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Serum multiple cytokines for the prediction of spontaneous preterm birth in asymptomatic women: A nested case-control study

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ABSTRACT

Background: Current biomarkers such as fetal fibronectin and cervical length are accurate predictors of spontaneous preterm birth (sPTB) in women with clinically suspected preterm risk; however, these are not effective for predicting the risk of sPTB in asymptomatic women. Therefore, we performed this study with the objective of determining whether the combinations of specific serum cytokines could accurately predict the sPTB risk in asymptomatic women.

Methods: We conducted a nested case-control study with 129 incident sPTB cases and 258 individually matched controls who participated in an ongoing birth cohort study. The maternal serum levels of the selected 35 cytokines were measured. We evaluated the relationship between the multiple cytokines and sPTB risk using conditional logistic regression and elastic net model.

Results: A panel of cytokines was significantly associated with an increased risk of sPTB. The odds ratio (OR) of sPTB per standard deviation (SD) increase of the predictive model score was 1.57 (95% CI 1.25–1.97) for the cytokines model. The combination of the selected serum cytokines was substantially more effective in predicting the risk for sPTB, as the receiver–operator characteristic curve (AUC) values were 0.546 and 0.559 in the single cytokine model and it improved to 0.642 in the multiple cytokines model ($P_{\text{AUC difference}} = 0.02$ for TNF-α vs. multiple cytokines; $P_{\text{AUC difference}} = 0.05$ for TRAIL vs. multiple cytokines). Moreover, the prediction was more accurate in overweight pregnant women, with an AUC = 0.879.

Conclusions: The current study suggested that the combination of selected serum cytokines can more effectively predict the risk of sPTB in asymptomatic women compared with the use of single cytokine.

1. Introduction

Preterm birth is the major cause of neonatal death worldwide, with approximately 15 million babies born preterm each year [1,2], and it is a heterogeneous phenotype with many biological pathways. Most preterm births are because of the spontaneous onset of labor without a known cause or effective prevention. The identification of risk factors is important for the prediction of spontaneous preterm birth (sPTB); however, the low sensitivities of the current methods, based on the demographic and behavioral risk factors, make it non-effective for the

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identification of the pregnant women who are at increased risk of sPTB. The use of biomarkers for the prediction of sPTB has been widely investigated.

The previous history of sPTB is the single strongest predictor of subsequent sPTB [3,4]. However, it is not applicable for primipara. Several studies have shown that biomarkers such as fetal fibronectin and cervical length are effective for the prediction of sPTB in symptomatic women [5]. These biomarkers have limited accuracy in predicting sPTB in pregnant women without the clinical manifestations of premature delivery. For asymptomatic women, a reliable evaluation of the risk factors of sPTB using biomarkers is beneficial in deciding whether further screening and clinical intervention are required.

Although there are multiple causes underlying sPTB, inflammation is the major risk factor for preterm birth [6,7]. Pregnancy has been considered as a state of special maternal–fetal immune tolerance, and cytokines play an important role in the maintenance of immune homeostasis [8]. An early study of circulating cytokines is promising for the identification of simple blood-based biomarkers for possible clinical use. Several studies have reported the relationship between maternal serum levels of cytokines, including IL-6, IL-8, IL-10, IL-16, IL-17A, TNF-α, MCP-3, IFN-γ, and MMP-9 and sPTB risk [9–12]. Although the previous studies have identified several different biomarkers, no single biomarker can accurately predict the risk of sPTB in asymptomatic women [13].

We conducted a nested case-control study with 129 incident sPTB cases and 258 individually matched controls who participated in an ongoing birth cohort study to identify whether the combination of specific maternal serum cytokines could early predict the onset of sPTB.

2. Methods

2.1. Subjects

This was a nested case-control study using the data of the Guangxi Birth Cohort Study (GBCS). The detailed descriptions of the GBCS have been previously reported [14]. A total of 6203 pregnant women were included at the baseline of the cohort study between July and September 2015. After follow-up through June 2016, 5541 (89.33%) participants were included, whereas 662 (10.67%) participants were excluded as they were lost to follow-up. A total of 206 participants with sPTB (< 37 weeks) were identified among the 5541 eligible participants (exclusion criteria: full-term low birth weight, macrosomia, stillbirth, deformity, multiple pregnancies, and medically premature delivery). In the current study, we focused on 129 participants at < 36 weeks of gestation to decrease the confounder of the last menstrual period. 129 singleton cases of sPTB were matched to 2 control term deliveries by maternal age, GBCS center, gestational age at baseline blood draw, and the gender of the fetus. Thus, a sample of 129 cases and 259 controls constituted the present study. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Number: 2015(028)).

Detailed information on the pregnant women and the infants were obtained from standardized face-to-face interviews and the Guangxi Maternal and Child Health Information System, including demographic characteristics (maternal age, occupation, education level, family income per year, and pre-pregnancy height and weight), lifestyle habits (smoking, alcohol use pre-pegnancy), pregnancy history, pregnancy complications, medication history, menstrual history, infant's gender, Apgar score, the infant's weight, length, and head and chest circumference.

2.2. Cytokines measurements

All the participants provided baseline blood samples that were centrifuged, separated, and stored at −80 °C. Multiplex assays on a Bio-Plex 200 system, Bio-Plex Pro Human Cytokine 8-plex Assay and Bio-Plex Pro Human Cytokine 2 8plx EXP (Bio-Rad Laboratories, Hercules, CA, USA), were used for the measurement of several cytokines including IL-1β, IL-1Rα, IL-2, IL-2Rα, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, Eotaxin, FGF-basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, PDGF-β, MIP-1β, RANTES, TNF-α, VEGF, MIF, TRAIL, MCP-3, TNF-β, and HGF. Cytokine concentrations below the limits of detection (LOD) were estimated with a parametric model-based procedure [15]. Using the measurements among controls for a target cytokine, we applied maximum likelihood approaches to estimate the parameters for the log-normal distribution. A value from the appropriate log-normal distribution was randomly sampled as the input value for each cytokine measurement < LOD. Masked replicate samples (N = 78) were interspersed to evaluate quality control. The intraclass correlation coefficients were > 0.87 (Supplementary Table 1).

2.3. Statistical analyses

We performed descriptive analyses to summarize the population characteristics by using t tests for continuous variables and Chi-square tests for categorical variables. The correlations among the 35 cytokines were calculated by Spearman's rank correlation analysis. We performed the conditional logistic regression analysis to estimate the odds ratios (OR) and 95% confidence intervals (CI) of sPTB, and cytokine concentrations were categorized using control quartiles as cut-points for the analyses of all sPTB. Estimates for trend were performed by modeling the median values of each quartile as a continuous variable.

The elastic net regression model was used to build a multivariate prediction model for the incident sPTB [16]. It is a regularized regression based on the combined penalty of Lasso and Ridge regression penalties [17]. This method aims to improve the prediction accuracy and model interpretation by decreasing over-fitting, and therefore it is ideal for researches that examine the highly correlated variables or rich set of variables. The prediction performance based on the parameters of lambda.min (optimal value of regularization parameter) was assessed. The predictive serum cytokine model scores were calculated as the weighted sum of all variables with weights equal to the regression coefficients according to the predictive models. The ORs of sPTB per standard deviation (SD) increase of predictive model score were computed by logistic regression models.

We performed a 10-fold cross validation to acquire an unbiased and robust estimation of prediction accuracy. In each run, the elastic net approach was utilized to 90% of the samples and the model obtained was utilized to the remaining 10%. The area under the receiver operating characteristic (ROC) curve (AUC) was calculated using the prediction accuracy of cytokine biomarkers for sPTB and the true status of sPTB for each sample. We further compared the prediction accuracy of different prediction models: (1) by BMI (< 18.5, 18.5–23.9, ≥ 24 kg/m^2). (2) by gestational age at sample collection (first trimester: ≤ 13 weeks, second and third trimesters: > 13 weeks). (3) by gestational age at preterm birth (< 34 weeks and ≥ 34 weeks).

Analyses were performed using IBM SPSS software version 22.0 (SPSS Inc. software, Chicago, Illinois, USA) and R version 3.4.2. All the reported *P* values were two-sided, with a significance level of 0.05.

3. Results

3.1. Baseline characteristics

Table 1 presents the demographic and clinical characteristics of 129 sPTB cases and 258 individually matched controls. Except for pre-pregnancy BMI ($P = 0.02$), maternal age, race, family income, educational levels, drinking, smoking, medication use, infant's gender, gestational age at enrollment, and the history of gestation were similar in both groups. The majority of sPTB subtypes were preterm labor (N = 63, 48.8%). Others were preterm prelabor rupture of membranes

Table 1
Characteristics of spontaneous preterm birth (sPTB) cases and matched controls selected from the Guangxi Birth Cohort Study.

Characteristics	Cases (N = 129)	Controls (N = 258)	*P*
	N (%) or mean ± SD	N (%) or mean ± SD	
Maternal age (years)	28.5 ± 5.5	28.3 ± 5.0	0.69
Race			0.93
Han	103 (79.8)	205 (79.5)	
other	26 (20.2)	53 (20.5)	
Pre-pregnancy BMI(kg/m^2)			0.02
Underweight (< 18.5)	34 (26.6)	59 (23.0)	
Normal (18.5–23.9)	73 (57.0)	178 (69.3)	
Overweight (≥24)	21 (16.4)	20 (7.8)	
Missing	1	1	
Family income (¥)			0.48
< 50,000	56 (43.4)	123 (47.7)	
≥50,000	50 (38.8)	84 (32.6)	
Missing	23 (17.8)	51 (19.8)	
Education			0.12
High school or less	81 (62.8)	182 (70.5)	
College or more	48 (37.2)	76 (29.5)	
Alcohol use pre-pregnancy			0.68
Yes	30 (23.3)	65 (25.2)	
No	99 (76.7)	193 (74.8)	
Current or ex-smoker			0.22
Yes	2 (1.6)	1 (0.4)	
No	127 (98.4)	257 (99.6)	
Medication use			0.75
Yes	2 (1.6)	3 (1.2)	
No	127 (98.4)	255 (98.8)	
Infant gender			1.00
Male	72 (55.8)	144 (55.8)	
Female	57 (44.2)	114 (44.2)	
GBCS Center			1.00
Guigang	15 (11.6)	30 (11.6)	
Liuzhou	22 (17.1)	44 (17.1)	
Nanning	13 (10.1)	26 (10.1)	
Qinzhou	26 (20.2)	52 (20.2)	
Wuzhou	10 (7.8)	20 (7.8)	
Yulin	43 (33.3)	86 (33.3)	
Gestational age at sample collection			1.00
≤13 weeks	46 (35.7)	93 (36.0)	
14–27 weeks	74 (57.4)	147 (57.0)	
≥28 weeks	9 (7.0)	18 (7.0)	
Gravid			0.79
Primigravid	39 (29.7)	80 (31.0)	
Multigravid	90 (70.3)	178 (69.0)	
Parity			0.61
Primiparity	79 (61.2)	151 (58.5)	
Multiparity	50 (38.8)	107 (41.5)	
Preterm birth			
< 34 weeks	29 (22.5)	–	–
≥34 weeks	100 (77.5)	–	
sPTB subtype			
PPROM	56 (43.4)	–	–
Preterm labor	63 (48.8)	–	
Missing	10 (7.8)	–	

Abbreviations: BMI, body mass index; GBCS, Guangxi Birth Cohort Study; PPROM, preterm prelabor rupture of membranes.
**P*-value from *t*-test for continuous variables and Chi-square test for categorical variables.

(PPROM) (N = 56, 43.4% of cases), and 10 cases remained unknown (7.8%). In addition, most of the 35 cytokines were correlated with each other (Supplementary Fig. 1).

3.2. Selection of single biomarker and prediction of sPTB

Table 2 shows the adjusted ORs for sPTB according to the quartiles of cytokine concentration. After adjustment for age, BMI (< 18.5, 18.5–23.9, ≥24 kg/m^2), race, medication use, income, and education,

Table 2
Associations between serum levels of selected cytokines and risk of spontaneous preterm birth.

Cytokines	Levels (pg/ml)	Number of cases/ controls	OR* (95%CI)	P_{Trend}
IL-10				0.123
	≤9.64	22/64	1.00 (Reference)	
	9.65–14.50	35/65	2.31 (1.02–5.22)	
	14.51–20.16	38/64	2.74 (1.16–6.51)	
	> 20.16	34/65	2.56 (1.03–6.37)	
MCP-3				0.083
	≤30.95	24/65	1.00 (Reference)	
	30.96–64.32	29/64	1.51 (0.72–3.18)	
	64.33–115.12	39/66	2.60 (1.13–5.98)	
	> 115.12	37/63	2.43 (1.04–5.70)	
TRAIL				0.014
	≤45.83	24/65	1.00 (Reference)	
	45.84–62.17	33/63	1.46 (0.71–2.99)	
	62.18–84.79	24/65	1.20 (0.57–2.53)	
	> 84.79	48/65	2.37 (1.16–4.82)	
TNF-α				0.005
	≤49.21	28/66	1.00 (Reference)	
	49.22–57.74	27/66	1.18 (0.57–2.44)	
	57.75–69.73	30/63	1.64 (0.76–3.55)	
	> 69.73	44/63	2.84 (1.28–6.29)	

Only the statistically significant results for cytokines were presented.
* Computed using conditional logistic regression modeling adjusted for age, BMI (< 18.5, 18.5–23.9, ≥24 kg/m^2), race, medication use, income and education.

the statistically significant associations between the highest levels of cytokine and increased sPTB risk were observed: 4th quartile vs. 1st quartile: OR = 2.56 for IL-10 (95% CI: 1.03–6.37, P_{Trend} = 0.123), OR = 2.43 for MCP-3 (95% CI: 1.04–5.70, P_{Trend} = 0.083), OR = 2.37 for TRAIL (95% CI: 1.16–4.82, P_{Trend} = 0.014), and OR = 2.84 for TNF-α (95% CI: 1.28–6.29, P_{Trend} = 0.005). We did not observe statistically significant associations between sPTB and other serum levels of cytokines (results not shown). We also evaluated the utility of serum levels of TNF-α and TRAIL to predict sPTB and performed an ROC curve analysis. These biomarkers cannot be used separately for the reliable prediction of sPTB with AUC 0.546 and 0.559, respectively (Fig. 1A). Thus, exploring multiple cytokine markers was indicated for this purpose.

3.3. Selection of multiple biomarkers and prediction of sPTB

The coefficients of all the models after running the elastic net model 10 times are given in Table 3. The predictive models were constructed with the selected cytokines by elastic net models. The OR of sPTB per SD increase of predictive model score was 1.57 (95% CI: 1.25–1.97). When the analysis was restricted to a subset with BMI between 18.5 and 23.9, the OR of sPTB per SD increase was 1.63 (95% CI: 1.23–2.16). The OR for subset with BMI < 18.5 was 2.13 (95% CI: 1.31–3.46). The OR for subset with BMI ≥ 24 could not be assessed owing to the limited sample size. When the analyses were categorized by gestational age at sample collection, the ORs of sPTB per SD increase were 2.22 (95% CI: 1.58–3.13) for early trimester and 1.95 (95% CI: 1.51–2.51) for the second and third trimester, respectively. In analyses stratified by gestational age at preterm birth, increase in the cytokine predictive scores were associated with the OR of 3.04 (95% CI: 1.86–4.97) for before 34 weeks and 1.53 (95% CI: 1.20–1.94) for preterm birth after 34 weeks.

To evaluate the predictive performance of the established models, ROC curve analyses were conducted (Fig. 1). The AUCs were 0.546 and 0.559 in the single cytokine model and significantly improved to 0.642 in the multiple cytokines model, which was estimated based on the 10-fold cross-validation (*P* for AUC difference = 0.02 for TNF-α vs.

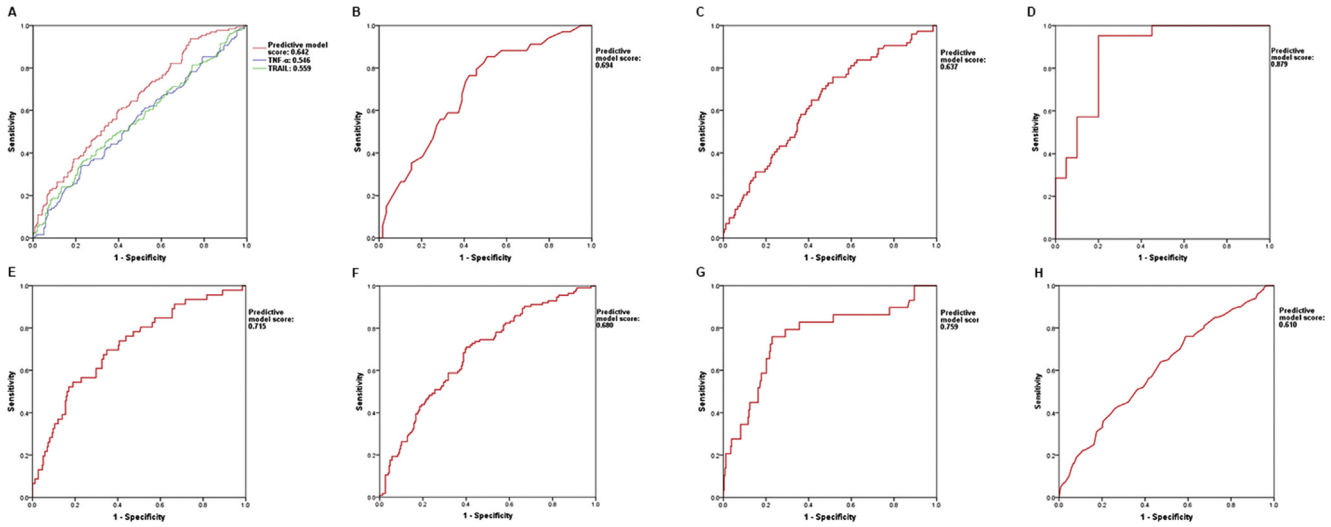

Fig. 1. ROC curves of the corresponding cytokines (selected by elastic net model) to predict sPTB in different models. (A) All individuals (129 sPTB cases and 258 term controls). (B) Individuals with BMI < 18.5 kg/m^2 (34 sPTB cases and 59 term controls). (C) Individuals with 18.5 ≤ BMI ≤ 23.9 kg/m^2 (74 sPTB cases and 179 term controls). (D) Individuals with BMI ≥ 24 kg/m^2 (21 sPTB cases and 20 term controls). (E) Individuals with gestational age ≤ 13 weeks at sample collection (46 sPTB cases and 93 term controls). (F) Individuals with gestational age > 13 weeks at sample collection (83 sPTB cases and 165 term controls). (G) Individuals with preterm birth before 34 weeks (29 sPTB cases and 258 term controls). (H) Individuals with preterm birth after 34 weeks (100 sPTB cases and 258 term controls).

multiple cytokines; *P* for AUC difference = 0.05 for TRAIL vs. multiple cytokines). The combinations of cytokines determined in the multiple cytokines model were IL-8, IL-9, IL-10, IL-13, IL-18, Eotaxin, IP-10, TNF-α, VEGF, TRAIL, MCP-3, TNF-β, and HGF. Next, the analysis was restricted to subsets stratified by BMI, gestational age at sample collection, and gestational age at preterm birth. The multiple cytokines had an AUC of 0.694, 0.637, and 0.879 for the prediction of sPTB in BMI < 18.5, BMI 18.5–23.9, and BMI ≥ 24, respectively. The combinations of cytokines with IL-9, IL-10, IL-13, Eotaxin, IP-10, TNF-α, VEGF, and TRAIL performed the best in women (BMI ≥ 24). In subanalyses with sample collection, the AUC of multiple cytokines performed better for the samples collected at ≤13 weeks than those at > 13 weeks (AUC, 0.715 vs. 0.680), and the combinations of cytokines were IL-8, IL-9, IL-13, Eotaxin, IP-10, VEGF, TRAIL, MCP-3, TNF-β, and HGF. Furthermore, certain cytokine such as IL-8 presented inverse association between the two models. When the subjects were stratified by preterm gestational age, the better performance was observed for the preterm < 34 week, AUC = 0.759. The combinations were IL-8, IL-9, IL-10, Eotaxin, TNF-α, VEGF, MCP-3, TNF-β, and HGF.

4. Discussion

In this nested case-control study, we have investigated the association between a panel of cytokines and the risk of sPTB, and found that these cytokines have improved prediction accuracy for incident sPTB compared with the single cytokine. Moreover, the predictions of sPTB were accurate in overweight pregnant women and in the early trimester, and it was also effective for the identification of

Table 3
Selected models and risk of spontaneous preterm birth.

Cytokines	All	BMI			Gestational age at sample collection		Preterm birth	
	(N = 129/258)	< 18.5 (N = 34/59)	18.5–23.9 (N = 74/179)	≥24 (N = 21/20)	≤13 weeks (N = 46/93)	> 13 weeks N = (83/165)	< 34 weeks N = (29/258)	≥34 weeks N = (100/258)
	Coefficients	Coefficients	Coefficients	Coefficients	Coefficients	Coefficients	Coefficients	Coefficients
IL-8	0.0193	–	0.1092	–	−0.1966	0.1426	0.1260	–
IL-9	0.0055	–	0.0202	0.0809	0.0381	0.0353	0.0672	–
IL-10	0.0461	–	0.1935	0.0176	–	0.0728	0.1263	–
IL-13	0.0100	–	–	0.6731	0.2976	0.0910	–	–
IL-18	0.0252	–	0.1012	–	–	0.1413	–	–
Eotaxin	−0.1205	–	−0.1180	−0.9015	−0.2279	−0.2739	−0.4479	–
IP-10	0.0943	–	0.0819	0.4476	0.0908	0.2095	–	0.0795
TNF-α	0.1431	0.1403	0.0342	0.8605	–	0.1813	0.5624	–
VEGF	−0.1893	–	−0.3526	−1.0247	−0.5622	−0.4646	−0.2342	−0.0896
TRAIL	0.1566	0.2724	0.0287	0.4606	0.3840	0.2387	–	0.1795
MCP-3	0.1367	–	0.2106	–	0.2134	0.2142	0.3579	–
TNF-β	−0.0822	−0.1267	−0.1044	–	−0.0057	−0.2438	−0.1001	–
HGF	−0.0147	–	–	–	−0.0127	−0.0846	−0.0427	–
Number of included -predictors	13	3	11	8	10	13	9	3
OR (95%CI)*	1.57 (1.25–1.97)	2.13(1.31–3.46)	1.63(1.23–2.16)	#	2.22 (1.58–3.13)	1.95 (1.51–2.51)	3.04 (1.86–4.97)	1.53(1.20–1.94)

The cytokines were not to be selected in the corresponding predictive models.

* The predictive model scores were computed as the weighted sum of all covariates with weights equal to the regression coefficients from the predictive models built by the elastic net regression model. OR, per SD increase of predictive model score computed using logistic regression.

\# The ORs could not be calculated due to the small sample sizes.

asymptomatic pregnant women who are at increased risk for sPTB before 34 weeks.

In terms of single cytokine, the findings of top predictors (TNF-α and TRAIL) derived from the elastic model were consistent with the findings from conditional logistic regression model. According to the findings from conditional logistic regression models, TNF-α and TRAIL might play a causal role in sPTB, as evident from the observed dose-response reaction across the quartiles (Table 3). TNF-α, being a cell signaling protein, is involved in systemic inflammation and is one of the cytokines that constitute the acute phase response. Inflammation at the maternal–fetal interface is well defined because of sPTB [18,19]. Systemic or local infection can trigger a cascade of events resulting in preterm labor [19,20]. Previous studies have suggested that a severe inflammation is incompatible with a normal pregnancy [21–23]. Abnormal increases in pro-inflammatory cytokines increase the risk of preterm labor [24–26]. Excessive cytokines can accelerate preterm delivery by triggering uterine contractions and activating cervical ripening [27,28]. Brou et al. found that higher concentration of TNF-α in African-American women contributed to the higher incidence of sPTB compared to the Caucasian women who were presented with a low concentration of TNF-α [29]. Mbongo et al. [10] reported that the women who delivered preterm had significantly higher levels of TNF-α compared with the women who delivered at term. Moreover, a murine model of lipopolysaccharide -induced preterm birth has shown that anti-TNF-α therapy might decrease fetal deaths and preterm labor [30]. TRAIL is a member of the TNF family. It can trigger apoptotic cell death by binding to certain death receptors. In our study, the elevated level of TRAIL was associated with sPTB risk. But the exact mechanism is unclear. Apoptosis plays an important role in the normal development of placenta, and an imbalance between proliferation and apoptosis of villous trophoblast is associated with abnormal pregnancy [31,32]. Studies suggested that elevated expression of inflammatory cytokines such as TNF-α, increased activity of proteases, dissolution of extracellular matrix components, and apoptosis are involved in the abnormal pregnancy process [33,34]. As a tumor necrosis factor-related apoptosis-inducing ligand, the potential pro-apoptotic effects support the hypothesis that TRAIL may increase the risk of sPTB.

Despite the significant associations between TNF-α and TRAIL in our results, the use of a single top cytokine in predicting sPTB is limited, and the previous study using the specific candidate cytokines failed to accurately predict the risk for sPTB in asymptomatic women, with AUC ranged from 0.48 to 0.54 for IL-2, IL-6, TNF-α, GM-CSF, and IFN-g [35]. The differences in study design and statistical analyses can explain, to some extent, the discrepancy in results. The major cause was that the single cytokine failed to accurately predict sPTB. Although the unique biomarker such as fetal fibronectin has an overall accuracy in identifying the risk for sPTB, the systematic review suggested that further researches in different subset of women (symptomatic or asymptomatic) and at different gestational age are required to determine whether the combination of biomarkers can improve the prediction of sPTB [36]. In our study, introducing multiple cytokines to the predictive models considerably improved the predictive ability for sPTB than single risk factors such as TNF-α or TRAIL selected by conditional logistic regression. Cytokines with diverse functions operate in a complex, tightly regulated network, which control the immune system function. In addition, multiple cytokines network can accurately predict the risk for sPTB than the single cytokine. As sPTB is a physiologically heterogeneous syndrome, it is linked to several molecular inflammatory mechanisms, which include cytokines and contraction-associated proteins [37].

Even with the relatively small sample size of the BMI categories, some interesting associations between multiple cytokines and maternal BMI were observed. Obvious differences were found in the effect of cytokine levels on sPTB depending on the level of pre-pregnancy BMI. AUC performance was better in the overweight women than in the normal and underweight women. Physiologically, pro-inflammatory cytokines such as TNF-α levels positively correlate with BMI [38,39]. Jelliffe-Pawlowski et al. found that combined elevated TNF-α and hyperlipidemia result in early PTB, suggesting the potential role of obesity [40]. Although few studies have investigated the relationship between BMI, serum cytokines, and sPTB [41], the effect of BMI on cytokine levels could partially explain the improved predictive ability with BMI stratification. Our findings indicate that selected multiple cytokines are a useful marker for the detection of sPTB in overweight pregnant women.

For the early prediction of sPTB in asymptomatic pregnant women, we investigated multiple cytokine patterns stratified on gestational age at sample collection with early, second, and third trimesters. Consequently, the AUC improved for the samples collected in the first trimester than those in second and third trimesters. Interestingly, we found that certain cytokines presented an inverse association in two models: for first trimester, IL-8 was negatively associated with sPTB, whereas for second and third trimesters, it was positively associated with sPTB. It suggested that the effect of certain cytokines dynamically changes during pregnancy. To date, only a few studies have investigated the longitudinal changes in the peripheral cytokines of sPTB during pregnancy [42]. Moreover, our findings were consistent with the previous studies. Both preterm and term labor have a “common pathway” of labor: increased uterine contraction, cervical dilatation, and chorioamniotic membranes rupture [43]. The switch of the myometrium from a quiescent to a contractile status is accompanied by the shift of the signal from anti-inflammatory to pro-inflammatory pathways, which include cytokines [37]. The next step involves the investigation of the upstream factors that cause the shift. We have also focused on the relationship between preterm birth and gestational age, and found that the markers are more predictive of preterm birth before 34 weeks’ gestation (marked by considerable neonatal morbidity) than the subsets preterm birth after 34 weeks’ gestation. Therefore, the multiple cytokines may be a useful biomarker to identify asymptomatic pregnant women at risk of more extreme sPTB, and also to determine whether further screening and clinical intervention are required.

An important strength of our study is the use of an elastic net regression model to select the variable and construct the model, which performed ideally in terms of both predictive accuracy and sparsity for the high-dimensional datasets [16]. As a result, the apparent predictive effect of multiple cytokine combinations was determined. In addition, maternal cytokines confer racial difference in the risk of sPTB. To our knowledge, our study was the first (in Chinese) to investigate the relationship between multiple cytokines and sPTB risk. Further studies on gene regulation may help to elucidate the ethnic disparity of sPTB [44,45].

This study also has some limitations. First, the measurements of some cytokines such as IL-2, IL-10, IL-15, GM-CSF, and MCP-1 were below the LOD for 40% of all the samples, which can be attributed to degradation. We have only compared the samples from the cases and controls from whom blood was drawn at about the same time, and the samples were stored under the same conditions for the same time. Thus, it is reasonable to assume that the level of degradation of the cytokine is the same. Moreover, the results were reliable as the cytokine levels were categorized into quartiles for analysis, and hence relative (but not absolute) concentrations were compared. It has been assumed that the rate of change in concentration does not differ between cases and controls, and therefore the resulting misclassification bias will probably be non-differential and hence not influence the observed associations. Second, although we have noted the importance of the gestational age at sampling, the collection of blood samples at multiple time points during pregnancy to examine the longitudinal changes in cytokine levels may not be feasible for an exploratory study. Third, our analysis failed to control the possible influence of air pollution, as air pollution might have influenced the serum cytokine expression through systemic inflammation [46]. In addition, we did not exclude the five women who were taken anti-inflammatory drugs or steroids for three months

immediately before inclusion in the study, as the effect of medication on serum cytokine levels might be limited after a certain time. We have also adjusted the potential confounder in our analysis. Finally, the sample size was relatively small. To overcome these shortcomings, we performed 10-fold cross-validation, for the assessment of the stability of the models to evaluate the potential generalization of these results to other datasets and also as a final forward classification step to avoid the overfitting of the predictive models. Even though our findings were internally cross-validated, it is crucial to independently validate in different cohorts.

5. Conclusion

The current study found that the combination of selected serum cytokines are effective for the prediction of sPTB in asymptomatic women when compared with the single cytokine, suggesting the potential use of multiple cytokines as the early predictors in sPTB risk assessment. The predictions were particularly accurate in overweight pregnant women and those in the early trimester. Multiple cytokines can accurately identify the asymptomatic pregnant women who are at increased risk of sPTB before 34 weeks of gestation, which is beneficial in planning further preventive screening and clinical intervention.

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Declarations of interests

None.

CRediT authorship contribution statement

Lulu Huang: Software, Methodology, Writing - original draft, Writing - review & editing. **Qingzhi Hou:** Formal analysis, Methodology, Software, Writing - original draft, Writing - review & editing. **Yaling Huang:** Investigation, Formal analysis, Methodology. **Juan Ye:** Investigation. **Shengzhu Huang:** Formal analysis, Methodology. **Jiarong Tian:** Investigation. **Ruiqiang Tang:** Formal analysis, Methodology. **Chaoqun Liu:** Writing - review & editing. **Yu Long:** Writing - review & editing. **Xiaolian Qin:** Investigation. **Xunjin Weng:** Investigation. **Yifeng Huang:** Investigation. **Mujun Li:** Conceptualization, Validation. **Xiaobo Yang:** Conceptualization, Validation, Project administration, Supervision, Data curation, Writing - review & editing. **Zengnan Mo:** Conceptualization, Validation, Supervision, Data curation, Writing - review & editing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.02.007.

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