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Performance of a proteomic preterm delivery predictor in a large independent prospective cohort

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BACKGROUND: Preterm birth remains a common and devastating complication of pregnancy. There remains a need for effective and accurate screening methods for preterm birth. Using a proteomic approach, we previously discovered and validated (Proteomic Assessment of Preterm Risk study, NCT01371019) a preterm birth predictor comprising a ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin.

OBJECTIVE: To determine the performance of the ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin to predict both spontaneous and medically indicated very preterm births, in an independent cohort distinct from the one in which it was developed.

STUDY DESIGN: This was a prospective observational study (Multicenter Assessment of a Spontaneous Preterm Birth Risk Predictor, NCT02787213) at 18 sites in the United States. Women had blood drawn at $17^{0/7}$ to $21^{6/7}$ weeks' gestation. For confirmation, we planned to analyze a randomly selected subgroup of women having blood drawn between $19^{1/7}$ and $20^{6/7}$ weeks' gestation, with the results of the remaining study participants blinded for future validation studies. Serum from participants was analyzed by mass spectrometry. Neonatal morbidity and mortality were analyzed using a composite score by a method from the PREGNANT trial (NCT00615550, Hassan et al). Scores of 0–3 reflect increasing numbers of morbidities or length of neonatal intensive care unit stay, and 4 represents perinatal mortality.

RESULTS: A total of 5011 women were enrolled, with 847 included in this planned substudy analysis. There were 9 preterm birth cases at $<32^{0/7}$ weeks' gestation and 838 noncases at $\geq 32^{0/7}$ weeks' gestation; 21 of 847 infants had neonatal composite morbidity and mortality index scores of ≥ 3, and 4 of 21 had a score of 4. The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio was substantially higher in both preterm births at $<32^{0/7}$ weeks' gestation and there were more severe neonatal outcomes. The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio was significantly predictive of birth at $<32^{0/7}$ weeks' gestation (area under the receiver operating characteristic curve, 0.71; 95% confidence interval, 0.55–0.87; $P=.016$). Stratification by body mass index, optimized in the previous validation study ($22<$body mass index≤ 37 kg/m^2), resulted in an area under the receiver operating characteristic curve of 0.76 (95% confidence interval, 0.59–0.93; $P=.023$). The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio predicted neonatal outcomes with respective area under the receiver operating characteristic curve of 0.67 (95% confidence interval, 0.57–0.77; $P=.005$) and 0.78 (95% confidence interval, 0.63–0.93; $P=.026$) for neonatal composite morbidity and mortality scores of ≥ 3 or 4. In addition, the ratio of insulin-like growth factor-binding protein 4 to sex hormone binding globulin significantly stratified neonates with increased length of hospital stay (log rank $P=.023$).

CONCLUSION: We confirmed in an independent cohort the ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio as a predictor of very preterm birth, with additional prediction of increased length of neonatal hospital stay and increased severity of adverse neonatal outcomes. Potential uses of the ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin predictor may be to risk stratify patients for implementation of preterm birth preventive strategies and direct patients to appropriate levels of care.

Key words: biomarker, insulin-like growth factor-binding protein 4, IGFBP4, neonatal morbidity and mortality, pregnancy, prematurity, preterm birth, proteomics, sex hormone-binding globulin

Preterm birth (PTB) remains a common and devastating pregnancy complication, accounting for more than 10% of all births in the United States.[1] Prematurity is the second leading cause of neonatal death in the United States and the leading direct cause of neonatal death worldwide.[2,3] Infants who are born as very preterm ($<32^{0/7}$ weeks' gestation) are at greatest risk of lifelong disabilities.[4]

EDITOR'S CHOICE

Application of interventions to prevent PTB is hindered by the inability to adequately predict individuals at greatest risk for very preterm delivery. A history of previous PTB is a powerful predictor of recurrent PTB but is seen in only approximately 10% of all PTBs.[5,6] Similarly, a short cervical length measured by transvaginal ultrasound between $16^{0/7}$ and $22^{6/7}$ weeks' gestation is a predictor of PTB, but accounts for only 7-10% of all PTBs."[7,8]

Medical indications are responsible for approximately 40% of all PTBs.[9] For a risk assessment tool for PTB to be the most clinically effective, it should predict spontaneous and iatrogenic PTB. Furthermore, it must identify women at risk for early PTB, because their neonates have the highest likelihood for severe morbidity and mortality. An example of a biomarker that did not meet this requirement is salivary estriol. This biomarker predicted late spontaneous PTB well, but was too variable and not efficacious for early PTB, and has been regarded as ineffective.[10] A test that reliably predicts a woman's risk for

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AJOG MFM at a Glance

IBP4/SHBG predicts very preterm birth ($<32^{0/7}$weeks), severe neonatal morbidity and mortality and length of neonatal hospital stay

multiple causes of PTB, particularly early PTB, and identifies neonates at risk for severe postnatal complications would be of great value to clinicians and families. Caregivers could tailor care or initiate interventions to extend gestation or improve neonatal outcomes.

The need for effective and accurate screening methods for PTB has driven interest in the discovery of new biomarkers, such as omics-based approaches. Saade and colleagues[11] described a novel serum proteomic spontaneous PTB predictor based on the ratio of insulin-like growth factor-binding protein 4 (IBP4, gene symbol IGFBP4) to sex hormone-binding globulin (SHBG).[11] This earlier study was not powered to investigate the performance of the predictor for PTB at $<32^{0/7}$ weeks' gestation and did not investigate sequelae of PTB, such as length of hospital stay and neonatal adverse outcomes.

This study aimed to expand the clinical utility of the previously validated IBP4/SHBG PTB biomarker to predict both spontaneous and medically indicated very PTBs, neonatal morbidity and/or mortality, and length of hospital stay of the neonate.

Materials and Methods

Study

The Multicenter Assessment of a Spontaneous Preterm Birth Risk Predictor (TREETOP) was a prospective observational study at 18 sites across the United States (ClinicalTrials.gov identifier: NCT02787213). The TREETOP study was designed for multiple assessments of IBP4/SHBG predictor performance over a range of outcomes of clinical importance and biomarker discovery. Patient biomarker results were not distributed to caregivers, and patients were not participating in protocols prescribing interventions to prevent PTB. The study was approved by the Institutional Review Board at each site. The study enrolled women at low risk for PTB at the age of 18 years and older with singleton pregnancies experiencing no symptoms of preterm labor or membrane rupture. Women with planned delivery before $37^{0/7}$ weeks' gestation, major anomalies or chromosomal disorders, planned cerclage, or progesterone use after $13^{6/7}$ weeks' gestation were excluded. Women were enrolled from $17^{0/7}$ to $21^{6/7}$ weeks' gestation with gestational age confirmed by a first trimester ultrasound and determined by the American College of Obstetricians and Gynecologists guidelines.[12]

This is the first phase of a planned 2-phase study. The IBP4/SHBG biomarker was previously found to predict spontaneous[11] and medically indicated[13] PTB at $<37^{0/7}$ weeks' gestation. The purpose of this first phase of the TREETOP study is to evaluate the ability of the IBP4-to-SHBG ratio to predict early PTB, both spontaneous and medically indicated, neonatal morbidity and/or mortality, and length of hospital stay of the neonate. Very PTB was defined as gestational age of $<32^{0/7}$ weeks.[1,4] Following the National Academy of Medicine guidelines,[14] the forthcoming second phase is reserved for validation studies of IBP4/SHBG risk stratification at clinically relevant thresholds, with assessment of sensitivity, specificity, likelihood ratios (LRs), odds ratios (ORs), and negative and positive predictive values (PPVs). Importantly, the separation of the study population into 2 phases was prespecified in the study protocol.

Selection of participants

Participants were randomly assigned by a third-party statistician to the first phase, approximately 30% of the study population, and the second phase, approximately 70% of the study population. Each phase reflected the TREETOP study population in both clinical and demographic factors as a whole. The prespecified range of gestational ages at blood draw for this substudy was limited to the previously validated blood draw range ($19^{1/7}$–$20^{6/7}$ weeks).[11]

Clinical data collection

Clinical data were recorded as prespecified on 4 occasions across pregnancy by qualified study coordinators using electronic case report forms. Collected data were monitored centrally and onsite and were subject to source document verification. Body mass index (BMI) was calculated using self-reported prepregnancy weight. Outcomes plus any complications were recorded. Deliveries were classified as term ($\geq 37^{0/7}$ weeks) or preterm ($<37^{0/7}$ weeks) with the specific gestational age at birth captured. Neonatal outcomes were collected through 28 days of life. Before database lock, classification of deliveries was confirmed by 3 board-certified maternal-fetal medicine specialists not involved in the study.

Sample collection

Maternal whole blood was processed to serum for no more than 2 hours after collection. Serum aliquots were barcoded and frozen at −80°C or maintained on dry ice within 2.5 hours. Samples were shipped overnight on dry ice in a temperature-monitored shipper. Thawed or hemolyzed (≥100 mg/dL hemoglobin, per a standardized color scale) samples were not accepted.

Laboratory methods

Samples were analyzed in the Sera Clinical Laboratory, a Clinical Laboratory Improvement Amendments (CLIA)- and College of American Pathologists (CAP)-accredited laboratory, using an analytically validated method.[15] Prospective analysis was continual in accordance with a commercial process intended to report results within 7 business days of sample receipt. Briefly, serum was depleted of abundant proteins, trypsin-digested, fortified with stable isotope standard (SIS) peptides, desalted, and analyzed using liquid chromatography-multiple reaction monitoring mass spectrometry. Response ratios (RR) were calculated by dividing the peak area of the endogenous peptide by that of the SIS peptide. The predictor score is the

natural logarithm of RRs of IBP4 and SHBG as follows:

$$S = \ln\left(\frac{RR_{\mathrm{IBP4}}}{RR_{\mathrm{SHBG}}}\right).$$

Aliquots of pregnant and nonpregnant pooled serum were included for quality control.[15] Routine clinical testing quality metrics monitoring the analytical performance were applied to all samples.[15]

Statistical methods

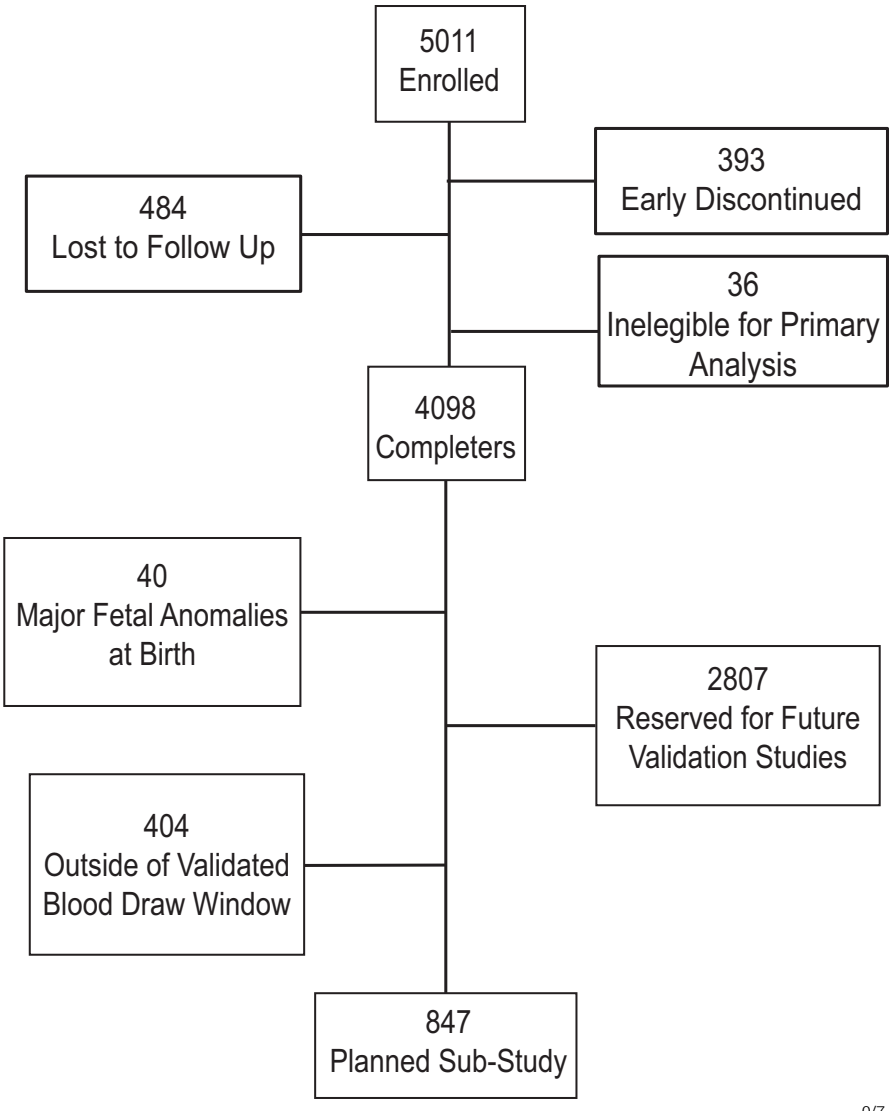
Best practices were employed to prevent bias[14] such as restricted access databases, blinding, and use of third-party statisticians for cohort selection. Except for the Clinical Operations personnel, Sera employees were blinded to all clinical data. Clinical Operations staff were blinded to the mass spectrometry data. Digital time stamping was utilized to provide an audit trail from subject level data through all analyses.

A published index scoring system (NMI), "0 to 4 scale with neonatal intensive care unit (NICU)"[8] measured neonatal composite morbidity and mortality. Within this scale, score increases by 1 with each diagnosis of respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage grade III or IV, all stages of necrotizing enterocolitis, periventricular leukomalacia, or proven severe sepsis (a clinically ill infant with positive culture, cardiovascular collapse, or unequivocal X-ray finding). Contribution of diagnoses was capped at NMI=3. NICU stays determined the NMI irrespective of concomitant diagnoses as follows: 1−4 days gave a score of 1, 5−20 days a score of 2, and >20 days a score of 3. Perinatal mortality (intrauterine fetal demise or neonatal mortality) was scored as 4. Data collection through 28 days of life allowed for confirmation of all conditions contributing to NMI. Severe NMI was defined as those with scores of 3−4, with mild to moderate NMI defined as scores of 1−2.

Demographic and clinical variables were compared between cases and noncases. Continuous data were examined for normality and transformed or assessed nonparametrically.

All statistical tests were 2-tailed at significance of 5% and, unless stated otherwise, performed in R (3.5.1 or higher, Comprehensive R Archive Network or Microsoft R Application Network). Count differences in categorical variables were assessed with chi-

FIGURE 1
Distribution of subjects in the TREETOP database



A total of 5011 participants were enrolled in the TREETOP study at gestational age between $17^{0/7}$ and $21^{6/7}$ weeks; 393 participants were discontinued, another 484 participants were lost to follow-up, and an additional 36 participants were ineligible for the primary analysis. Of the remaining 4098 women completing the study with eligibility for analysis, 40 were excluded from these analyses owing to the presence of major fetal anomalies detected at birth. Of the 4058 eligible women remaining, 2807 women were reserved for future validation studies whereas 1251 were randomly assigned to this first phase. A total of 847 cases with blood drawn within the previously validated window ($19^{1/7}$ and $20^{6/7}$ weeks' gestation) comprise this planned substudy; 404 with blood drawn outside this window are assigned to future studies for discovery across a broader blood draw window.

TREETOP, Multicenter Assessment of a Spontaneous Preterm Birth Risk Predictor.

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TABLE 1
Demographic and clinical characteristics of cases and noncases

Cases and noncases, n (%)	Delivery at <32 weeks' gestation, 9 (1.1)	Delivery at ≥32 weeks' gestation, 838 (98.9)	*P* value
Maternal age			.719
Median	27	30	
IQR	23–33	26–33	
BMI			.453
Median	28.9	25.9	
IQR	23.6–32.5	22.6–30.9	
Maternal race			.054
Black	3 (33%)	170 (20%)	
White	2 (22%)	507 (60%)	
Other	4 (44%)	161 (19%)	
Maternal ethnicity			.947
Hispanic	4 (44%)	331 (40%)	
Non-Hispanic	5 (56%)	505 (60%)	
Maternal education level			.067
No high school graduation	0 (0%)	128 (15%)	
High school degree/GED	8 (89%)	373 (44%)	
College degree	1 (11%)	334 (40%)	
Unknown	0 (0%)	3 (0.4%)	
Parity			.820
Nulliparous (*P*=0)	3 (33%)	358 (43%)	
Parous (*P*≥1)	6 (67%)	480 (57%)	
Previous PTB at <37 weeks' gestation			.052
Yes	2 (22%)	32 (43%)	
No	7 (78%)	806 (57%)	
Gestational age at birth (d)			<.001
Median	211	275	
IQR	184–214	269–281	
Neonatal hospital stay (d)			<.001
Median	28	2	
IQR	28–28	2–3	
Neonatal gender			.943
Female	4 (44%)	418 (50%)	
Male	5 (56%)	419 (50%)	

Shown are counts and percentages for categorical variables and medians with IQRs for continuous variables. Comparisons between cases (PTB at <32 weeks' gestation) and noncases were performed using Wilcoxon or Fisher's exact tests, as appropriate. Missing values are excluded in the frequency tables. Collection of neonatal hospital stay was capped at 28 days as per study protocol. A total of 8 of 9 cases were medically indicated for the following conditions: preeclampsia (5), HELLP (1), nonreassuring fetal testing (1), and intrauterine fetal demise (1).

BMI, body mass index; *IQR*, interquartile range; *GED*, general education diploma; *HELLP*, hemolysis, elevated liver enzymes, and low platelets; *PTB*, preterm birth.

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squared test. Median differences in continuous variables were assessed with the Wilcoxon test. Predictor performance was assessed by area under the receiver operating characteristic curve (AUC) with direction of effect prespecified, significance assessed by a 1-sided Wilcoxon test, and confidence intervals (CIs) calculated by the DeLong's

TABLE 2
Clinical characteristics of the case population (preterm birth at ≤32 weeks' gestation)

Patient number G/P	Gestational age delivered (wk/d)	Spontaneous vs medically indicated birth	Low-dose aspirin use before screening	Complications
1 5/0	23 3/7	miPTB	No	AMA, BMI<18.5 kg/m^2, ART conception, history of multiple SAB, IUGR (confirmed by BW), Doppler reversal of umbilical artery end-diastolic flow, oligohydramnios, IUFD
2 3/1	24 4/7	sPTB	No	Late second trimester heavy vaginal bleeding, acute-onset PPROM, PTL on the day of delivery
3 4/1	26 2/7	miPTB	Yes	Obesity, history of multiple SAB, severe preeclampsia, TTP, vaginal spotting during late second trimester, factor V Leiden mutation carrier
4 5/2	29 6/7	miPTB	Yes	Obesity, previous pregnancy with preeclampsia, severe preeclampsia, IUGR (confirmed by BW)
5 1/0	30 1/7	miPTB	No	HELLP syndrome
6 3/2	30 2/7	miPTB	No	AMA, obesity, preeclampsia, vaginal spotting during the third trimester
7 3/2	30 4/7	miPTB	No	Severe preeclampsia, nonreassuring fetal testing (acute onset), previous sPTB
8 2/1	30 4/7	miPTB	Yes	Chronic hypertension, preexisting diabetes, previous pregnancy with preeclampsia, previous miPTB, preeclampsia, hypothyroidism
9 2/1	31 0/7	miPTB	Yes	Preeclampsia; previous IUFD with preeclampsia

AMA, advanced maternal age; *ART*, assisted reproductive technology; *BMI*, body mass index; *BW*, birthweight; *G/P*, gravidity/parity; *IUFD*, intrauterine fetal demise; *IUGR*, intrauterine growth restriction; *HELLP*, hemolysis, elevated liver enzymes, and low platelets; *miPTB*, medically indicated preterm birth; *PPROM* preterm premature rupture of membranes; *PTL*, preterm labor; *SAB*, spontaneous abortion; *sPTB*, medically indicated preterm birth; *TTP*, thrombotic thrombocytopenic *purpura*.

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method.[16] Controls were defined as all subjects who were not cases.[17] Association between predictor scores and length of neonatal hospital stay was assessed by Kaplan–Meier analysis where death or discharge was the event and significance was assessed by the log-rank statistic.

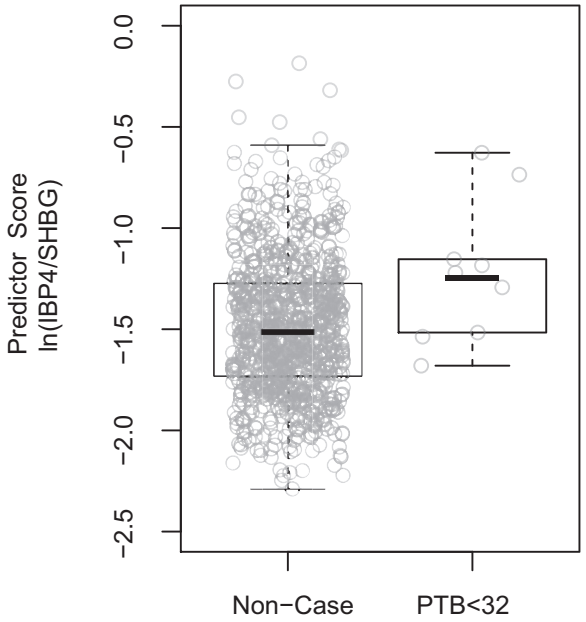
To evaluate association of predictor score to risk of both PTB at $<32^{0/7}$ weeks gestation and severe NMI, we assessed PPVs, positive LRs (LR+), and ORs using all possible thresholds with a minimum of 10 participants (cases or noncases) on either side. As TREETOP is representative of the US population as a whole (eg, 1.2% PTB at $<32^{0/7}$ weeks' gestation),[1] calculations were performed without prevalence adjustment. For calculation of ratios, counts of zero cases or noncases were conservatively substituted with a count of 1.

Results

A summary of the distribution of study participants in TREETOP is indicated in Figure 1. Of the 5011 women enrolled, 4098 completed the study and 4058 delivered babies without major fetal anomalies. As described in Materials and Methods, eligible participants were randomly assigned into 1 of 2 phases with 847 forming the planned substudy of IBP4/SHBG predictor performance in the previously validated blood draw window of $19^{1/7}$ to $20^{6/7}$ weeks' gestation. The 2 phases did not differ by demographic and clinical parameters (maternal BMI, age, race, ethnicity, education, obstetrical history, neonatal gestational age at birth, and gender; $P>.05$). Approximately 70% (2807) were reserved for future validation of novel predictors of adverse pregnancy outcomes (Figure 1).

Demographics, maternal characteristics, and delivery information were compared between cases and noncases (Table 1). Cases (n=9) were defined as those delivering at $<32^{0/7}$ weeks' gestation for any cause; noncases (n=838) delivered at $\geq 32^{0/7}$ weeks' gestation. All 9 case patients underwent midtrimester cervical length screening by transabdominal or transvaginal ultrasound and were found to have no shortening (defined as cervical length <25mm). A total of 8 of 9 cases resulted from medically indicated deliveries, further characteristics of which are summarized in Table 2. There were no significant differences in demographics or maternal characteristics between cases and noncases (Table 1). By design, the gestational age at birth was lower in cases. In addition, hospital stay of the neonate was

FIGURE 2
IBP4/SHBG predictor scores for PTB cases at <32 weeks' gestation vs noncases



Comparison of IBP4/SHBG predictor scores between cases who delivered preterm at <32 weeks' gestation (mean, −1.22) vs noncases who delivered at ≥32 weeks' gestation (mean, −1.48; t-test P=.032). Predictor score distributions are shown by box plots (box, interquartile range; line, mean; whiskers, remaining range of scores to a maximum of 1.5 box widths) and by a scattergram of all individual subjects.

IBP4/SHBG, insulin-like growth factor-binding protein 4/sex hormone-binding globulin; *Ln*, natural logarithm; *PTB*, preterm birth.

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longer (P<.001) in PTBs at <32 weeks' gestation (median of 28 days, the limit of collection) than births at ≥32 weeks' gestation (median of 2 days).

IBP4/SHBG scores were higher in PTB cases than in noncases (mean, −1.22 vs −1.48; P=.032) (Figure 2) and predictive of PTB cases vs noncases (AUC, 0.71; 95% CI, 0.55−0.87; P=.016). Increasing IBP4/SHBG scores were associated with decreasing gestational age at birth across all subjects (linear regression, P<.001). Prespecified stratification by BMI, as was performed in the previous validation study[11] (22<BMI≤37 kg/m^2), resulted in an AUC of 0.76 (95% CI 0.59−0.93; P=.023).

A total of 21 of 847 infants had severe NMI (scores≥3); 4 of 21 experienced mortality (score=4). Note that 7 of 9 cases of PTB at <32$^{0/7}$ weeks' gestation had NMI scores of 3. Neonatal death occurred in the other 2 PTB cases at <32$^{0/7}$ weeks' gestation (NMI score=4). The remaining 12 of 21 subjects with NMI≥3 included 6 moderate PTBs between 32$^{0/7}$ and 34$^{6/7}$ weeks' gestation (3 indicated deliveries for preeclampsia), 3 late PTBs between 35$^{0/7}$ and 36$^{6/7}$ weeks' gestation (1 intrauterine fetal demise), and 3 term births (1 intrauterine fetal demise). As expected, gestational age and weight at birth (linear regression, P<.001) were each correlated to NMI.

The IBP4/SHBG predictor score was positively correlated with NMI score (linear regression, P=.02) across all subjects (Figure 3). The IBP4/SHBG ratio was predictive of severe vs nonsevere NMI with an AUC of 0.67 (95% CI, 0.57−0.77; P=.005). Furthermore, IBP4/SHBG scores predicted severe vs mild to moderate NMI (scores of 3−4 vs 1−2), with an AUC of 0.65 (95% CI, 0.52−0.77; P=.02) and predicted mortality (scores of 4 vs 0−3) with an AUC of 0.78 (95% CI, 0.63−0.93; P=.026). IBP4-to-SHBG ratios associated with severe NMI (score≥3) do not differ between subjects with or without PTB at <32$^{0/7}$ weeks' gestation (P>.5), implying prediction of severe NMI beyond those caused by early PTB.

Clinical risk for PTB at <32$^{0/7}$ weeks' gestation and severe NMI (≥3) as measured by PPV is illustrated at a range of predictor scores (Figure 4, **A**). The risks of PTB at <32$^{0/7}$ weeks' gestation and severe NMI (≥3) rise smoothly as the predictor score increases, with a steeper rise in risk occurring between scores of −1.5 and −1.0 (Figure 4, **A**). ORs are indicated for PTB at <32$^{0/7}$ weeks' gestation and severe NMI (≥3) over the same range of predictor scores (Figure 4, B), demonstrating an association between increasing predictor scores and PTB at <32$^{0/7}$ weeks' gestation and severe NMI, along with reduced risk at low predictor scores. In the upper quartile, predictor scores range from −1.3 to −0.6 (median, −1.1), ORs from 4.5−20 (median, 6.6), positive LRs from 2.5−17 (median, 4.6), and PPVs from 2.3% to 7.0% (median, 3.0%), corresponding to 1.9x to 5.8x increased risk over baseline (median, 2.5x).

Finally, we noted a significant relationship (linear regression, P<.001) between predictor score and length of hospital stay of the neonate. Gestational age at birth was also inversely associated with length of stay (linear regression, P<.001). Kaplan−Meier analysis (Figure 5) found longer lengths of stay for infants delivered to women with an IBP4/SHBG predictor score in the upper quartile vs women with lower predictor scores. Various thresholds of predictor score (20th through 80th percentiles of predictor score, P=.001−.05) separated subjects by longer vs shorter lengths of stay. Prediction of length of stay by IBP4/SHBG remained significant when cases of PTB at <32$^{0/7}$ weeks' gestation (linear regression, P=.003) and severe NMI outcomes (linear regression, P=.016) were excluded, indicating the predictor is sensitive to additional adverse neonatal outcomes associated with longer neonatal hospital stay.

Comment

Principal findings

We report that the IBP4-to-SHBG ratio predicts risk of delivery before $32^{0/7}$ weeks' gestation caused by either spontaneous preterm labor or membrane rupture or medical indications. Furthermore, the IBP4-to-SHBG ratio is predictive of severe neonatal morbidity and mortality and increased length of hospital stay of the neonate. Prediction of severe neonatal adverse outcomes further reinforces and extends our understanding of this biomarker.

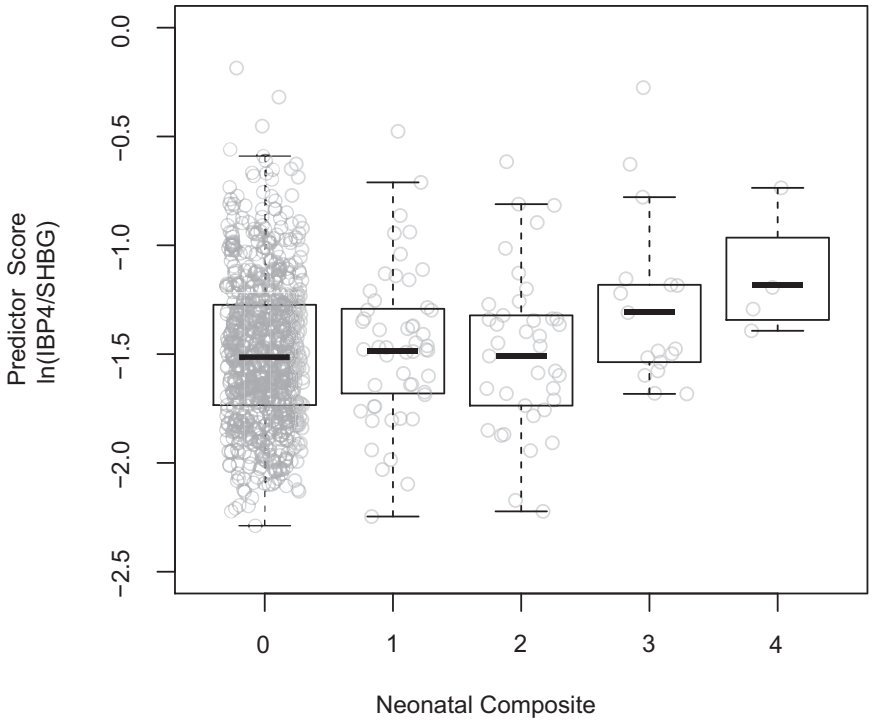
Results in the context of what is known

We elected to analyze all PTBs at $<32^{0/7}$ weeks' gestation and, more importantly, measures of neonatal health such as NMI and length of hospital stay of the neonate for several reasons.

Evidence exists that both spontaneous and iatrogenic PTBs share common pathways.[18] Misclassification of the type of PTB has been reported to occur in 5% to 15% of PTBs,[19] suggesting nonspecific features of clinical presentation. Furthermore, as previously reported, the IBP4/SHBG biomarker has indicated predictive performance in both PTB phenotypes.[13]

The performance of the IBP4/SHBG biomarker in PTB prediction suggests its connection to pathways of prematurity. Notably IBP4 regulates insulin-like growth factors involved in maintaining adequate nutrient delivery to the fetal compartment.[20] IBP4 is expressed by the placenta[21,22] and has been reported to be upregulated (increased in the circulation) in women with growth-restricted fetuses,[22] upregulated in the placentas of small-for-gestational-age neonates,[23] and downregulated in the placentas of large-for-gestational-age neonates.[23] These observations suggest IBP4 may be a biomarker for conditions of uteroplacental insufficiency. SHBG regulates levels of free and biologically active sex steroids,[24] is placentally expressed,[25] and is reported to be downregulated by proinflammatory cytokines such as tumor necrosis factor alpha and interleukin 1beta.[26] Clinically meaningful prediction of PTB risk may require that biomarkers be sensitive to conditions of placental dysfunction and inflammation. Ultimately, predicting risk of adverse neonatal outcomes is more beneficial and effective than predicting surrogate measures such as a gestational age below a threshold.

FIGURE 3
IBP4/SHBG predictor scores stratified by neonatal composite outcome score



Comparison of IBP4/SHBG predictor scores between participants assigned to each level of neonatal composite outcome score (NMI) as described in Materials and Methods (NMI 0=mean, −1.48; NMI 1=mean, −1.46; NMI 2=mean, −1.48; NMI 3=mean, −1.28; NMI 4=mean, −1.15; regression P=.02). Neonatal composite outcome scores of 0–3 reflect increasing numbers of morbidities or length of NICU stay, and 4 represents perinatal mortality. Predictor score distributions are shown by box plots (box, interquartile range; line, mean; whiskers, remaining range of scores to a maximum of 1.5 box widths) and by a scattergram of all individual subjects.

IBP4/SHBG, insulin-like growth factor-binding protein 4/sex hormone-binding globulin; *Ln*, natural logarithm; *NICU*, neonatal intensive care unit.

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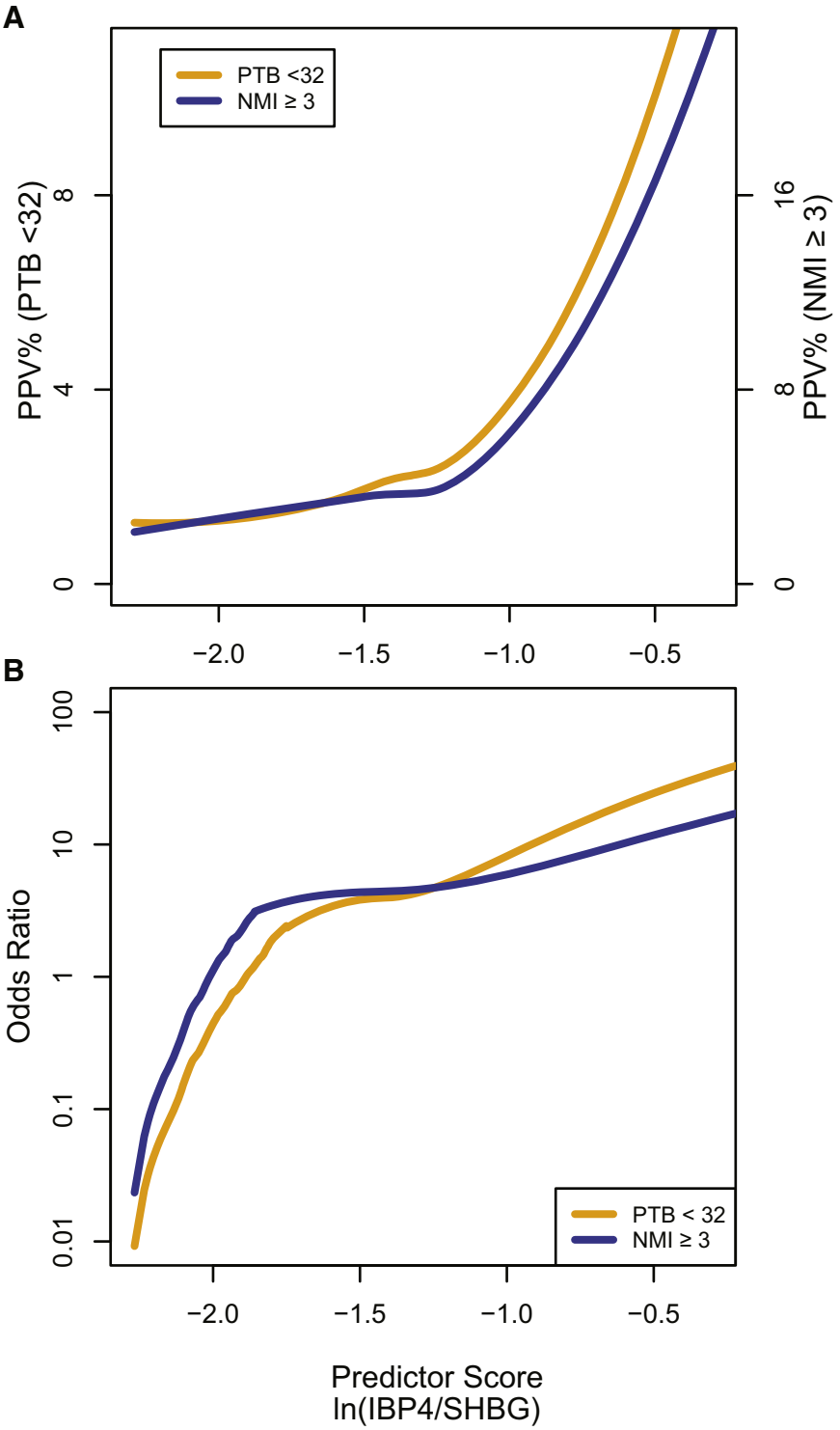
Clinical implications

Midtrimester quantification of risk for early PTB and adverse neonatal outcomes by established biomarkers offers intriguing opportunities for investigating benefits of patient stratification. Biomarker-based risk stratification may prove to be clinically and economically effective if paired with currently utilized interventions. A decision-analytic model[27] has predicted health and economic benefits of risk stratification of pregnancies using a hypothetical test paired with published interventions. Current literature supports the utility of additional surveillance and testing (ie, more frequent ambulatory visits and cervical length measurements) toward reduction of PTB rates.[28–30] Interventions such as corticosteroids and magnesium sulfate for a woman exhibiting signs and symptoms of preterm labor have well-established benefits to neonatal health.

Research implications

Direct measures of health and economic benefit are underway in trials pairing the IBP4/SHBG predictor with current interventions (PREVENT PTB NCT03530332 and AVERT PRETERM NCT03151330).

FIGURE 4



A, Stratification of risk of PTB at <32 weeks' gestation and neonatal morbidity and mortality by IBP4/SHBG predictor score. Positive predictive value (PPV) is plotted as a function of IBP4/SHBG predictor score for preterm delivery at <32 weeks' gestation (PTB at <32 weeks' gestation) and for neonatal composite outcome score (NMI≥3). **B,** Odds ratio for PTB at <32 weeks' gestation and neonatal morbidity and mortality by IBP4/SHBG predictor score. Odds ratio (OR) is plotted as a function of IBP4/SHBG predictor score for preterm delivery at <32 weeks' gestation (PTB at <32 weeks' gestation) and for neonatal composite outcome score (NMI ≥3).

IBP4/SHBG, insulin-like growth factor-binding protein 4/sex hormone-binding globulin; *Ln*, natural logarithm; *OR*, odds ratio; *PPV*, positive predictive value; *PTB*, preterm birth.

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Assessment of novel or improved interventions for prevention of early PTB and severe neonatal outcomes may benefit from a risk stratification tool like the IBP4/SHBG biomarker. Thus, the IBP4/SHBG biomarker may affect pregnancy and neonatal outcomes not only directly but through improvement of therapeutic trials.

Strengths and limitations

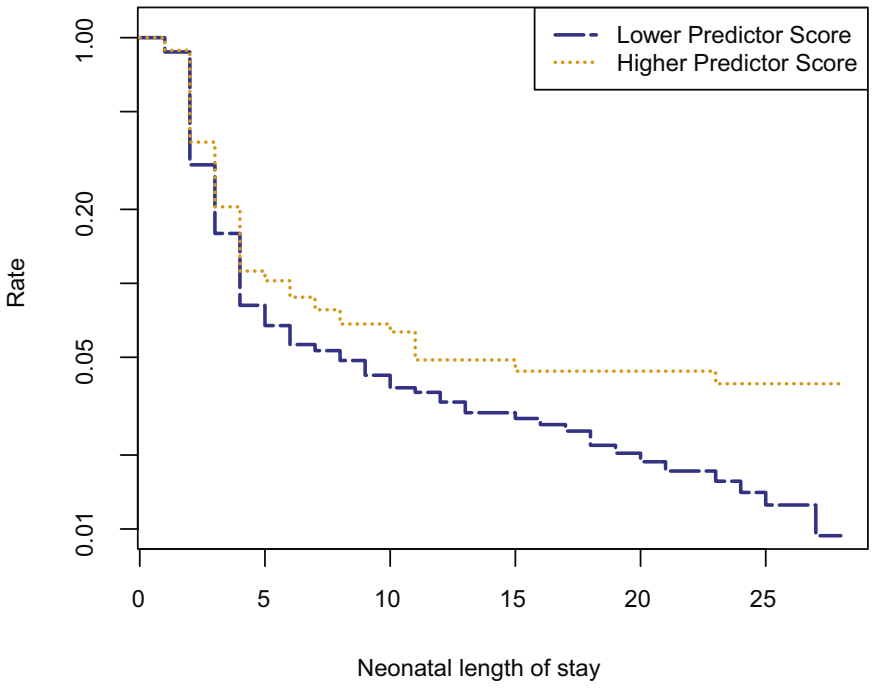
This study has several strengths. Women were enrolled at 18 sites across the United States to capture the diversity of the population. Samples were analyzed prospectively over an 18-month period using a validated method in a CLIA- and CAP-accredited laboratory, emulating clinical use of the test. A blinding protocol, study plan detailing the division of subjects into first and second phase analyses, and criteria for assessment of IBP4/SHBG PTB predictor performance were prespecified. Randomly selected substudy subjects were representative of the study as a whole. All were assigned either case or noncase status avoiding artifactual inflation of test performance caused by gapping.[17]

One limitation of the current confirmation study was low power for precise determination and optimization of threshold parameters, related to the number of early PTB cases. We also observed an excess of iatrogenic over spontaneous PTBs. Although our previous study reported the ability of the IBP4-to-SHBG ratio to predict spontaneous PTB, this study was limited in evaluating this marker for spontaneous birth at <32 weeks' gestation. Additional limitations were that the strongest predictor performance occured in a relatively narrow blood draw range ($19^{1/7}$ to $20^{6/7}$ weeks)[11] and that the predictor used only 1 clinical covariate (BMI) associated with PTB risk (eg, omitting maternal age, history of PTB, cervical length). The reserved cohort for the second phase of the TREETOP study may address these limitations.

Conclusion

This study reports the utility of the IBP4/SHBG ratio measured in serum drawn from asymptomatic women in the midtrimester to predict PTB at $<32^{0/7}$ weeks' gestation and measures of neonatal health such as extended hospital stays of the neonate and severe neonatal complications. ■

FIGURE 5
Kaplan–Meier analysis of neonatal hospital stay stratified by IBP4/SHBG predictor score



These curves indicate the rate of neonatal hospital discharge or mortality as a function of days in hospital for neonates stratified by IBP4/SHBG predictor score. Participants were separated into high- and low-scoring groups by the upper quartile of the predictor score. Neonates born to mothers with higher scores (in the upper quartile) have longer lengths of stay (P=.024) than neonates born to mothers with lower scores (in the lower 3 quartiles). Neonatal hospital stay includes all levels of care. Collection of neonatal hospital stay was capped at 28 days as per study protocol. Event rate is shown in log scale.

IBP4/SHBG, insulin-like growth factor-binding protein 4/sex hormone-binding globulin.

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