Combined Proinflammatory Biomarkers Have Better Predictive Value for Term Labor than Single Markers

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Background: The timing of parturition is an important determinant of labor and delivery care. Early parturition is associated with increased neonatal morbidity and mortality. Most existing studies analyzed a single factor for the initiation of parturition, and the role of multiple factors in initiating parturition has not been comprehensively analyzed.

Material/Methods: We measured the levels of proinflammatory mediators, hypoxia factor, matrix metalloproteinases, hormones, and oxytocin, as well as fetal umbilical blood flow, before and after labor, and their associations with parturition. We also built a statistical model to predict the timing of parturition based on the measurement data.

Results: IL-1β, IL-6, TNF-α, MMP-9, and HIF-1α concentrations significantly increased from full term to labor. The PRL level significantly decreased from full term to parturition. There was no significant change in MCP-1, E3, and OT concentrations from full term to parturition. IL-1β, IL-6, TNF-α, and MMP-9 concentrations were negatively correlated with the initiation of parturition. There was a small but nonsignificant increase in umbilical venous blood flow before parturition. Multiple factors showed a close correlation with the initiation of parturition, and area under the curve analysis showed that a multiple factor model was superior to single factors in the establishment of a model to predict initiation of parturition; however, these results need further confirmation.

Conclusions: Combined proinflammatory biomarkers have better predictive value for term labor than single biomarkers.

MeSH Keywords: **Interleukins • Parturition • Tumor Necrosis Factors**

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Background

Early or late parturition increases morbidity and mortality in newborns, increasing the financial burden on the healthcare system and causing social problems for families [1–3]. Premature infants are prone to respiratory distress syndrome (due to fetal lung immaturity), intracranial hemorrhage, necrotizing minor inflammation, neonatal sclera, and other serious complications and often need to be transferred to a neonatal intensive care unit for treatment. The incidence of other long-term complications that increase neonatal mortality, such as cerebral palsy and visual impairment, is significantly higher in premature infants than in full-term mature infants [4]. A recent study showed that from 2003 to 2008, 33.6–40.9% of neonatal deaths in China were directly or indirectly related to preterm birth. Prolonged pregnancy can also lead to increased neonatal morbidity and perinatal mortality. Studies on the mechanism of labor initiation help find suitable biomarkers to predict and prevent preterm birth, reduce intervention for late pregnancy, and more accurately predict the timing of labor induction to reduce the number of cesarean sections, thus improving outcomes for mothers and children.

Although the precise mechanism by which labor starts is still not clear, research has shown that childbirth is regulated by multiple pathways, including endocrine factors, hormones, and proinflammatory reactions, among other factors, and is a complex physiological phenomena [5,6]. Emerging studies suggest that proinflammatory mediators may be critically involved in the initiation of labor [7,8]; however, other factors, such as endocrine factors, hormones, and hypoxia-induced factors (including hypoxia inducible factor HIF-a), are also involved [9–12].

There are reports that myometrial and placental HIF-1α mRNA expression is increased after delivery and that placental insufficiency is common in pregnant women who deliver early [10]. This study also aimed to detect changes in HIF-1α and umbilical venous blood flow before and after childbirth to determine whether hypoxia is involved in birthing initiation and if so, to explore its role.

Since no literature has systematically analyzed the abovementioned factors in a clinical setting, we aimed to systematically measure the changes in these various factors before and after the onset of labor and to comprehensively analyze whether these factors could be used to predict the initiation of labor.

To assist in clinical treatment and improve the outcome for pregnant women, it is necessary to establish a predictive model. However, there are very few studies on birthing initiation, and these tend to be limited to a mathematical model that initiates labor by the single factor of proinflammatory mediators. The purpose of this study was to investigate the effects of 3 kinds of parameters related to labor: 1) proinflammatory cytokines, including interleukin 1 beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1]), matrix metalloproteinases (MMP-9), and hormones; 2) endocrine factors, including estrogen 3 (E3), oxytocin (OT), and prolactin 1 alpha (PRL-1α); and 3) hypoxia-related parameters, including HIF-1α and umbilical vein blood flow, in pregnant women before and after labor initiation and then, based on individual results, forming a mathematical model for predicting the start of labor based on the results of individual factors.

Material and Methods

Patients selection and grouping

The study was approved by the ethics committee of West China Second University Hospital, and informed consent was signed by every participant. The study conforms to standards required by Declaration of Helsinki. We recruited consecutive pregnant women who registered at the West China Second University Hospital between January 2016 and September 2016, were in their first singleton pregnancy, and planned for vaginal delivery. We collected blood samples and ultrasound measurements at full term (38–39 weeks, with no significant contractions) and after labor initiation (12 contractions per hour with 2 cm os opening). To analyze the time and start to take measurements at childbirth, we used 3 days as a cutoff value for grouping and chose the factors that are mostly closely related to the initiation of childbirth.

ELISA methods

Serum samples were separated and frozen immediately for storage after blood was drawn. IL-1β, IL-6, TNF-α, MCP-1, MMP-9, HIF-1α, E3, oxytocin, and prolactin were measured using standard ELISA tests. We examined the association between lab measurements (absolute value at full term and changes between full term and delivery) and time to human parturition. We also evaluated the predictive values of respective laboratory measurements for the timing of delivery in regression models.

Umbilical vein blood flow data measurement

Umbilical vein blood flow data were extracted using a GE E8 type, GE Voluson 730 expert type, Philips iU22 color Doppler ultrasound diagnostic convex probe (probe frequency 3.5–5.0 MHz). Three experienced sonographers performed the testing. Pregnant women were asked to lay in the supine position and maintain calm breathing to allow for routine examination of fetal structure, measure fetal growth indicators, and select the placental face of the umbilical vein. The sonographer

Table 1. The proinflammation cytokines before and after labor onset.

	Before labor		Labor onset		P value
	Means	SEM	Means	SEM	
IL-1β (pg/ml)	1.26	1.98	6.98	6.89	<0.05
IL-6 (pg/ml)	6.32	6.05	23.83	21.63	<0.05
TNF-α (pg/ml)	1.13	1.47	5.37	3.17	<0.05
MCP-1 (pg/ml)	106.11	30.64	108.47	31.69	0.161

then identified the largest long axis of the umbilical vein section and enlarged the image to measure the diameter of the umbilical vein; 3 continuous measurements were averaged. Once the diameter was recorded, pulsed wave Doppler ultrasound was used to measure the umbilical vein when no obvious fetal activity could be detected. This procedure involved making a sample line parallel to the long axis of the umbilical vein and calculating the maximum flow rate, minimum flow rate, and average flow rate over time of the umbilical vein to ascertain the umbilical vein blood flow parameters.

Statistical analysis

We used two-sample Student's t-test to compare 2 independent continuous variables; the Chi-square test was used to compare categorical variables. We used a paired t-test to compare measurements from the same individuals. For the labor inspection data and delivery index start waiting time correlation analysis, we used a Pearson correlation coefficient analysis. The absolute values of the Pearson coefficient showed a strong correlation between 0.8–1.0, whereas 0.6–0.8 was strongly correlated, 0.4–0.6 was moderately correlated, 0.2–0.4 was weakly correlated, and 0.0–0.2 was uncorrelated.

We used area under the curve (AUC) analysis to assess the predictive value of individual lab measurements, which were calculated via receiver operating characteristic (ROC) curve analyses. We performed multifactorial analysis by including multiple lab measurements in the logistic regression model as continuous predictors. We evaluated the value of multiple predictors by comparing the AUC of the predicted probabilities estimated by the multifactorial model. The statistical significance level was set at p<0.05. We performed all statistical analyses using Stata 13.

Results

ELISA results for the cytokines

The concentrations of serum cytokines from full term to labor onset are listed in Table 1. The concentrations of IL-1β, IL-6, and TNF-α were significantly increased at onset of labor. Before delivery, IL-1β, IL-6, and TNF-α concentrations were 1.16±1.98 pg/ml, 6.32±6.05 pg/ml, and 1.13±1.47 pg/ml, respectively. The labor onset IL-1β, IL-6, and TNF-α concentrations were 6.98±6.89 pg/ml, 23.83±21.63 pg/ml, and 5.37±3.17 pg/ml, respectively. The differences between these prepartum and labor onset values were statistically significant (P<0.05) (Figure 1A–1C). The difference in the concentration of MCP-1 was not significant (Figure 1D).

Three days was used as the cutoff time from initial measurement to delivery; 25 mothers delivered their children within 3 days, and 75 mothers delivered after 3 days. Cytokine concentrations were compared between the 2 groups based on whether the baby was delivered within 3 days. The concentrations of IL-1β, IL-6, and TNF-α in the >3 days group were 0.83±1.20 pg/ml, 4.99±4.93 pg/ml, and 0.75±1.01 pg/ml, respectively; in the ≤3 days group, these concentrations were 2.54±3.07 pg/ml, 10.32±7.33 pg/ml, and 2.26±2.00 pg/ml. The differences between the 2 groups were statistically significant (P<0.05) (Figure 2A–2D).

Further analysis showed negative correlations between serum IL-1β, IL-6, and TNF-α concentrations and the time to the labor initiation; that is, the higher the concentration was, the shorter the time to the labor initiation (Pearson coefficients were –0.310, –0.230, and –0.409, respectively). This finding suggests that the proinflammatory mediators (IL-1β, IL-6, and TNF-α) are closely related to the onset of labor and may be involved in the initiation of labor (Figure 3).

Other parameters related to onset of labor

Hypoxia-related indicators (such as HIF-1α and umbilical blood flow) were also measured in 100 pregnant women before and after labor initiation, and the results show that HIF-1α production before and after labor initiation was significantly different (P<0.05). Umbilical pulse blood flow in labor before and after the start of delivery was not statistically significant (P=0.17). However, correlation analysis of umbilical vein blood flow and birth weight showed a positive correlation with birth weight (r^2=0.461; Figure 3D).
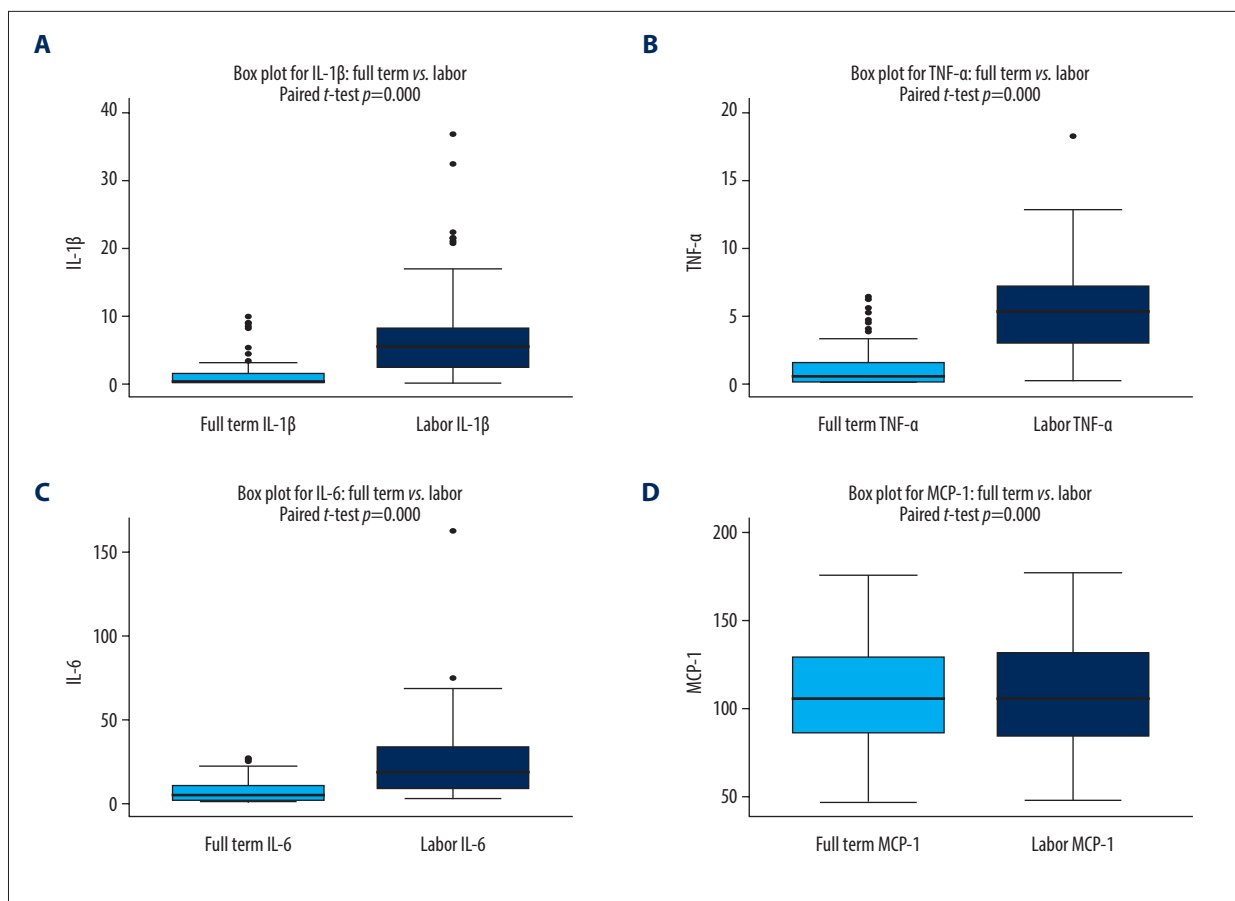
Figure 1. (**A**) The concentration of IL-1β before and after labor initiation, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**B**) The concentration of IL-6 before and after labor initiation, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**C**) The concentration of TNF-α and IL-1β before and after labor initiation, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**D**) The concentration of MCP-1 before and after labor initiation, the units are reported as pg/ml, and a statistically significant difference was found, P=0.161.

When other parameters were measured, including MMP-9 and endocrine hormones (including E3, OT, and PRL), there were no significant changes in the parameters, with the exception of MMP-9 (Pearson coefficients were –0.264).

Mathematic model to predict labor within 3 days

We used ROC curve analysis to analyze IL-1β, IL-6, TNF-α, or MMP-9 as single indexes to predict the probability of delivery within 3 days (0 to 1 interval). Taking TNF-α as an example, when a cutoff concentration of >0.7 pg/ml was used, prediction of the onset of childbirth within 3 days had a sensitivity of 76% and specificity of 68%. The lower the concentration of TNF-α was, the less likely it was that the mother would give birth within 3 days, also suggesting that the inflammatory response may play an important role in the initiation of full-term pregnancy delivery.

Based on the presumptive assumption of multiple factors and the initiation of labor within 3 days, a multifactor logistic regression analysis was used, and the resulting probability value was used to construct the ROC curve to obtain the AUC value (Figure 4). The results show that the combined effect of multiple factors has greater predictive power than a single indicator, such as the probability of labor initiation (Figure 4 and calculation of the area under the curve). The model including a single indicator predicts the onset of labor within 3 days, with AUC values of 0.7395, 0.7240, 0.7536, and 0.6360 for IL-1β, IL-6, TNF-α, and MMP-9, respectively. The single highest predictive value was for TNF-α. Serum IL-1β, IL-6, TNF-α, and MMP-9 concentrations were significantly different when predicting the onset of delivery (sensitivity, specificity, positive predictive value, and negative predictive value of labor) within 3 days. To effectively predict the onset of labor within 3 days, the predicted AUC for TNF-α was 0.820; when combined with
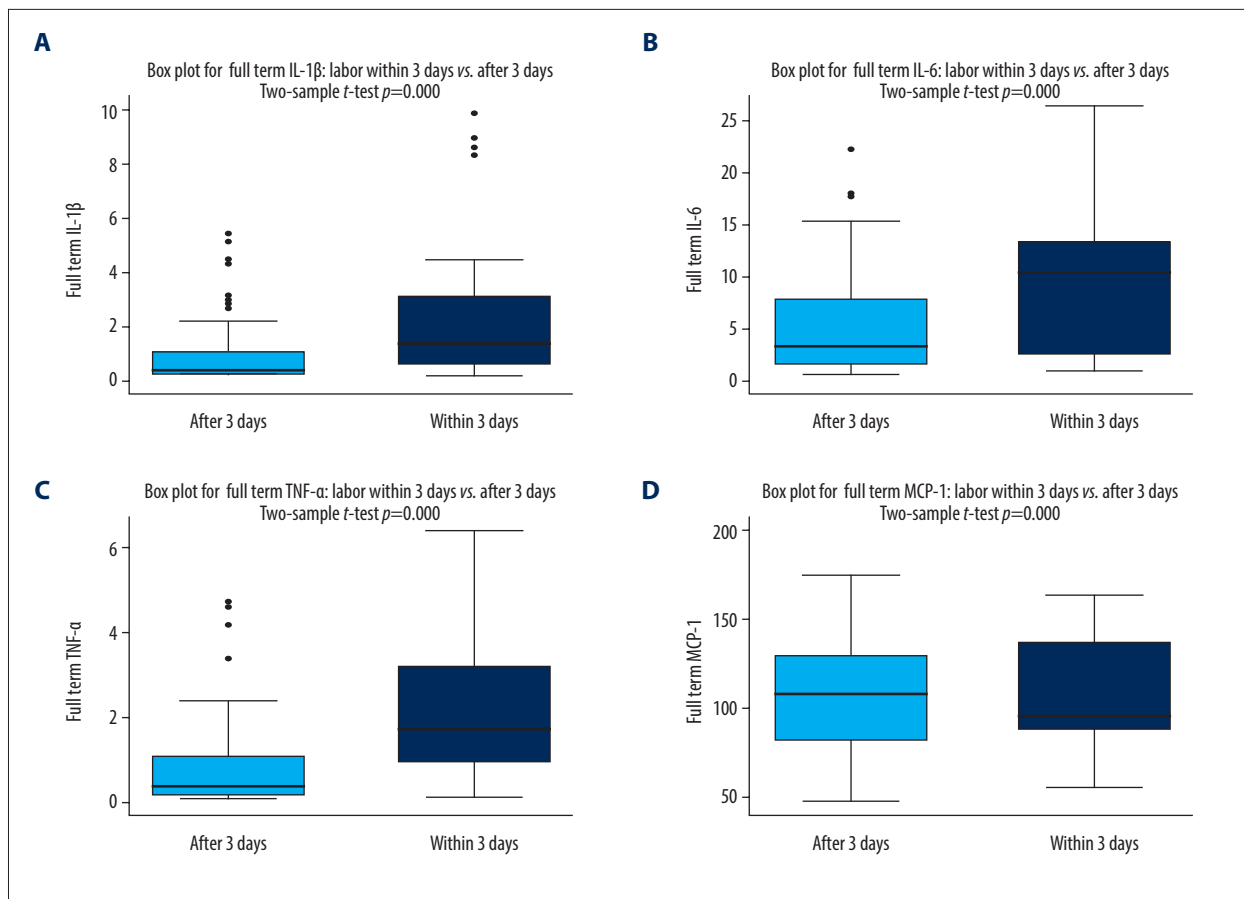
Figure 2. (**A**) The concentration of IL-1β in the group within 3 days and the group after 3 days, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**B**) The concentration of TNF-α in the group within 3 days and the group after 3 days, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**C**) The concentration of IL-6 in the group within 3 days and the group after 3 days, the units are reported as pg/ml, and a statistically significant difference was found, P<0.05. (**D**) The concentration of MCP-1 in the group within 3 days and the group after 3 days, the units are reported as pg/ml, and a statistically significant difference was found, P=0.901.

IL-1β, when IL-1β, IL-6, TNF-α, and MMP-9 were combined, the predicted AUC was 0.870 (Figure 4).

Discussion

The initiation of parturition is a complex process that involves interactions between multiple molecules, and the major players include proinflammatory mediators, endocrine factors, hormones, and multichannel regulation [13,14]. However, the exact initiator of birth is still unclear. Although there are some theories on the start of childbirth, such as the formation of a lower uterine segment and cervical maturation theory, neurotransmitters, immunological factors, mechanical theory, and endocrine control, no single factor can explain how labor starts [15–17]. Existing reports tend to be limited to single factors and are often confined to the single factor of proinflammatory mediators. This study used a multifactorial approach

to detect proinflammatory cytokines (IL-1β, IL-6, TNF-α, and MCP-1), matrix metalloproteinases (MMP-9), hormones and endocrine factors (E3, OT, PRL), and hypoxia (HIF-1α and umbilical venous blood flow) to combine systemic information on the differences between before and after the start of parturition and a possible mechanism involving multivariate biomarkers in labor initiation.

According to the results of the correlation analysis, a predictive model of labor initiation was established. In line with a previous study, the current study showed that serum proinflammatory mediators (IL-1β, IL-6, and TNF-α) show some ability to predict the onset of delivery for pregnant women [18–20]. Previous studies have shown that other biochemical proteins, such as MMP-9 and prolactin, are altered during the labor process. Our study found a similar change, but other factors, such as ethnic differences and analysis methods, may account for the difference [21–23]. Since this study included a relatively
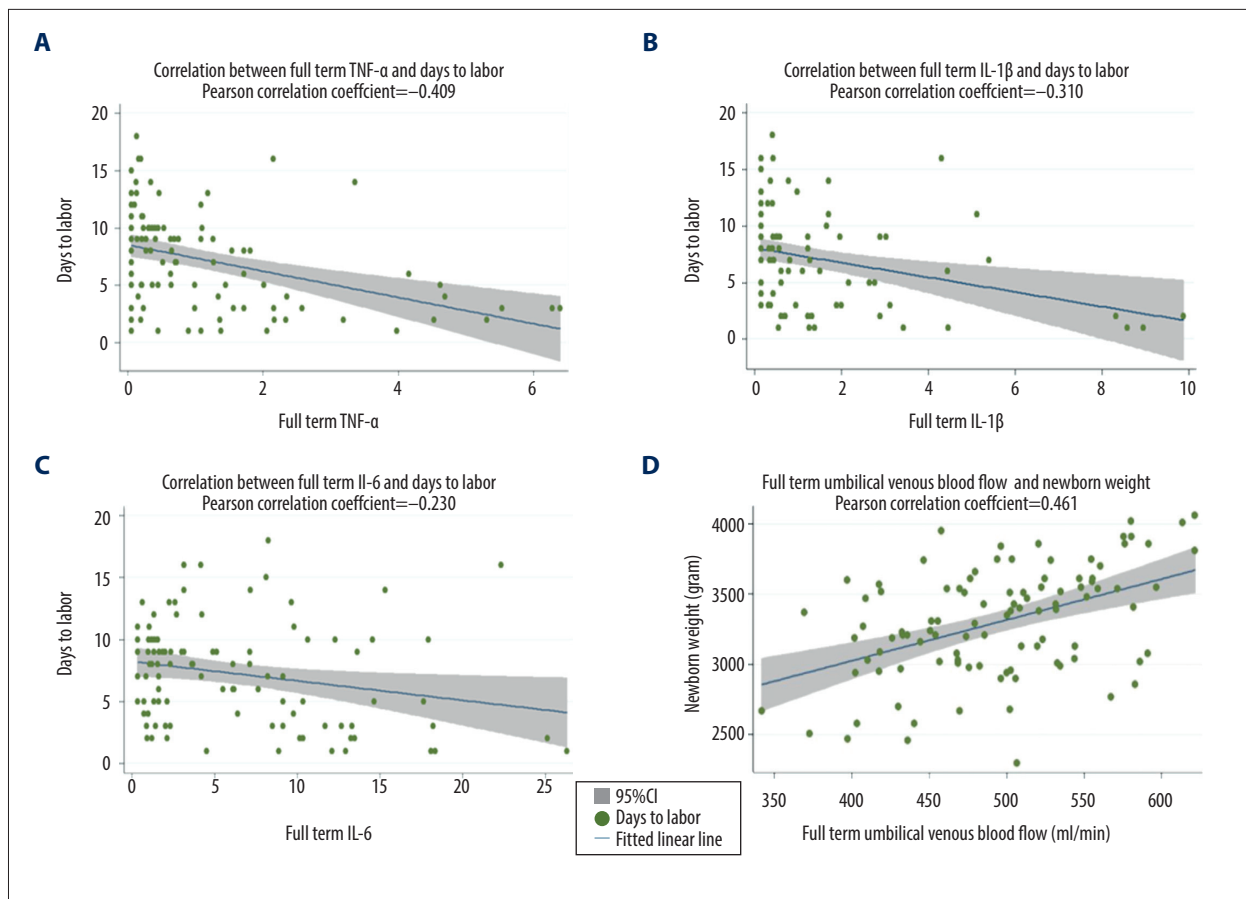
Figure 3. (**A**) The relationship between the concentration of TNF-α and the time of labor initiation, the X-axis shows concentration (pg/ml), the Y-axis shows the number of days of labor initiation, and the Pearson value was –0.409. (**B**) The relationship between the concentration of IL-1 and the time of labor initiation, the X-axis shows concentration, (pg/ml), the Y-axis shows the days of labor initiation, and the Pearson value was –0.310. (**C**) The relationship between the concentration of IL-6 and the time of labor initiation, the X-axis shows concentration, (pg/ml), the Y-axis shows the days of labor initiation, and the Pearson value was -0.23. (**D**) Correlation analysis of umbilical vein blood flow and birth weight showed a positive correlation with birth weight (r^2=0.461).

large sample and the findings are consistent with the existing literature, this conclusion could result in a single indicator being used to estimate delivery; however, the sensitivity and specificity of the predictions are unsatisfactory. Therefore, in the present study, we further established a multifactorial logistic regression to form a predictive model to improve predictive performance. Thus, the multivariate model of this study was established.

Maternal peripheral blood, fetal umbilical cord blood, cervical secretions, amniotic fluid, placental tissue, decidua, and myometrium have been used in previous birthing studies. In these studies, maternal peripheral blood and fetus-free DNA samples are increasingly being studied since they are easy to extract and analyze [24–26]. Furthermore, the present study intends to use pregnant women as their own controls in the research sample to exclude, as much as possible, differences

between individual analysis and multifactorial changes in labor before and after birthing and to explore their roles. Therefore, our data are of great importance for follow-up studies on biomarker discovery.

There are several limitations to the current study. First, due to the sample size, this multivariate linear model does not directly identify the best cutoff value for each test. In the future, experimental analysis and a corresponding sensitivity analysis including a larger sample are needed to calculate the appropriate predictive index. Second, this study included only pregnant women at 38–39 weeks, and investigation of women at other gestational weeks is needed to analyze the test results and their respective predictive performance for further verification. To achieve this goal, we need to expand the sample size to collect samples from different gestational weeks and may even need to include nonpregnant women. Collecting the
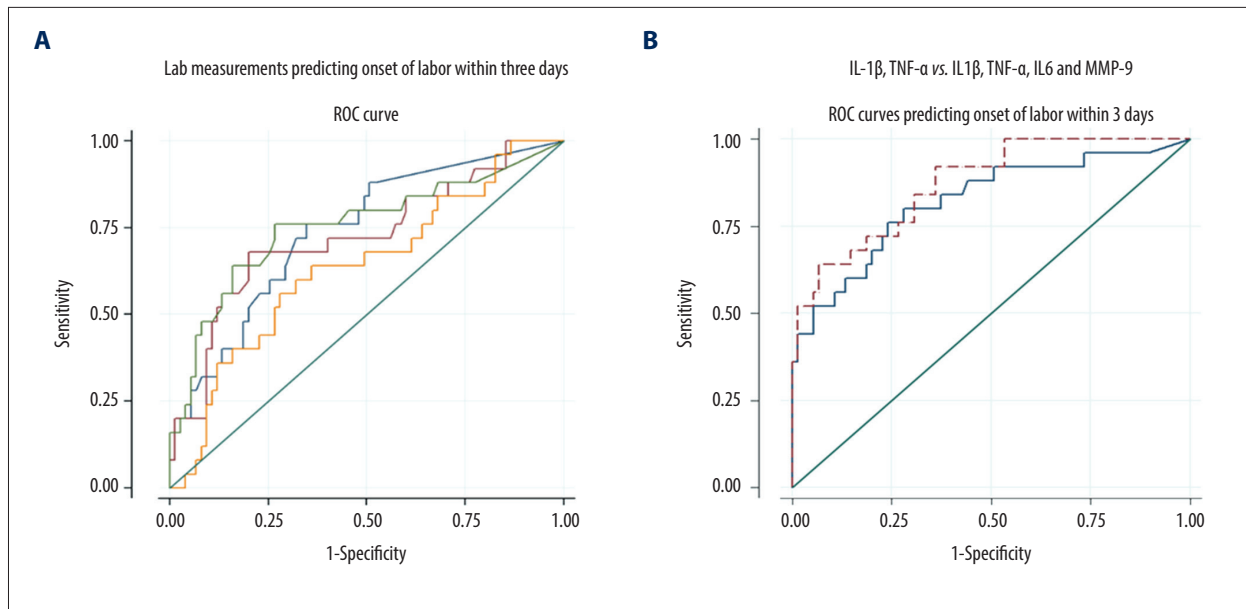
A

Lab measurements predicting onset of labor within three days

ROC curve

B

IL-1β, TNF-α *vs.* IL1β, TNF-α, IL6 and MMP-9

ROC curves predicting onset of labor within 3 days

Figure 4. (**A**) Single factors for predicting the possibility of labor in 3 days. For the AUC value, IL-1β was 0.7395, IL-6 was 0.724, TNF-α was 0.7536, and MMP-9 was 0.636. (**B**) Multiple factors for predicting the possibility of labor in 3 days, which shows that the AUC for IL-1β and TNF-α were both 0.82, and the 4 factors IL-1β, IL-6, TNF-α, and MMP-9 together have an AUC value of 0.8704.

data for these different periods as well as the baseline concentration of cytokines are essential for building a probability model of natural labor for different gestational weeks and to provide satisfactory early warning of premature delivery. Last but not least, to better understand the mechanism of how inflammation signaling initiates the labor process, animal modeling is warranted for further biomarker discovery.

Conclusions

Combined proinflammatory biomarkers have better predictive value for preterm labor than single biomarkers.

Acknowledgment

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