.2	2.1	99.1
Low uE3 (n = 4471)	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 = 1526)	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 and hCG; 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 and INH; 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 and INH; 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, and INH; low uE3 (n = 60)	5 (8.3)	12.0	34.2 (9.9–117.9)[d]	0.4	100.0	8.3	99.1

AFP, alpha-fetoprotein; *hCG,* human chorionic gonadotropin; *INH,* inhibin-A; *uE3,* unconjugated estriol.

"High" biomarker: multiple of the median ≥95th percentile; "at risk" biomarker: any "at risk" biomarker multiples of the median >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 mellitus (all dichotomized as yes vs no); [b] AFP, hCG, uE3, and INH multiples of the median 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 who had neither "at risk" biomarkers nor "no abnormal" biomarkers were not included; [c] Any high biomarker and/or low uE3 (all biomarkers found to be predictive in Table 2); [d] $P < .001$; [e] Crude model (insufficient power to adjust for other factors listed in footnote a); [f] $P < .05$.

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of "mild" vs "severe" preeclampsia diagnoses. Given that the study included >130,000 pregnancies and that our findings for early-onset preeclampsia are consistent with studies with more clinical definitions of preeclampsia,[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 preeclampsia, observed risks can be used to identify at-risk pregnancies. Such information may be especially useful for nulliparous women for whom no pregnancy history is available. The information could also be used to further target an at-risk population and to assist in risk stratification. Importantly, because we know that aspirin, when started in the early second-trimester in a higher risk population, reduces the risk of the development of 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 addressed specifically the clinical treatment 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 are secreted by the placenta, and uE3 is secreted by a combination of the fetal-placental unit.[33] Further investigation of biomarker patterns for

severe preeclampsia, 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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