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Validating the ratio of insulin like growth factor binding protein 4 to sex hormone binding globulin as a prognostic predictor of preterm birth in Viet Nam: a case-cohort study

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ABSTRACT

Objective: To validate a serum biomarker developed in the USA for preterm birth (PTB) risk stratification in Viet Nam.

Methods: Women with singleton pregnancies ($n = 5000$) were recruited between 19^{+0}-23^{+6} weeks' gestation at Tu Du Hospital, Ho Chi Minh City. Maternal serum was collected from 19^{+0}-22^{+6} weeks' gestation and participants followed to neonatal discharge. Relative insulin-like growth factor binding protein 4 (IGFBP4) and sex hormone binding globulin (SHBG) abundances were measured by mass spectrometry and their ratio compared between PTB cases and term controls. Discrimination (area under the receiver operating characteristic curve, AUC) and calibration for PTB <37 and <34 weeks' gestation were tested, with model tuning using clinical factors. Measured outcomes included all PTBs (any birth ≤37 weeks' gestation) and spontaneous PTBs (birth ≤37 weeks' gestation with clinical signs of initiation of parturition).

Results: Complete data were available for 4984 (99.7%) individuals. The cohort PTB rate was 6.7% ($n = 335$). We observed an inverse association between the IGFBP4/SHBG ratio and gestational age at birth ($p = 0.017$; AUC 0.60 [95% CI, 0.53-0.68]). Including previous PTB (for multiparous women) or prior miscarriage (for primiparous women) improved performance (AUC 0.65 and 0.70, respectively, for PTB <37 and <34 weeks' gestation). Optimal performance (AUC 0.74) was seen within 19-20 weeks' gestation, for BMI >21 kg/m2 and age 20-35 years.

Conclusion: We have validated a novel serum biomarker for PTB risk stratification in a very different setting to the original study. Further research is required to determine appropriate ratio thresholds based on the prevalence of risk factors and the availability of resources and preventative therapies.

Introduction

Preventing preterm birth (PTB, defined as delivery <37 weeks' gestation) is a global priority. Of the estimated 15 million babies born preterm each year [1,2], approximately one million will die from complications, and many more will be left with lifelong disabilities [3].

PTB prediction in otherwise low-risk women could improve neonatal outcomes by targeting evidence-based strategies such as dedicated PTB clinics, vaginal progesterone supplementation, cervical cerclage or pessary, nutritional supplementation, and/or improved parental education and birth preparedness [4–7].

To date, preventative interventions for PTB have only been partially effective. A heterogenous syndrome, PTB involves both known and unknown pathways [8,9]. Better targeting of preventative strategies could mean fewer premature babies, with cost savings to society and the health system. Given the importance of environmental, nutritional, genetic, medical, and infectious exposures to PTB [10], protein expression associated with PTB could vary by context. Exclusion of populations living in high-burden settings from PTB biomarker discovery and validation work reduces the global impact of screening and therapeutic insights. External validation should be performed, using data from patients who are at risk for the same disease/event, are not included in model development, and, ideally, are from a different population. This step should be performed before evaluating the biomarker's clinical impact in the intended population [11].

In a previous discovery and validation study [12] in a United States (US) population, two placentally expressed proteins involved in uteroplacental insufficiency and inflammation were identified as biomarkers of spontaneous PTB (sPTB) when measured at 19^{+0}-20^{+6} weeks' gestation. The ratio of serum insulin-like growth factor-binding protein 4 (IGFBP4) to sex hormone-binding globulin (SHBG) was shown to be predictive of sPTB in asymptomatic women, with an area under the receiver operating characteristic curve (AUC) of 0.75 (95% confidence interval [CI], 0.56-0.91) and improved performance (e.g. AUC = 0.80) with more accurate gestational age (GA) dating [13], when case/control assignments are more certain. Importantly, Markenson et al. (2020) [14] demonstrated that IGFBP4/SHBG predicts not only sPTB, but also very early PTB (medically indicated PTB [miPTB] +sPTB) and more direct measures of adverse neonatal outcomes associated with prematurity. The predictor performed better in women with body mass index (BMI) between 22-37 kg/m^2 and in identifying those at risk of birth at earlier GAs, when the clinical consequences of prematurity are greatest. Highest predictive performance was seen for deliveries at <32 weeks' gestation, with deliveries including both sPTB and miPTB.

When adjusted for BMI and gestational age at blood draw (GABD), IGFBP4/SHBG significantly predicted sPTB in a subsequent study [15] of pregnant women recruited from sites in Bangladesh, Pakistan, and Tanzania as part of the Alliance for Maternal and Newborn Health Improvement (AMANHI) biorepository study [16]. However, AMANHI was not designed to evaluate all-cause PTB predictive performance or different sPTB etiologies, and subject numbers limited further stratification (e.g. geography-specific variation).

Predictor performance outside a US population needs better characterization, as it is possible that pathways contributing to PTB vary globally.

Here, we aimed to explore the hypothesis that the IGFBP4/SHBG predictor would stratify PTB risk in a larger, non-US population by: (1) externally validating the IGFBP4/SHBG predictor for all-cause PTB, in addition to sPTB, in a Vietnamese population; (2) tuning the test using clinical factors available at screening; and (3) identifying sub-populations in whom the test's discriminatory value is highest.

Methods

The Viet Nam Preterm Birth Biomarker study was a case-cohort study of PTB and term deliveries. The study was conducted with a prospective protocol and reported in line with TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis) recommendations [17].

Study population

Pregnant women presenting to the ultrasound department at Tu Du Hospital, Ho Chi Minh City, for their mid-pregnancy anomaly ultrasound scan from September 27, 2016-May 9, 2018 were invited to participate in the study. Tu Du Hospital is one of the largest maternity hospitals in Southeast Asia, conducting around 60000 deliveries per year. It serves as a local hospital for the inner city population, as well as a referral hospital for higher-risk cases from the greater city area and surrounding provinces.

Eligible participants were ≥18 years of age with a viable singleton pregnancy at 19^{+0}-22^{+6} weeks' gestation, with BMI <35 kg/m^2. Individuals were ineligible if they planned to deliver at another hospital, had evidence of major fetal abnormality, had active bleeding at the time of enrollment, were taking heparin during the pregnancy, had received blood products during pregnancy, or were clinically jaundiced. Individuals taking progesterone in pregnancy were initially excluded. This exclusion resulted in lower recruitment and raised the possibility that the cohort would not be representative of the eventual target population. Thus, following a protocol amendment in October 2017, progesterone intake was no longer an exclusion. Specifically, progesterone use recorded in this study was for women who experienced threatened miscarriage or any bleeding in the first trimester. These women were prescribed 20 mg of micronized desogestrol orally, taken daily for 2-4 weeks before 16 weeks' gestation.

Baseline measurements

Medical and obstetric histories obtained from participants included gravidity and parity, previous PTB, prior low birth weight baby (defined as birth weight <2500 g), previous miscarriages, and previous pregnancy terminations. Crown-rump length measurement from an ultrasound performed <14 weeks' gestation was used to estimate GA at recruitment and delivery.

Outcome measurements

After birth, information was collected on pregnancy complications; initiation and mode of delivery; neonatal size and status (liveborn, stillborn); and neonatal complications in babies admitted to the neonatal intensive care unit (NICU). Primary outcomes included all-cause PTBs (all-PTB), defined as any birth ≤37 weeks' gestation, and sPTBs, defined as birth ≤37 weeks' gestation with clinical signs of initiation of parturition, as per Villar et al. [18]. Two obstetricians (JEH and LPD), blinded to IGFBP4/SHBG predictor results, independently reviewed all PTB case notes and assigned sPTB and medically or obstetrically indicated PTB (miPTB) categorization. Birth weight percentiles and small for GA were defined using INTERGROWTH-21st standards for GA and sex [19]. Stillbirth was defined as any baby born with no signs of life after 20 weeks' gestation. Neonatal deaths were captured up until the time of hospital discharge only.

Case cohort selection

Within the full cohort of 5000 participants, all-PTB was compared to 500 participants selected randomly (case cohort design) (Supplemental Figure 1). Twenty random samples of 500 participants (which also included those delivering preterm) were drawn from the full cohort using adaptive minimization. Subjects most closely matching the study population prevalence on key variables (BMI, maternal age, GABD, and GA at birth) were selected. Laboratory testing was performed on this cohort of 500 individuals and all remaining PTB cases from the full cohort ($n = 297$) (Supplemental Figure 1). The adaptive minimization process was then repeated to partition PTB cases and controls into three equally sized sub-cohorts (A, B, and C), which were matched across the same key exposure and outcome variables (Supplemental Table 1). For prediction of all-cause PTB, controls were defined as term births, and PTBs from the 500-subject cohort were grouped with cases. For sPTB prediction,

controls were defined as both term births and the miPTBs selected as a part of the cohort, and cases excluded miPTBs.

IGFBP4/SHBG predictor performance was first tested in sub-cohort A. Sub-cohorts A and B were then combined to identify clinical factors that complemented the IGFBP4/SHBG predictor using the method described below. The modified predictor was trained on sub-cohorts A and B, and performance was validated on the held-out sub-cohort C. The three sub-cohorts were then combined to demonstrate performance of the final tuned models of the IGFBP4/SHBG predictor + clinical variables and to identify subpopulations with maximal performance.

Sample collection and processing

Venous blood samples were collected in serum separator tubes. Following clot formation at room temperature and centrifugation at 3000 rpm, serum was aliquoted within 2 h of collection for −80 °C storage at the Oxford University Clinical Research Unit (Ho Chi Minh City, Viet Nam). At the end of the study, samples were shipped to Sera Prognostics in two batches in liquid nitrogen shipping cannisters at −150 °C. In a US Clinical Laboratory Improvement Amendments- and College of American Pathologists-certified laboratory, samples were analyzed by liquid chromatography-multiple reaction monitoring mass spectrometry following a previously described procedure [12,21]. Individual samples were randomized to assay batches that also contained replicates of two pooled quality control sera samples: QC1, from non-pregnant women aged 21-33; and QC2, from pregnant women at 17-27 weeks' gestation (Golden West Biologicals, Temecula, CA, USA). All batches were required to meet Westgard criteria for acceptance based on QC samples [21,22].

Clinical factor selection and development of ObRisk variable

We performed a statistical search for clinical factors predictive of PTB using the Least Absolute Shrinkage and Selection Operator (LASSO) method [23]. Two hundred bootstrapped samples from sub-cohorts A and B with replacement were created, and the models were cross validated on leftover samples in each bootstrap iteration. Clinical factors selected via LASSO were recorded for each iteration. This process was repeated for nulliparous and multiparous subgroups. We created a new variable, ObRisk, with a score of 1 for multiparous women with previous PTB, and 1 for nulliparous

women with prior miscarriage. Women not meeting either criterion were assigned a score of 0.

Predictor models

To calculate the predictor score, relative IGFBP4 and SHBG levels were expressed as response ratios (RR) of the peak area for the endogenous peptide divided by the peak area of its corresponding SIS peptide [12,21]. The base IGFBP4/SHBG predictor score was calculated as the natural logarithm of (RR_{IGFBP4}/RR_{SHBG}).

Sample size estimation

Sample size was estimated using NCSS PASS statistical software [24]. Prior to the study, we assumed an sPTB rate of 2% based on historical data, with a total PTB rate of 10% due to provider-initiated deliveries from other causes. The recruitment target of 5000 women was selected to provide at least 100 sPTB cases and 400 PTB cases from other causes. This allowed testing of the null hypothesis: AUC = 0.5 with 90% power, assuming a 2:1 control-to-case allocation ratio.

Missing data

Owing to low (<5%) missing data across all clinical variables and outcomes, no imputation was performed, and a complete-case analysis is presented.

Blinding

Sera Prognostics personnel were blinded to clinical endpoints. Analyses were performed by an independent, third-party statistician, who initially received only mass spectrometry data and outcomes for sub-cohort A. Following verification, testing of ranked hypotheses, classifier tuning, and generation of new ranked hypotheses, this statistician was unblinded to the validation cohort.

Statistical analysis

All statistical tests were two-tailed and performed at the 5% significance level unless stated otherwise. Analyses were performed using SAS Version 9.3 (or higher) [25] or R Version 3.1.3 (or higher) [26]. Demographic and baseline variables were compared between cohort participants using chi-squared tests for categorical variables and Kruskal-Wallace tests for continuous variables [27].

We assessed models to predict sPTB or all-PTB, <37 or <34 weeks' gestation. Discriminative performance of the model was assessed using the C-statistic and presented as the AUC. AUC was assessed directionally, with cases hypothesized to have higher predictor scores than non-cases. Significance was tested *via* the one-tailed Wilcoxon-Mann-Whitney test, and 95% CIs were generated using DeLong's method [28]. Sensitivity, specificity, and positive and negative likelihood ratios (LR±) were calculated at thresholds corresponding to screen-positive rates (SPR) of 10%, 20% and 30%. Accurate reporting of a test's clinical predictive performance requires demonstration of discrimination and calibration, and avoidance of pitfalls such as selective subject exclusion by GA [29]. Calibration, defined as the agreement between the observed and the predicted outcomes, was assessed [30,31]; a slope of 1.0 represented a perfectly calibrated test.

Subgroup analysis and differences between development and validation populations

The IGFBP4/SHBG predictor was identified and validated originally in a US population. A key difference in the present study is a shift in the gestational blood draw window to align it with the Viet Nam mid-trimester ultrasound scan. Optimal performance of the US test was originally established as 19^{+0}-20^{+6} weeks [12]. In Viet Nam, the mid-trimester ultrasound scan is between 21^{+0}-22^{+6} weeks. Additionally, prior data indicated that IGFBP4/SHBG predictive performance has optimal BMI and GA ranges [12]. Performance was assessed by AUC for IGFBP4/SHBG + ObRisk for sliding windows across ranges of BMI, maternal age, and GABD. Calculation of prevalence-dependent performance metrics used the PTB prevalence in the analyzed study population.

Results

Cohort characteristics

Of 10711 women approached, 5000 were eligible and consented to participate. Reasons for nonparticipation are shown in Figure 1. Complete end-point data were available for 4984 (99.7%) participants (Table 1). Median age was 28 years, and median GA at recruitment and blood draw was 21^{+2} weeks. Just over half (2666/4984) of participants were in their first pregnancy; amongst multiparous women, 11.6% (268/2318) reported a previous PTB. Median BMI was 22.5 kg/m2, and median time in formal education was 13 years.
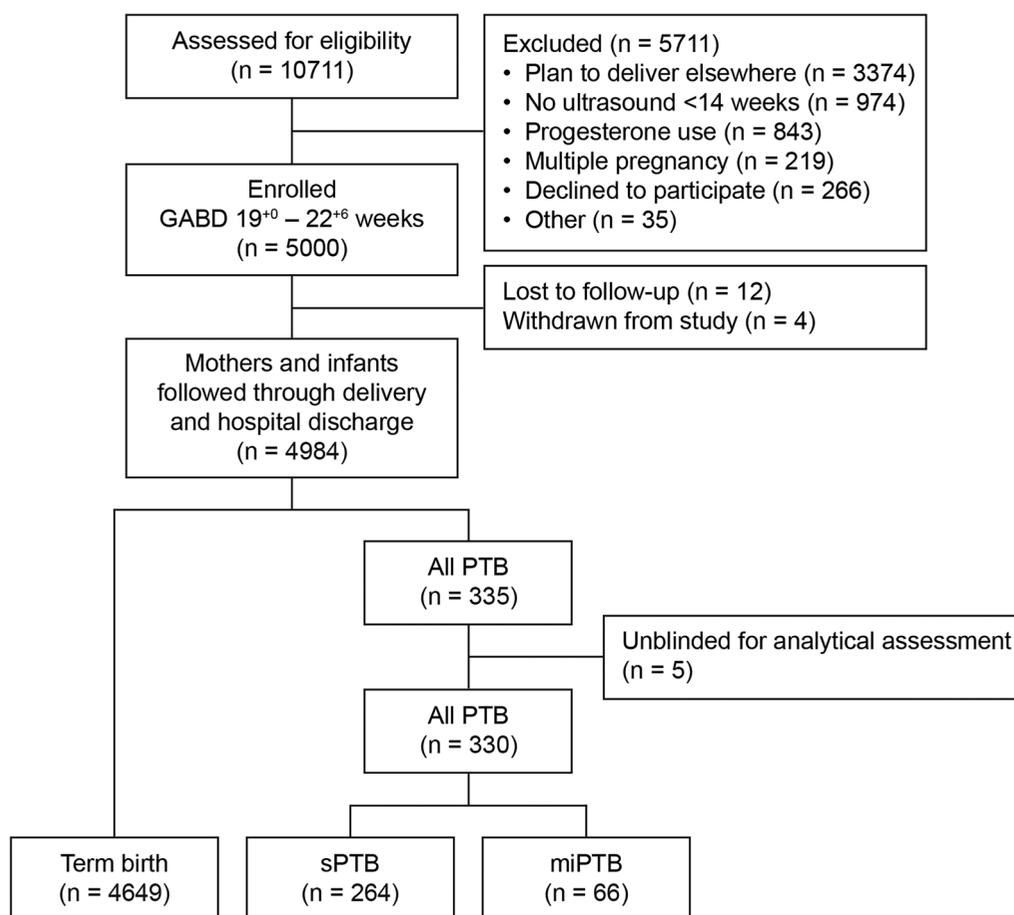
Figure 1. Study overview.
SGABD, gestational age at blood draw; miPTB, medically indicated preterm birth; PTB, preterm birth; sPTB, spontaneous preterm birth.

There were 4968 live births and 16 stillbirths. Thirteen babies died after birth and before discharge. Within the cohort, 335 participants delivered preterm (PTB rate 6.7%): 66 were miPTB, and 269 were sPTBs. Five sPTBs delivered outside the hospital and were removed from the primary analysis. Nearly one-third (30.5%; 1522/4984) of participants and 44.5% (149/335) of those delivering preterm reported taking progesterone prior to 16 weeks during the index pregnancy.

Preterm birth prediction based on IGFBP4/SHBG

We observed an inverse association between IGFBP4/SHBG and GA at birth ($p = 0.017$). The predictor significantly differentiated participants who delivered sPTBs from the background cohort population; however, overall discrimination was modest (AUC 0.60; 95% CI, 0.53-0.68), with similar performance in predicting any PTB (0.60; 0.53-0.67) (Table 2). Performance was better for predicting PTB at lower GA thresholds: for example, for birth <34 weeks' gestation, AUCs for sPTB and all-cause PTB were 0.67 (0.53-0.81) and 0.68 (0.56-0.79), respectively (Table 2).

IGFBP4/SHBG predictor tuning

We explored the additive value of clinical factors predictive of PTB. Bootstrapped sampling using LASSO regression repeatedly identified the number of prior miscarriages for nulliparas as a strong clinical factor (Supplemental Figure 2A), whereas for multiparas, the number of previous PTBs was selected repeatedly (Supplemental Figure 2B). The ObRisk variable improved predictive performance of IGFBP4/SHBG for PTB (Table 2).

The fully specified predictor developed for this population and its associated score is defined as:.

$$0.8 + 0.81 * \ln\left(RR_{IGFBP4} / RR_{SHBG}\right) + 1.43 * ObRisk$$

Validation on the independent sub-cohort C showed that prediction of all-PTB <37 and <34 weeks' gestation produced AUCs of 0.62 (0.55-0.69) and 0.67 (0.55-0.79), respectively.

Table 1. Maternal characteristics and pregnancy outcomes in the Viet Nam study cohort.

	Total	Term birth (delivery ≥37 weeks' gestation)	miPTB (delivery <37 weeks' gestation)	sPTB (delivery <37 weeks' gestation)	All-PTB (miPTB + sPTB)
Cases and noncases, n (%)	4984 (100.0)	4649 (93.3)	66 (1.3)	269 (5.3)	335 (6.7)
Maternal age					
Median	28	28	30.5	28	29
IQR	26 – 32	26 – 32	27 – 33	26 – 32	26 – 32
BMI at enrollment					
Median	22.5	22.5	23.3	22.2	22.5
IQR	20.8 – 24.3	20.8 – 24.3	21.8 – 25.2	20.5 – 24.4	20.7 – 24.8
Marital status					
Married	4973 (99.8)	4638 (99.8)	66 (100.0)	269 (100.0)	335 (100.0)
Separated/divorced	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Single	10 (0.2)	10 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal education level					
No school attended	6 (0.1)	6 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Primary	81 (1.6)	77 (1.7)	2 (3.0)	2 (0.7)	4 (1.2)
Secondary	1472 (29.5)	1353 (29.1)	19 (28.8)	100 (37.2)	119 (35.5)
Professional technical training	1332 (26.7)	1242 (26.7)	18 (27.3)	72 (26.8)	90 (26.9)
University	2093 (42.0)	1971 (42.4)	27 (40.9)	95 (35.3)	122 (36.4)
Occupational status					
Managerial/professional/ technical	1768 (35.5)	1658 (35.7)	25 (37.9)	85 (31.6)	110 (32.8)
Clerical support, service, or sales	1394 (28.0)	1307 (28.1)	18 (27.3)	69 (25.7)	87 (26.0)
Housework	924 (18.5)	860 (18.5)	10 (15.2)	54 (20.1)	64 (19.1)
Student	7 (0.1)	7 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Skilled manual work	345 (6.9)	313 (6.7)	7 (10.6)	25 (9.3)	32 (9.6)
Unskilled manual work	374 (7.5)	342 (7.4)	4 (6.1)	28 (10.4)	32 (9.6)
Other	172 (3.5)	162 (3.5)	2 (3.0)	8 (3.0)	10 (3.0)
Parity					
Nulliparous ($p = 0$)	2666 (53.5)	2497 (53.7)	26 (39.4)	143 (53.2)	169 (50.4)
Parous ($p \geq 1$)	2318 (46.5)	2152 (46.3)	40 (60.6)	126 (46.8)	166 (49.6)
Gravidity					
Multigravida	3021 (60.6)	2809 (60.4)	53 (80.3)	159 (59.1)	212 (63.3)
Primigravida	1963 (39.4)	1840 (39.6)	13 (19.7)	110 (40.9)	123 (36.7)
Prior preterm birth					
0	2050 (88.4)	1944 (90.3)	30 (75.0)	76 (60.3)	106 (63.9)
≥1	268 (11.6)	208 (9.7)	10 (25.0)	50 (39.7)	60 (36.1)
Prior stillbirth					
0	2270 (97.9)	2115 (98.3)	35 (87.5)	120 (95.2)	155 (93.4)
≥1	48 (2.1)	37 (1.7)	5 (12.5)	6 (4.8)	11 (6.6)
Prior miscarriage					
0	4492 (90.1)	4195 (90.2)	57 (86.4)	240 (89.2)	297 (88.7)
≥1	492 (9.9)	454 (9.8)	9 (13.6)	29 (10.8)	38 (11.3)
On progesterone <16 weeks' gestation					
No	3462 (69.5)	3276 (70.5)	39 (59.1)	147 (54.6)	186 (55.5)
Yes	1522 (30.5)	1373 (29.5)	27 (40.9)	122 (45.4)	149 (44.5)
Gestational diabetes					
No	4074 (81.7)	3820 (82.2)	42 (63.6)	212 (78.8)	254 (75.8)
Yes	910 (8.3)	829 (17.8)	24 (36.4)	57 (21.2)	81 (24.2)
Pregnancy-induced hypertension					
No	4895 (98.2)	4569 (98.3)	63 (95.5)	263 (97.8)	326 (97.3)
Yes	89 (1.8)	80 (1.7)	3 (4.5)	6 (2.2)	9 (2.7)
Preeclampsia					
No	4908 (98.5)	4596 (98.9)	46 (69.7)	266 (98.9)	312 (93.1)
Yes	76 (1.5)	53 (1.1)	20 (30.3)	3 (1.1)	23 (6.9)
Impaired fetal growth					
No	4665 (93.6)	4370 (94.0)	51 (77.3)	244 (90.7)	295 (88.1)
Yes	319 (6.4)	279 (6.0)	15 (22.7)	25 (9.3)	40 (11.9)
Mode of delivery					
Assisted breech or breech extraction	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cesarean section	2188 (43.9)	2057 (44.2)	45 (68.2)	86 (32.0)	131 (39.1)
Vaginal assisted	248 (5.0)	242 (5.2)	0 (0.0)	6 (2.2)	6 (1.8)
Vaginal spontaneous	2547 (51.1)	2349 (50.5)	21 (31.8)	177 (65.8)	198 (59.1)
Gestational age at birth (weeks^{+days})					
Median	39^{+1}	39^{+2}	34^{+6}	35^{+5}	35^{+5}
IQR	38^{+3} – 39^{+6}	38^{+4} – 39^{+6}	32^{+4} – 36^{+3}	34^{+3} – 36^{+3}	34^{+1} – 36^{+3}

(Continued)

Table 1. Continued.

	Total	Term birth (delivery ≥37 weeks' gestation)	miPTB (delivery <37 weeks' gestation)	sPTB (delivery <37 weeks' gestation)	All-PTB (miPTB + sPTB)
Neonate sex					
Female	2420 (48.6)	2265 (48.7)	30 (45.5)	125 (46.5)	155 (46.3)
Male	2564 (51.4)	2384 (51.3)	36 (54.5)	144 (53.5)	180 (53.7)
Neonate weight (grams)					
Median	3200	3200	2150	2500	2500
IQR	3000 − 3500	3000 − 3500	1600 − 2600	2200 − 2900	2100 − 2900
Neonate status at birth					
Alive	4968 (99.7)	4646 (99.9)	53 (80.3)	269 (100.0)	322 (96.1)
Antepartum death	14 (0.3)	3 (0.1)	11 (16.7)	0 (0.0)	11 (3.3)
Intrapartum death	2 (0.0)	0 (0.0)	2 (3.0)	0 (0.0)	2 (0.6)
Neonate status at hospital discharge					
Alive	4939 (99.1)	4634 (99.7)	49 (74.2)	256 (95.2)	305 (91.0)
Alive − referred to high-level care	16 (0.3)	11 (0.2)	2 (3.0)	3 (1.1)	5 (1.5)
Deceased	29 (0.6)	4 (0.1)	15 (22.7)	10 (3.7)	25 (7.5)
Newborn intensive special care					
No	4695 (94.5)	4505 (97.0)	25 (47.2)	165 (61.3)	190 (59.0)
Yes	272 (5.5)	141 (3.0)	28 (52.8)	103 (38.3)	131 (40.7)
Unknown	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.3)
Length of newborn intensive special care stay (days)					
Median	5	4	9.5	7	7
IQR	2 − 9	2 − 6.2	5 − 13.2	3 − 12	4 − 12

Table 2. Performance of the IGFBP4/SHBG predictor alone and when applying the clinical ObRisk factor, evaluated for preterm births at <37 and <34 weeks' gestation.

Cohort	Outcome (weeks)	Predictor Model	Cases	Cohort Controls	AUC	95% CI	P value
A	All-PTB <37	IGFBP4/SHBG	110	156	0.60	0.53 − 0.67	0.003
A	All-PTB <34	IGFBP4/SHBG	23	165	0.68	0.56 − 0.79	0.003
A	sPTB <37	IGFBP4/SHBG	88	159	0.60	0.53 − 0.68	0.004
A	sPTB <34	IGFBP4/SHBG	15	167	0.67	0.53 − 0.81	0.014
A + B	All-PTB <37	IGFBP4/SHBG + ObRisk	220	312	0.65	0.60 − 0.70	<0.001
A + B	All-PTB <34	IGFBP4/SHBG + ObRisk	46	332	0.70	0.61 − 0.80	<0.001
A + B	sPTB <37	IGFBP4/SHBG + ObRisk	176	317	0.66	0.60 − 0.71	<0.001
A + B	sPTB <34	IGFBP4/SHBG + ObRisk	31	335	0.71	0.59 − 0.82	<0.001

AUC, area under the receiver operating characteristic curve; CI, confidence interval; IGFBP4, insulin-like growth factor binding protein 4; ObRisk, a dichotomous clinical factor based on prior miscarriage (nulliparas) or prior preterm birth (multiparas); PTB, preterm birth; sPTB, spontaneous preterm birth; SHBG, sex hormone binding globulin.

Clinical predictive performance

Both IGFBP4/SHBG alone and IGFBP4/SHBG + ObRisk demonstrated good calibration, with predicted probabilities falling on the diagonal (Figure 2). Adding ObRisk led to tighter CIs and improved calibration at extremes of risk (Figure 2B). Women were assigned as being high- or low-risk according to a predictor score threshold corresponding to an SPR of 20%. Kaplan-Meier plots of GA at birth demonstrate that women identified as high-risk delivered earlier than those at low-risk, using both the base IGFBP4/SHBG ($p = 0.031$) and IGFBP4/SHBG + ObRisk ($p < 0.001$) predictors (Figure 3). Diagnostic accuracy for risk of all-PTB <37 or <34 weeks' gestation at different thresholds is summarized for combined Cohorts A, B and C in Table 3. We assessed threshold performance at three cutoff points

defined by calling either 10%, 20%, or 30% of the population positive for risk of all-PTB. Specificities ranged from 70-90% at sensitivities of 25-64%. PPV and NPV ranged from 0.4-16% and 94-99%, respectively, consistent with the low prevalence of these outcomes. LRs ranged as high as 3.0 and as low as 0.5, and ORs reached 4.3. Predictive performance for sPTB and miPTB risk <37 weeks' gestation in combined cohorts A, B, and C was similar to that for all-PTB (Supplemental Table 2).

In addition to prior PTB and miscarriage, the clinical variable search identified associations of BMI and maternal age with PTB (Supplemental Figure 2). IGFBP4/SHBG and IGFBP4/SHBG + ObRisk predictive performance improved as BMI and maternal age increased and when blood was drawn between
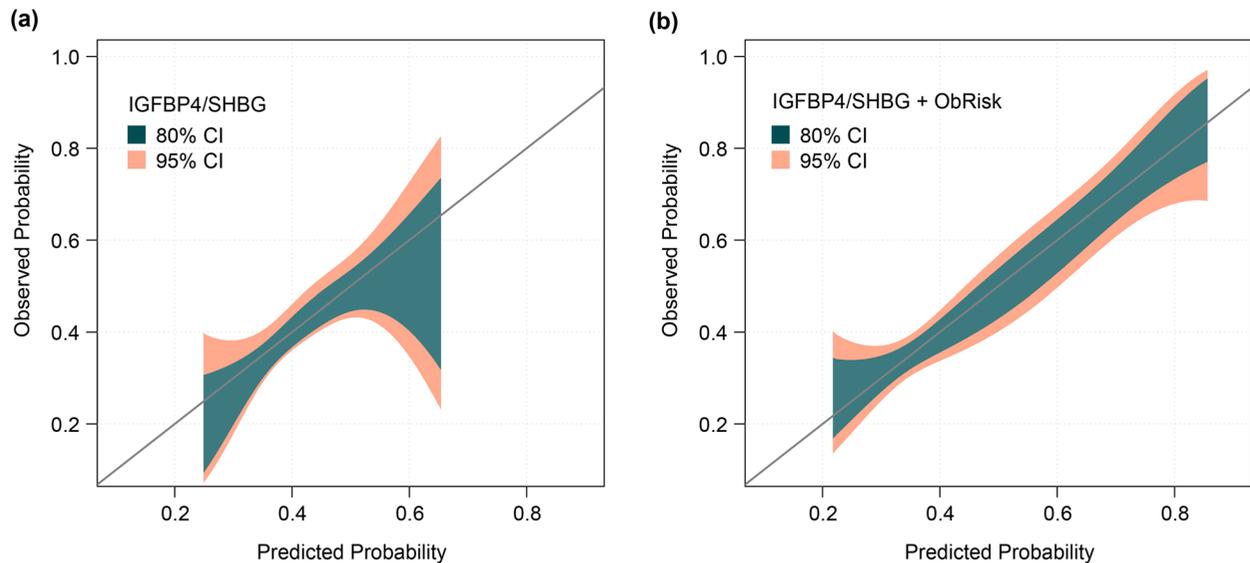
Figure 2. Calibration plots for (a) IGFBP4/SHBG and (b) IGFBP4/SHBG + ObRisk.
CI, confidence interval; IGFBP4, insulin-like growth factor-binding protein 4; ObRisk, a dichotomous clinical factor based on prior miscarriage (nulliparas) or prior preterm birth (multiparas); SHBG, sex hormone-binding globulin.

19^{+0}-20^{+6} weeks' gestation, as originally described in the US validation study. Based on this, we assessed discrimination in a subgroup analysis of participants meeting certain stratifications of these characteristics (Supplemental Table 3). The model performed superiorly for women with BMI >21 kg/m^2, age 20-35 years, and GABD 133-146 days, with AUC 0.74 for all-PTB <37 and <34 weeks' gestation. LRs ranged as high as 5.5 and low as 0.5, respectively, with ORs reaching 11 for prediction of PTB <34 weeks' gestation.

Discussion

We present an updated PTB prediction model using the ratio of serum IGFBP4/SHBG, measured around 20 weeks' gestation in asymptomatic women in Viet Nam. Adding ObRisk, a simple clinical variable incorporating prior PTB in multiparous women or history of miscarriage in those in their first ongoing pregnancy, improved model performance. Importantly, the strongest predictive performance was seen in those delivering <34 weeks' gestation, when prematurity complications are most severe. Predictor performance was greatest in women with BMI >21 kg/m^2, aged 20-35 years, with GABD 19^{+0}-20^{+6} weeks' gestation. Performance in this subgroup was moderate, similar to that observed in the US population [12]. This is likely an underestimation of actual predictive performance, as it does not account for the impact of GA imprecision on the assignment of "true" and "false" cases and controls. For example, we have demonstrated previously that use of only those subjects dated by early

ultrasound (e.g. prior to 9^{+0} weeks) improves predictor performance [13]. Our study reaffirms the need for external validation, model tuning and testing of predictive models before implementation in a new population/geography – a step frequently missing from predictive models developed for pregnancy care [32].

The IGFBP4/SHBG predictor appeared to perform better in Viet Nam than in the AMANHI cohort [15]. A major difference in study design may largely explain this difference: Blood samples from the AMANHI cohort were drawn between 17^{+0}-20^{+6} (median 18^{+2}) weeks' gestation, i.e. outside the optimal window of 19^{+0}-20^{+6} weeks. GABD was, therefore, included in the linear regression, although modeling cannot fully compensate for this difference. AMANHI participants also had a lower median BMI (21.8 kg/m^2) than both the US and Viet Nam populations. Adding BMI to the model improved predictive performance in AMANHI participants, but more than half of the cohort had a BMI below the optimum defined for the US population (>22 kg/m^2). Lastly, cohort stratification was not performed due to limited sample numbers, and incorporation of clinical variables was not tested.

Notably, despite the population-level differences between Viet Nam and the USA, including the lower rate of PTB in Viet Nam (6.5% vs. 10%), a significant IGFBP4/SHBG signal persists, suggesting a common underlying biological pathway for certain PTB cases. IGFBP4 regulates insulin-like growth factors involved in sustaining adequate nutrient delivery to the fetus [33]. It is expressed by the placenta [34,35] and has been reported to be upregulated (increased in circulation) in
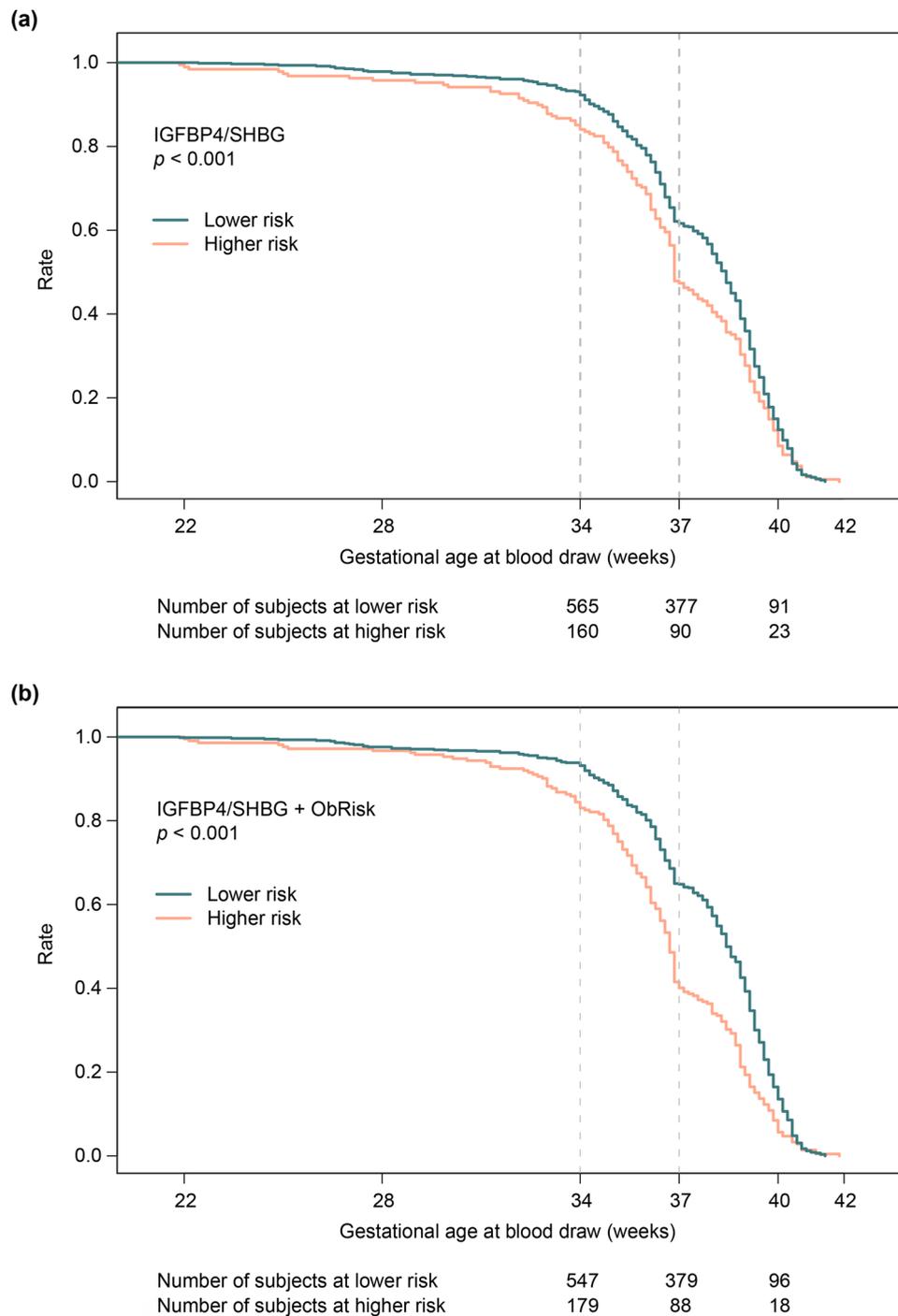
Figure 3. Kaplan-Meier plots of gestational age at birth for subjects evaluated to be at lower or higher PTB risk, stratified by (a) IGFBP4/SHBG ratio and (b) IGFBP4/SHBG ratio + ObRisk, at a screen-positive rate of 20%.
IGFBP4, insulin-like growth factor-binding protein 4; ObRisk, clinical factor based on prior miscarriage (nulliparas) or prior preterm birth (multiparas); SHBG, sex hormone binding globulin.

pregnancies with growth-restricted fetuses [34,35], upregulated in the placentas of small-for-gestational-age babies [36], and downregulated in the placentas of large-for-gestational-age babies [36]. These observations may indicate that IGFBP4 is a biomarker of utero-placental insufficiency. SHBG regulates levels of free/ biologically active sex steroids [37], is expressed by the placenta [38], and is reported to be suppressed by proinflammatory cytokines such as tumor necrosis factor alpha and interleukin 1 beta [39]. Biomarkers for both placental insufficiency and inflammation may combine for clinically meaningful sPTB risk prediction.

Table 3. IGFBP4/SHBG + ObRisk predictor performance for all-cause preterm birth under selected testing scenarios in combined cohorts A, B, and C.

Outcome	Stratification	Number of Cases	Number of Controls	SPR (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	OR
All-PTB <37 weeks gestation	None	330	467	10	24.5	91	16.2	94.5	2.74	0.83	3.30
				20	37.9	81.3	12.5	94.9	2.02	0.76	2.65
				30	49.7	71.4	10.9	95.3	1.74	0.7	2.47
	BMI + MAGE + GABD	59	78	10	44.1	90.4	5.2	99.3	4.63	0.62	7.49
				20	60.1	80.5	3.5	99.4	3.08	0.50	6.22
				30	61.8	70.4	2.4	99.4	2.09	0.54	3.85
All-PTB <34 weeks gestation	None	70	491	10	31.4	90.3	4.4	98.9	3.24	0.76	4.27
				20	48.6	80.4	3.4	99.1	2.48	0.64	3.88
				30	64.3	70.5	3.0	99.3	2.18	0.51	4.30
	BMI + MAGE + GABD	11	79	10	54.5	90.1	1.2	99.9	5.57	0.50	11.06
				20	54.5	80.1	0.6	99.9	2.74	0.57	4.83
				30	54.5	70.1	0.4	99.9	1.82	0.65	2.81

All-PTB, all-cause PTB; BMI, body mass index; GABD, gestational age at blood draw; LR±, positive and negative likelihood ratios; MAGE, maternal age; NPV, negative predictive value; ObRisk, clinical factor based on prior miscarriage (nulliparas) or prior preterm birth (multiparas); OR, odds ratio; PTB, preterm birth; PPV, positive predictive value; SPR, screen-positive rate.

Strengths and limitations

Key strengths of this study are the rigorous clinical and biological data collection, few losses to follow-up, and standardized definitions of sPTB and miPTB based on signs of initiation of parturition rather than eventual mode of birth. The random and representative nature of the case-cohort design enables its application to future outcome studies [20]. The study was rigorously conducted with regular external quality monitoring. GA was established in all participants using ultrasound before 14 weeks' gestation, minimizing the risk of misclassifying PTB and term births. Blood draw timing, 19^{+0}-22^{+6} weeks' gestation, was matched to the routine mid-pregnancy anomaly scan in Viet Nam, demonstrating how the test might perform if incorporated into routine practice. All laboratory analyses were conducted blinded to the clinical outcome, and in developing the ObRisk predictor, care was taken to select variables that could be easily collected at the time of blood draw.

Limitations of the study include the lower-than-anticipated PTB rate in the study population (6.5% vs 10.0%); however, this rate is in keeping with international estimates for Viet Nam (6.5%) published after our study concluded [2]. It is noteworthy that women with multiple gestations were excluded from this study. Given the limited study scope and budget, we were unable to validate the predictor for multifetal pregnancies in this study, but this aspect should be examined. A motivation for this study was to validate the test in a high-burden setting, which was not reflected in the lower PTB prevalence observed. Nevertheless, the consequences for babies born preterm remain concerning, and PTB prevention is a national priority: UNICEF estimates that 45% of newborn deaths in Viet Nam are related to complications secondary to PTB [40]. Our study was a hospital-based sample, and thus performance could differ at population level. Processing of all samples was performed in the US in a private laboratory, which would not be feasible or affordable should the predictor be used commercially in Viet Nam. We are developing a low-cost enzyme linked immunosorbent assay (ELISA) that, after validation, might be used in other settings. Finally, demonstration of clinical utility of the IBP4/SHBG biomarker in Viet Nam should precede its broad clinical use.

Conclusion

Despite an ever-increasing number of prediction models for pregnancy outcomes, very few are externally validated. Consequently, most published models are never used in clinical practice [41]. Arguably, it is as important to understand the implications of implementing of a positive (or negative) test [42], particularly when considering use in resource-limited settings. For Viet Nam, any health and financial benefits of prediction will need to outweigh the costs of the test and resources needed for treating those identified at high-risk – including those who go on to deliver at term (false positives). IGFBP4/SHBG could be useful in stratifying women to target specialized clinical care. Such benefits and costs, including the efficacy of lower-cost approaches, should be the focus of ongoing research.

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Ethics approval

Disclosure statement

Contribution to authorship

Study conceptualization was by JEH, JJB, DEH, SK, LQT. Data curation was performed by LPD, VKTT, TTD, BTHN, EK. Formal analysis was performed by JJB, AP, TCF. Funding acquisition was attained by SK. Investigations were performed by JEH JJB, LPD, TCF. Methodology was developed by JEH, JJB, LPD, AP, VKTT, TTD, SK, LQT. Project administration was performed by LPD, VKTT, TTD, BTHN, GT, EK. Project supervision was the responsibility of GT, EK, LQT and JEH. Validation of data was performed by JJB and AP. AP also performed the visualizations of data. Writing of the original draft was by JEH, JJB, AP, ACF, TCF. Writing - reviewing and editing of subsequent drafts was by all authors.

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Data sharing

Data supporting the findings of this study are present in the paper and/or the Supplemental Materials. Additional data related to this paper may be requested from the authors.

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