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Integration of Cervical Length, Inflammatory Marker, and Vaginal Biomarkers (PAMG-1 and fFN) in the Diagnosis of Threatened Preterm Labor

Jianfeng Lu, Juefei Lu, Xiaoli Zhang, Ling Mu, and Weiming Luo

Department of Obstetrics and Gynecology, Tongxiang Maternal and Child Health Hospital, Tongxiang, Zhejiang, China

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ABSTRACT

The aim of this research was to evaluate the diagnostic efficacy of integrating cervical length (CL), interleukin-6 (IL-6), placental alpha microglobulin-1 (PAMG-1), and fetal fibronectin (fFN) in predicting preterm birth among pregnant women with threatened preterm labor (TPL).

This study retrospectively analyzed clinical data from 150 pregnant women admitted for TPL between January 2021 and December 2024. Participants were divided into two groups based on pregnancy outcome: full-term delivery (n=85) and preterm birth (n=65). Additionally, 100 healthy pregnant women with no history of adverse pregnancy outcomes who underwent routine prenatal examinations during the same period were selected as the healthy control group. All participants underwent transvaginal ultrasound to measure CL, and venous blood samples were collected to assess serum IL-6 levels. PAMG-1 and fFN levels were measured in vaginal secretions.

There were no significant differences in baseline characteristics among the three groups. However, significant differences in CL, serum IL-6 levels, and positive rates of PAMG-1 and fFN were detected. Pearson correlation analysis showed significant associations between CL, IL-6, PAMG-1, fFN, and preterm birth. ROC curve analysis indicated that the AUC values for CL, IL-6, PAMG-1, and fFN alone were 0.798, 0.803, 0.753, and 0.754, respectively.

The combined application of these markers yielded an AUC of 0.920, significantly higher than any single marker. The combined use of CL, IL-6, PAMG-1, and fFN significantly enhances the diagnostic accuracy of preterm birth in patients with TPL.

Keywords: Biomarkers; Cervical length measurement; Human FFN protein; Interleukin-6; Placental hormones; Premature obstetric labor

INTRODUCTION

Preterm delivery, characterized as childbirth occurring prior to 37 weeks of pregnancy, continues to be a major global challenge in obstetrics.[1] The incidence of preterm birth has been increasing annually. According to the latest report from the WHO and UNICEF, the global preterm birth rate in 2020 was approximately 10%, meaning that about 1 in 10 newborns were born prematurely.[2] Preterm delivery is the primary contributor to adverse outcomes and fatalities during the perinatal period. It increases the risk of NICU admission, bronchopulmonary dysplasia,

Corresponding Author: Jianfeng Lu, MD;
Department of Obstetrics and Gynecology, Tongxiang Maternal and Child Health Hospital, Tongxiang, Zhejiang, China.
Tel/Fax: (+86 188) 5832 3956, Email: 18858323956@163.com

intraventricular hemorrhage, and necrotizing enterocolitis in the perinatal period, and children born prematurely also have significantly increased risks of cardiovascular diseases, metabolic disorders, and malignancies during adulthood.[3-5]

The cervical length (CL) serves as a vital parameter for evaluating cervical competence and estimating the likelihood of preterm delivery.[6] Numerous studies have consistently demonstrated that a shorter CL is associated with a greater risk of preterm birth. For example, a CL≤25 mm significantly increases the risk of preterm delivery, with the risk approaching 50% when the CL is≤15 mm.[7,8] Furthermore, the normal CL before 24 weeks of gestation is typically≥30 mm, and values below this threshold are considered high risk.[9] Transvaginal ultrasound is regarded as the gold standard for measuring the CL because of its high image clarity, reproducibility, and sensitivity.[10] In contrast, transabdominal or transperineal ultrasound methods yield lower image quality and sensitivity.[11] As a result, transvaginal ultrasound is widely recommended for assessing preterm birth risk. For expectant individuals exhibiting signs of impending preterm delivery, dynamic monitoring of the CL is of significant clinical importance. A decrease in CL signifies a heightened likelihood of preterm delivery, necessitating timely interventions to mitigate this risk.

The detection of vaginal biomarkers has emerged as a significant approach for assessing preterm birth risk in contemporary research. The fetal fibronectin (fFN) is a glycoprotein situated at the junction between the fetal membranes and the decidual tissue. Under normal circumstances, fFN is absent after 20 weeks of gestation.[12] However, when a pregnant woman exhibits signs of preterm labor, fFN is released into the cervicovaginal secretions from the choriodecidual space, making it a useful marker for predicting preterm birth.[13] The fFN test demonstrated a robust negative predictive value ranging from 97% to 99%, suggesting that a negative outcome strongly implies a minimal probability of preterm delivery occurring within the subsequent week. However, its positive predictive value is relatively low, limiting its utility as a standalone predictor of preterm birth.[14] Placental alpha microglobulin-1 (PAMG-1), a protein present in amniotic fluid and fetal membranes, leaks into cervicovaginal secretions when there is membrane rupture or a heightened likelihood of premature delivery.[15] Research has demonstrated that PAMG-1 displays superior positive predictive value and a positive likelihood ratio relative to fFN, enhancing its precision in forecasting delivery within a week among individuals experiencing symptoms of impending preterm labor. Furthermore, the PAMG-1 test eliminates the need for a speculum, is straightforward to administer, and provides results in under 5 minutes.[16] Although the use of fFN or PAMG-1 alone has limitations, combining these biomarkers can significantly improve the accuracy of preterm birth prediction.

Laboratory markers, such as interleukin-6 (IL-6), also have predictive value for threatened preterm labor (TPL). IL-6 exerts its effects through cell surface receptors.[17] During pregnancy, IL-6 is produced by the amnion, chorion, and placental trophoblasts. It is present in the uterus, placenta, and amniotic fluid after the second trimester, where it is maintained at low levels. However, its concentration significantly increases before the commencement of labor.[18] IL-6 enhances the effects of colony-stimulating factor (CSF) and IL-3 in stimulating placental growth, making it essential for fetal development and facilitating delivery at appropriate concentrations.[19] IL-6, in conjunction with other intrauterine growth factors such as CSF and IL-3, also directly or indirectly supports the growth of hematopoietic stem cells, ensuring normal fetal growth and development.[19] Additionally, IL-6 is a physiological component of amniotic fluid, with low concentrations being necessary for fetal and placental growth.[20] Recent studies on the mechanisms of labor have revealed that IL-6 participates in multiple steps of arachidonic acid metabolism through autocrine or paracrine pathways. This process stimulates uterine tissues to synthesize prostaglandins, which contribute to the onset and progression of labor.[21,22]

Therefore, this study conducted a retrospective analysis of the clinical data of 150 pregnant women with TPL and 100 healthy pregnant women, aiming to explore the value of the combined application of CL, IL-6, PAMG-1, and fFN in diagnosing the risk of preterm delivery in pregnant women with TPL.

MATERIALS AND METHODS

Research Participants

A retrospective review of the medical records of 150 pregnant women diagnosed with impending preterm delivery who were hospitalized at our institution between January 2021 and December 2024 was

performed. The diagnostic criteria for TPL were as follows: gestation between 28 and 37 weeks; a uterine size consistent with the gestational age; minimal vaginal bleeding; the presence of low back pain or intermittent lower abdominal pain; and regular uterine contractions accompanied by progressive cervical shortening but with an unopened cervical os upon pelvic examination.

The inclusion criteria were as follows: complete and authentic medical records and imaging examination results; participants were required to have clear consciousness, normal cognition, and the ability to communicate effectively with health care providers; singleton pregnancy; regular menstrual cycle; no history of using specific medications (e.g., corticosteroids, immunosuppressants) use during pregnancy or before the blood tests; and normal amniotic fluid volume, normal placental position, and normal fetal morphology.

The exclusion criteria were as follows: oligohydramnios or polyhydramnios; abnormal placental position or fetal morphology; presence of severe organic lesions in vital organs; comorbidities such as hepatitis B or tuberculosis; history of cervical surgery; history of mental illness; presence of autoimmune or inflammatory diseases such as rheumatologic disorders, infections, diabetes, etc.; and poor cooperation during examinations.

The enrolled pregnant women were divided into a full-term delivery group (85 cases) and a preterm delivery group (65 cases) on the basis of their pregnancy outcomes. Additionally, 100 pregnant women with normal prenatal check-up results during the same period, who were at 28 to less than 37 weeks of gestation and receiving prenatal care at this hospital, were randomly selected as the healthy control group, with exclusion of those having a history of adverse pregnancy and childbirth.

Research Methods

CL testing

All pregnant women underwent transvaginal ultrasound examination to measure CL. Health care providers communicated with the pregnant women and their families in advance, informing them of the procedure, precautions, cooperation methods, purpose, and significance of the transvaginal ultrasound examination. This was done to ensure that the pregnant women understood the necessity and importance of the examination, thereby improving their cooperation during the process. Before the transvaginal ultrasound examination, the pregnant woman was instructed to empty her bladder. She was then positioned in the lithotomy position on the examination bed. A transvaginal ultrasound probe, covered with a sterile sheath and coated with coupling gel, was gently inserted into the anterior fornix of the vagina, taking care to avoid applying pressure to the cervix. The image was adjusted to display the midsagittal section of the cervix, clearly showing the internal os, external os, cervical canal, and mucosal layer, ensuring that the width of the anterior and posterior cervical lips was consistent. The straight-line distance from the internal os to the external os was measured. Owing to the dynamic changes in the cervix, measurements were taken at least three times within three minutes, and the shortest value was recorded. During the examination, care was taken to minimize pressure on the cervix and to avoid a full bladder or excessive pressure, which could elongate the cervix and lead to inaccurate measurements.

IL-6 Detection

Five milliliters of peripheral venous blood were collected and placed into EDTA anticoagulant tubes. After mixing thoroughly for 10–20 minutes, the tubes were stored in a refrigerator at 4 °C and then centrifuged at 2500 rpm for 20 minutes. The dual-antibody sandwich technique (IL6 kit from Thermo Fisher Scientific, USA) was employed to quantify the concentration of IL-6 in the human serum samples.

PAMG-1 Detection

PAMG-1 in vaginal secretions was measured (AmniSure PAMG-1 rapid immunoassay kit, USA). The procedure was as follows: the pregnant woman was positioned in the lithotomy position, and after the cervix was fully exposed with a speculum, a sterile swab was placed in the vaginal fornix for 10–15 seconds to collect vaginal secretions. Immediately afterward, the collected sample was quickly transferred to a reagent bottle, and the white area of the test strip was fully immersed in it. After 10 minutes, the test strip was removed, and the results were read. If both the control line and the reaction line appeared, the result was considered positive (PAMG-1≥5 ng/mL); if only one line appeared, it was judged as negative.

fFN Detection

The standardized procedure for collecting vaginal secretions (referencing the PAMG-1 testing steps) was

followed. The specific operations included properly placing a sterile swab inside the vagina to collect the sample and then immediately immersing the swab in a buffer solution. Next, an fFN rapid test strip (ADEZA, USA) was used to detect fFN in the sample. The criteria for interpreting the test results were as follows: if the test strip showed two lines (fFN≥50 ng/mL), it was judged as positive; conversely, if only one line appeared, it was judged as negative.

Statistical Analysis

Statistical analysis was performed with SPSS 25.0. Normally distributed continuous data are expressed as $\bar{x} \pm s$ and were analyzed by one-way ANOVA and LSD-t tests. Categorical data are shown as n (%) and were assessed by the χ^2 test. Pearson correlation was used to analyze significant indicators, and ROC curves were used to evaluate diagnostic performance; AUC was subsequently calculated. For the combined application of CL, IL-6, PAMG-1, and fFN, a logistic regression model was employed to integrate these biomarkers for predicting preterm birth. $p<0.05$ indicated significance.

RESULTS

Comparison of General Characteristics

There were no statistically significant differences in age, BMI, gestational week, number of pregnancies, or deliveries among the three groups of pregnant women (all $p>0.05$). See Table 1.

Comparison of CL

The CL in the preterm delivery group was 21.86 ± 2.88 mm, that in the full-term delivery group was 25.47 ± 3.41 mm, and that in the healthy control group was 33.67 ± 3.35 mm. There was a statistically significant difference among the three groups ($p<0.05$); see Table 2.

Comparison of Serum IL-6 Levels

The serum IL-6 level in the preterm delivery group was 85.76 ± 15.41 pg/mL, that in the full-term delivery group was 72.87 ± 10.15 pg/mL, and that in the healthy control group was 43.87 ± 14.15 pg/mL. There was a statistically significant difference among the three groups ($p<0.05$); see Table 3.

Comparison of the Positive Rate of PAMG-1 and fFN in Vaginal Secretions

The positive rate of PAMG-1 in the preterm delivery group was 75.38% (49/65), that in the full-term delivery group was 24.71% (21/85), and that in the healthy control group was 0.00% (0/100). There was a statistically significant difference among the three groups ($p<0.05$). The positive rate of fFN in the preterm delivery group was 90.78% (59/65), that in the full-term delivery group was 40.00% (34/85), and that in the healthy control group was 0.00% (0/100). There was a statistically significant difference among the three groups ($p<0.05$). See Table 4.

Table 1. Comparison of general characteristics among the preterm delivery group, full-term delivery group, and healthy control group ($\bar{x} \pm s$)

Groups	n	Age, y	BMI, kg/m^2	Gestational age, weeks	Number of pregnancies, times	Number of deliveries, times
Preterm delivery group	65	28.69 ± 2.22	23.66 ± 2.65	33.42 ± 2.14	1.54 ± 0.51	1.53 ± 0.51
Full-term delivery group	85	28.94 ± 2.32	23.46 ± 2.19	33.51 ± 2.41	1.59 ± 0.62	1.58 ± 0.59
Healthy control group	100	29.11 ± 2.41	23.57 ± 2.74	33.77 ± 2.23	1.74 ± 0.53	1.62 ± 0.57
F		0.669	0.130	0.564	1.547	0.222
p		0.513	0.879	0.570	0.217	0.802

BMI: body mass index

Table 2. Comparison of CL among the preterm delivery group, full-term delivery group, and healthy control group ($\bar{x} \pm s$, mm)

Groups	n	CL
Preterm delivery group	65	21.86 ± 2.88
Full-term delivery group	85	25.47 ± 3.41
Healthy control group	100	33.67 ± 3.35
F		292.169
p		<0.001

CL: cervical length

Table 3. Comparison of serum IL-6 levels among the preterm delivery group, full-term delivery group, and healthy control group ($\bar{x} \pm s$, pg/mL)

Groups	n	IL-6
Preterm delivery group	65	85.76 ± 15.41
Full-term delivery group	85	72.87 ± 10.15
Healthy control group	100	43.87 ± 14.15
F		207.200
p		<0.001

IL-6: interleukin-6

Table 4. Comparison of positive rate of PAMG-1 and fFN in vaginal secretions (n, %)

Groups	n	PAMG-1		fFN	
		Positive	Negative	Positive	Negative
Preterm delivery group	65	49	16	59	6
Full-term delivery group	85	21	64	34	51
Healthy control group	100	0	100	0	100
χ^2		111.740		139.365	
p		<0.001		<0.001	

fFN: fetal fibronectin; PAMG-1: placental alpha microglobulin-1

Pearson Correlation Analysis

Table 5 presents the correlations among changes in the CL and the IL-6, PAMG-1, and fFN levels and the occurrence of preterm birth in patients with TPL through Pearson correlation coefficient analysis. The results revealed that CL (r=-0.486, *p*=0.000), IL-6 (r=0.520, *p*=0.000), PAMG-1 (r=0.494, *p*=0.000), and fFN (r=0.494, *p*=0.000) were significantly correlated with the occurrence of preterm birth in patients with TPL.

The Diagnostic Efficacy of Each Index for Preterm Birth

ROC curve analysis revealed that the AUCs for predicting preterm birth using CL, IL-6, PAMG-1, and fFN were 0.798 (95% CI=0.725-0.859), 0.803 (95% CI=0.730-0.863), 0.753 (95% CI=0.676-0.820), and 0.754 (95% CI=0.677-0.820), respectively, indicating moderate predictive accuracy. The AUC for the combined use of CL, IL-6, PAMG-1, and fFN was 0.920 (95% CI=0.864–0.958), demonstrating significantly greater predictive accuracy than any single indicator.

These findings suggest that the combination of CL, IL-6, PAMG-1, and fFN can provide a more accurate prediction of whether preterm birth will occur.

Using the maximum cutoff point of the Youden index, located near the upper left corner of the ROC curve, as the optimal threshold, the optimal cutoff values were 24.23 mm for CL and 76.92 pg/mL for IL-6. The predictive sensitivity and specificity were 83.08% and 67.06% for CL, 76.92% and 78.82% for IL-6, 75.38% and 75.29% for PAMG-1, 90.77% and 60.00% for fFN, and 87.69% and 82.35% for their combined application. These results suggest that the combined application of these factors can improve the predictive accuracy over that of any of the individual indicators (see Table 6 and Figure 1).

Table 5. Correlation analysis between changes in CL, IL-6, PAMG-1, and fFN levels and the occurrence of preterm birth in patients with TPL

	CL	IL-6	PAMG-1	fFN
Pearson r	-0.486	0.520	0.494	0.494
p	<0.001	<0.001	<0.001	<0.001

CL: cervical length; fFN: fetal fibronectin; IL-6: interleukin-6; PAMG-1: placental alpha microglobulin-1; TPL: threatened preterm labor.

Table 6. Diagnostic value of CL, IL-6, PAMG-1, and fFN testing for predicting preterm birth in patients with TPL

Indicator	AUC	95% CI	Sensitivity, %	Specificity, %	Youden's Index	Best Cut-off Value	*p*
CL	0.798	0.725-0.859	83.08	67.06	0.501	≤24.23	<0.001
IL-6	0.803	0.730-0.863	76.92	78.82	0.558	>76.92	<0.001
PAMG-1	0.753	0.676-0.820	75.38	75.29	0.507	-	<0.001
fFN	0.754	0.677-0.820	90.77	60.00	0.508	-	<0.001
Joint Application	0.920	0.864-0.958	87.69	82.35	0.701	-	<0.001

AUC: area under the curve; CI: confidence interval; CL: cervical length; fFN: fetal fibronectin; IL-6: interleukin-6; PAMG-1: placental alpha microglobulin-1; TPL: threatened preterm labor

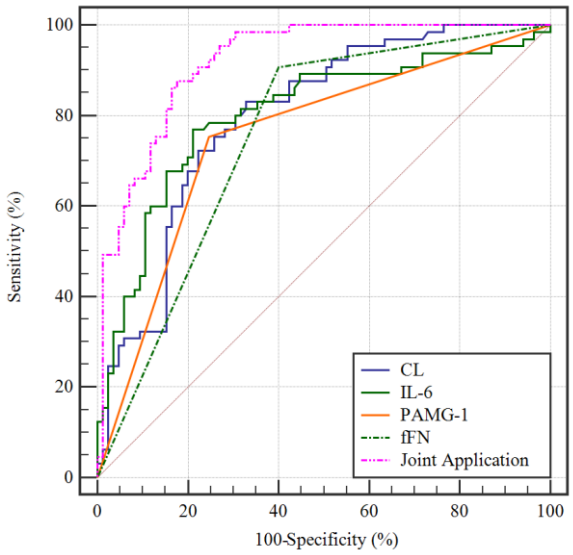

Figure 1. ROC Curves for Predicting Preterm Birth using CL, IL-6, PAMG-1, and fFN

DISCUSSION

Currently, the prediction of preterm birth still lacks specific indicators, and predicting preterm birth remains a clinical challenge. According to public health management protocols, preterm birth management can be categorized into three levels: primary prevention aims at early identification and reduction of preterm birth risk; secondary prevention focuses on screening high-risk populations for preterm birth and implementing early interventions; and tertiary prevention targets pregnant women who have already experienced preterm birth, employing pharmacological treatments and other interventions to increase the survival rate of preterm infants and improve their quality of life.[23] On this basis, exploring specific physicochemical indicators for predicting spontaneous preterm birth and taking effective measures to mitigate the impact of high-risk factors on pregnancy is clinically important. Importantly, premature delivery is the primary contributor to adverse outcomes and fatalities during the perinatal period.[24] Although current therapeutic and nursing interventions have significantly improved the survival outcomes of neonates born prematurely, the development of efficient strategies to prevent preterm delivery continues to be a pressing priority. The results of this study show that the incidence of spontaneous preterm birth in pregnant women with TPL is as high as 43.33%, further underscoring the importance of clinical preterm birth prevention.

During normal pregnancy, the CL is typically maintained above 30 mm, reaching its maximum during the second trimester (approximately 20–25 weeks).[25] However, starting from the late trimester (approximately 28–32 weeks), the cervix may gradually dilate and shorten.[26] This process varies among individuals and may be influenced by multiple factors, including hormonal fluctuations, mechanical uterine distension, and inflammatory responses. In clinical practice, methods for assessing CL primarily include transvaginal digital examination, transabdominal ultrasound, transperineal ultrasound, and transvaginal ultrasound. Traditionally, transvaginal digital examination has been commonly used to evaluate cervical status; however, this method has significant limitations. Digital examination can assess only the vaginal portion of the cervix and fails to accurately measure the CL, cervical contractions, and changes in the internal os.[27] Moreover, digital examination results are highly subjective and lack quantitative precision, and repeated examinations may stimulate uterine contractions, increasing the risk of premature rupture of membranes and preterm birth, thereby adversely affecting tocolytic therapy.[28]

With advancements in ultrasound technology, ultrasound assessment has become the preferred method for evaluating the CL. Ultrasound offers advantages such as noninvasiveness, strong reproducibility, and accurate measurement data. Transvaginal ultrasound, characterized by its simplicity, absence of bladder filling requirements, minimal maternal discomfort, and clear visualization of cervical morphology, has emerged as the most commonly used method for CL assessment.[29] In this study, transvaginal ultrasound measurements were performed on all pregnant women, revealing that the CL in the preterm birth group was 21.86 ± 2.88 mm, that in the full-term delivery group was 25.47 ± 3.41 mm, and that in the healthy control group was 33.67 ± 3.35 mm, with statistically significant differences among the three groups ($p<0.05$). The primary physiological function of the cervix is to maintain pregnancy, ensuring that the fetus is not delivered prematurely before term. However, some pregnant women may experience congenital or acquired cervical insufficiency, rendering the cervix unable to withstand the gradually increasing pressure during pregnancy, leading to its progressive shortening and dilation.[30]

A multitude of factors may contribute to the shortening of the CL. Infections or inflammatory conditions, such as chorioamnionitis, can trigger the release of local cytokines, which promote the remodeling and softening of cervical tissue, thereby leading to a reduction in the CL.[31] Second, factors such as multiple pregnancies, polyhydramnios, or fetal activity can increase the mechanical pressure exerted on the cervix, which similarly results in cervical shortening.[32] Additionally, an imbalance between progesterone and estrogen may affect the metabolism of cervical collagen, leading to cervical softening and subsequent shortening.[33]

In this study, the shortest CL was observed in the preterm birth group (21.86 ± 2.88 mm), indicating that significant structural and functional changes may have occurred in the cervix of these pregnant women, rendering it unable to effectively maintain pregnancy and thereby increasing the risk of preterm birth. The CL

in the full-term delivery group (25.47 ± 3.41 mm), although longer than that in the preterm birth group, was still significantly shorter than that in the healthy control group (33.67 ± 3.35 mm). These findings suggest that some pregnant women may exhibit mild cervical insufficiency without progressing to preterm birth. Despite having a shorter CL, these women were able to maintain pregnancy until term. Furthermore, some pregnant women may have successfully avoided preterm birth through interventions such as progesterone therapy, lifestyle modifications, or other measures. The longest CL was observed in the healthy control group (33.67±3.35 mm), providing important reference value for clinical practice. The typically longer CL in healthy pregnant women indicates an intact cervical structure capable of effectively supporting pregnancy. This finding further validates the reliability of the CL as a predictive indicator for preterm birth.

Additionally, the results of this study revealed that a transvaginal ultrasound-measured CL of≤24.23 mm predicted preterm birth, with an AUC of 0.798 (95% CI=0.725–0.859), a sensitivity of 83.08%, and a specificity of 67.06%. This finding indicates that the CL, as a single indicator, has moderate diagnostic value but is limited by a notable false-positive rate. Therefore, relying solely on the CL for preterm birth prediction may not meet the clinical demand for high diagnostic accuracy. To further increase the accuracy of preterm birth prediction, this study also measured other relevant indicators, including IL-6, PAMG-1, and fFN. These indicators reflect the potential risk of preterm birth from different perspectives, such as inflammatory responses, placental function, and fetal membrane integrity. By combining the analysis of these indicators, a more comprehensive assessment of preterm birth risk can be achieved, addressing the limitations of relying on a single indicator.

Studies have shown that infections or noninfectious inflammatory conditions (such as chorioamnionitis) can stimulate the release of large amounts of IL-6 from maternal or fetal tissues, triggering a cascade of pathophysiological changes. IL-6 promotes the secretion of matrix metalloproteinases (MMPs), accelerating the degradation of cervical collagen and leading to cervical softening, shortening, and dilation, thereby increasing the risk of preterm birth.[34] Additionally, IL-6 stimulates uterine smooth muscle cells to produce prostaglandins (PGs), enhancing uterine contractility and inducing preterm contractions.[35] Furthermore, IL-6 mediates inflammatory responses that weaken the structural stability of fetal membranes, increasing the likelihood of premature membrane rupture.[36]

In this investigation, the serum IL-6 concentrations in the preterm delivery group were markedly elevated compared with those in the full-term delivery group and the healthy control group (p<0.05), further validating the crucial role of IL-6 in preterm birth. ROC curve analysis revealed that IL-6 alone had an AUC of 0.803 (95% CI=0.730-0.863) for the prediction of preterm delivery, with a sensitivity of 76.92% and a specificity of 78.82%, highlighting its substantial diagnostic utility.

During normal pregnancy, fFN can be identified in cervical and vaginal secretions during the initial phases of gestation (before 20 weeks), but their levels are typically low (<50 ng/mL) during the mid-trimester (22–35 weeks), as the chorion and decidua gradually fuse.[37] Between 22 and 35 weeks of gestation, an fFN level of ≥50 ng/mL in cervical secretions often indicates compromised fetal membrane integrity or separation of the chorion from the decidua, which could be linked to a heightened likelihood of preterm delivery.[38] Elevated fFN levels typically reflect changes in the adhesive state between fetal membranes and the uterine wall, which may result from infections, inflammation, or mechanical stress causing microdamage to the membranes, leading to the release of fFN into cervical and vaginal secretions.[39] Moreover, recurrent uterine contractions may increase mechanical friction between the fetal membranes and the uterine wall, leading to fFN release.[40]

In this study, the fFN positive rate in the preterm delivery group was notably greater than that in the full-term delivery group and the healthy control group (p<0.05), further underscoring the predictive value of fFN for preterm birth. ROC curve analysis revealed that fFN alone achieved an AUC of 0.754 (95% CI=0.677-0.820) for predicting preterm delivery, with a sensitivity of 90.77% and a specificity of 60.00%. The high sensitivity of fFN makes it an effective screening tool, yet its relatively low specificity suggests the potential for false-positive results. Therefore, fFN should be used in combination with other indicators for early prediction of preterm birth to enhance diagnostic accuracy. Additionally, clinicians should interpret fFN results in conjunction with other clinical indicators and patient-specific factors to minimize the risk of unnecessary interventions.

Under typical pregnancy conditions, PAMG-1 is found in elevated concentrations within amniotic fluid but in minimal amounts in cervical and vaginal secretions.[41,42] When fetal membranes rupture, PAMG-1 from amniotic fluid can enter vaginal secretions through microscopic defects in the chorion or due to membrane degradation caused by inflammation.[43] Therefore, the detection of PAMG-1 in vaginal secretions can be used to diagnose PROM and serve as a predictive indicator for preterm birth risk. The results of this study revealed that the positive rate of PAMG-1 in the preterm birth group was significantly greater than that in the full-term delivery group and the healthy control group (p<0.05). Additionally, PAMG-1 alone had an AUC of 0.753 (95% CI=0.676-0.820) for the prediction of preterm birth, with a sensitivity of 75.38% and a specificity of 75.29%.

From a clinical application perspective, the combined detection of fFN and PAMG-1 may overcome the limitations of using a single biomarker. For example, a positive fFN result with a negative PAMG-1 result may indicate that fetal membrane integrity has not yet been fully compromised but still suggests an increased risk of preterm birth. Conversely, when both markers are positive, it may indicate a significantly greater risk of preterm birth. Furthermore, the integration of other indicators, such as CL, can further increase the accuracy of preterm birth prediction. The results of this study demonstrated that the combined application of CL, IL-6, PAMG-1, and fFN achieved an AUC of 0.920 (95% CI=0.864-0.958), with a sensitivity of 87.69% and a specificity of 82.35%, indicating significantly greater predictive accuracy than that of individual biomarkers. Our study demonstrates that the combined use of CL, IL-6, PAMG-1, and fFN significantly enhances the diagnostic accuracy of preterm birth in patients with TPL. This integrated approach achieves an AUC of 0.920, which is significantly higher than any single marker or previously studied combinations (e.g., CL + fFN or IL-6 + PAMG-1).[44,45] This comprehensive model provides a more accurate and reliable method for identifying high-risk pregnancies, potentially leading to better clinical outcomes through early intervention. These findings suggest that the combination of CL, IL-6, PAMG-1, and fFN can more accurately predict the likelihood of preterm birth. On the basis of this finding, clinicians should consider incorporating these biomarkers into routine monitoring protocols for pregnant women with risk factors for preterm birth. Through combined detection, high-risk populations can be identified earlier, providing a basis for personalized intervention strategies.

For example, for pregnant women identified as high risk on the basis of combined biomarker results, preventive measures such as bed rest and pharmacological treatments can be implemented to decrease the occurrence of preterm delivery. Additionally, this combined detection model can help optimize the allocation of medical resources by avoiding unnecessary interventions for low-risk pregnancies while ensuring timely and effective management of high-risk cases. Future research directions could focus on exploring the dynamic changes in these biomarkers across different gestational ages to achieve more precise preterm birth prediction. Moreover, the integration of emerging biomarkers and technologies, such as genetic testing and artificial intelligence-assisted diagnostics, holds promise for further improving the efficacy of preterm birth prediction. As combined detection techniques continue to advance and become more widely adopted, their potential to reduce maternal and neonatal complications associated with preterm birth and improve perinatal outcomes will be fully realized, providing robust support for maternal and infant health.

Limitations of the Study

While our study provides valuable insights into the combined use of CL, IL-6, PAMG-1, and fFN in predicting preterm birth, it is important to acknowledge several limitations. First, the optimal cut-off values for these biomarkers were derived from our specific study population and may not be universally applicable. External validation in diverse populations is needed to confirm their generalizability. Second, our study design was retrospective, which may introduce selection bias and limit causal inference. Third, the sample size, although substantial, may not be large enough to capture the full variability in biomarker levels across different clinical scenarios. Fourth, while the combined approach improves accuracy, the cost, accessibility, and feasibility of routinely measuring all four markers (especially in low-resource settings) are not discussed. The practical implementation of this combined approach may face challenges due to the need for specialized equipment and trained personnel, which may not be readily available in all healthcare settings. Future research should address these limitations by conducting prospective, multicenter studies with larger and more diverse cohorts.

Additionally, exploring the dynamic changes in these biomarkers over time could provide more nuanced predictive models. Lastly, the integration of emerging technologies, such as machine learning, could enhance the accuracy and robustness of preterm birth prediction models. Practical considerations, including cost-effectiveness and accessibility, should also be evaluated to ensure the feasibility of implementing these biomarkers in routine clinical practice, particularly in low-resource settings.

This study demonstrated that the management of preterm birth remains challenging, particularly due to the lack of specific predictive biomarkers and limited clinical research. Indicators such as the CL and the proinflammatory cytokines IL-6, fFN, and PAMG-1 each hold value in predicting preterm birth, but their individual application has certain limitations. CL, a critical indicator of cervical function, is closely associated with preterm birth when shortened; abnormal elevations in IL-6 are strongly linked to pathological inflammatory responses during preterm birth; and fFN and PAMG-1 are related to fetal membrane integrity and preterm birth risk. By combining these biomarkers, the accuracy of preterm birth prediction can be significantly improved, achieving an AUC of 0.920, with a sensitivity and specificity of 87.69% and 82.35%, respectively, demonstrating superior predictive performance compared with individual biomarkers. Therefore, clinicians should consider incorporating the CL and IL-6, PAMG-1, and fFN into routine monitoring protocols for pregnant women with risk factors for preterm birth. This approach can facilitate early identification of high-risk populations and provide a basis for personalized intervention strategies.

STATEMENT OF ETHICS

This experiment was approved by Tongxiang Maternal and Child Health Hospital Ethics Committee.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, Jianfeng Lu, upon reasonable request. Please contact via Tel: (+86) 18858323956.

AI ASSISTANCE DISCLOSURE

No AI tools were used in the preparation of this manuscript.

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