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RESEARCH ARTICLE

WILEY

Blood routine test is a good indicator for predicting premature rupture of membranes

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Objective: To assess the value of blood routine test (blood RT) in order to predict the occurrence of premature rupture of membranes (PROM).

Methods: A retrospective study was conducted to collect blood RT data from 100 cases of preterm premature rupture of membranes (pPROM), 70 cases of full-term premature rupture of membranes (fPROM), and 100 cases of full-term pregnancy (Normal). Nonparametric tests were performed for each blood routine parameter, the ROC curve was established for the parameters with significant difference, and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratios (+LR), and negative likelihood ratios (−LR) were further calculated.

Results: The statistical results showed that WBC, NE.%, LY.%, EO.%, BA.%, NE.#, EO.#, RBC, HGB, HCT, and NLR were significantly different between pPROM and fPROM ($P < 0.05$). There was a significant difference in WBC, NE.%, LY.%, NE.#, MO.#, RBC, HGB, HCT, and NLR between the pPROM and Normal groups ($P < 0.05$). Between the fPROM and Normal groups, only WBC was statistically significant ($P < 0.05$). By establishing ROC curve, the results showed that when the cutoff value of WBC was 9.63 and NEU# was 7.12, their combined detection had the best predictive value with a sensitivity of 73% and a specificity of 81%. In addition, Its PPV was 79.3%, NPV was 75%, +LR was 3.84, and -LR was 0.33.

Conclusion: The patient's blood RT results can be used to predict the risk of premature rupture of membranes, and in order to improve the sensitivity and specificity, multiple parameters can be combined.

KEYWORDS
blood routine test, NEU, prediction value, PROM, WBC

1 | INTRODUCTION

PROM refers to the fetal membrane unexpectedly ruptured before full-term delivery. In Europe, the incidence of PROM is about 5%-15%,[1] and the incidence in China is about 2.7%-17%.[2] Among them, the pPROM, which is less than 37 weeks of pregnancy, is the most harmful, with the incidence of 2.0%~3.5%.[3] Once the fetal membrane rupture occurred, barrier protection immediately disappears. Pregnant women will irreversibly incorporate amniotic infection, chorioamnionitis, fetal distress, and placental abruption until preterm birth. It has been reported in the literature that 30%-40% of preterm births are related to PROM,[4] and 75% of perinatal deaths are associated with premature birth.[5] PROM has obviously become an important cause of perinatal fetal death. In obstetrics, the treatment of PROM patients is quite tricky, and more use of single conservative treatment programs, including inhibition of contractions, the use of antibiotics to prevent infection, the injection of glucocorticoid to promote lung maturation, is witnessed.[6] However,

the traditional treatment effect is not satisfactory, and about 90% of pregnant women give birth within one week.[7] Therefore, the early diagnosis and the prevention of the occurrence of PROM are even more important.

Until now, the pathogenesis of PROM is not clear and is generally considered the result of multifactorial interactions. Upstream infection of the genital tract is the most important factor of concern, with more than 60% of PROM associated with infection and subsequent cascading inflammation, which is mainly referred to as chorioamnionitis. Traditional inflammatory markers are as follows: white blood cell (WBC), C-reactive protein (CRP), and procalcitonin (PCT). They are important for the diagnosis of chorioamnionitis, especially subclinical chorioamnionitis.[8,9]

Blood RT is a routine process for the hospitalized patients, which includes 24 important indicators, named white blood cell count (WBC), neutrophil percentage (NE.%), lymphocyte percentage (LY.%), eosinophil percentage (EO.%), basophil percentage (BA.%), monocyte percentage (MO.%), neutrophil count (NE.#), lymphocyte count (LY.#), eosinophil count (EO.#), basophil count (BA.#), monocyte count (MO.#), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), coefficient variation of the distribution width of the red blood cell (RDW-CV), standard deviation of the distribution width of the red blood cell (RDW-SD), platelet count (PLT#), thrombocytopenia (PCT), the proportion of large platelets (PLCR), mean platelet volume (MPV), and platelet distribution width (PDW).

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been shown to be useful in the early prediction and differential diagnosis of cancer, thrombus, and diabetics.[10,11] The aim of this study was to investigate its value in the diagnosis and prognosis of PROM.

2 | MATERIALS AND METHODS

2.1 | General information

Patients with PROM treated at Qilu Hospital of Shandong University from January to December 2017 were recruited. A total of 100 pPROM patients with gestational age less than 37 weeks and 70 fPROM patients with a pregnancy time greater than or equal to 37 weeks were included in the study, and all blood RT collection time must be within 2 hours after the rupture of membranes. At the same time, 100 cases of full-term pregnant women were selected as control group, and blood RT collection time must be completed before delivery. This study has been approved by the Medical Ethics Committee of Qilu Hospital of Shandong University. All patients whose data were collected were informed of this study and authorized consent was obtained. In statistical analysis, the patient's personal information was hidden and replaced by digital code.

2.2 | Diagnostic, inclusion, and exclusion criteria

Diagnostic criteria for PROM are as follows: (a)Excessive liquid flows out of the vagina itself, pH>7; (b) fetal hair is visible in the liquid sediment smear; after drying, the fern-like crystals can be seen.

Inclusion criteria are as follows: (a) All the selected patients did not have other diseases, including preeclampsia, eclampsia, urinary tract infection, pyelonephritis, common cold or any kind of viral infections, connective tissue disease, and other infectious diseases; (b) all were singleton pregnancies; and (c) no antibacterials were used in the past 2 weeks.

Exclusion criteria are as follows: (a) patients who did not meet the inclusion criteria; (b) specimen collection time does not meet the prescribed time limit.

2.3 | Specimen collection and detection

After routine skin disinfection, 2 mL of venous blood was collected into anticoagulant tubes with ethylenediaminetetraacetic acid (EDTA-K2; BD, USA), to mix lightly 5 times. Blood RT was measured by SYSMEX XN-3000 automatic five classifications hematology analyzer (SYSMEX, Kobe, Japan).

2.4 | Data entry

The EpiData 3.1 software was used to input the basic information and blood RT data of the study subjects. In order to further reduce the input errors, the double-entry method is used for data entry and logic error detection. After all the data are collated, it was imported into Excel 2010 software, and after proofreading, the database was established.

2.5 | Data analysis

The general scenario of the research object is described, mainly including age and days of pregnancy; according to the characteristics of the data, concentration trend and discrete trend were described. If the data were normally distributed, the mean and standard deviation would be used; if the data were non-normal distribution, the median and quartile spacing would be used. If the variables between the case group and the control group satisfied the homogeneity of the variance, the parameter test would be used; otherwise, the nonparametric test would have been used. The test results include its statistic and P value. Specifically, the Levene method is used to test the homogeneity of the variance of the two samples, $P > 0.1$ represents no statistical difference, and the data are consistent with variance. Kolmogorov-Smirnov test (K-S test) was used to test the normality of two samples. $P > 0.1$ represents no statistical difference, and the data are normal distribution. Nonparametric tests between case group and control group were performed using Kolmogorov-Smirnov Z-rank test (K-S Z-rank test); $P < 0.05$ represents significant difference.

2.6 | Establish ROC curve

Sensitivity measures the ability of an experiment to correctly identify a patient. Specificity measures the ability of an experiment to correctly identify nonpatients. Both can be used to evaluate the authenticity of the model. The positive predictive value is used to assess the likelihood of a target disease in screening test–positive people. The negative predictive value is the likelihood that screening test–negative people are free from the target disease. Both of them can evaluate the prediction ability of the model. Change in the cutoff point will get different index values; that is the greater the sum of sensitivity and specificity, the better the diagnosis of the model. In this study, we selected multiple cutoffs to calculate the sensitivity, specificity, positive predictive value, and negative predictive value, respectively, in order to find the best cutoff point. The ROC curve was established based on the sensitivity and specificity, and the model was evaluated comprehensively by ROC curve and area under the curve. When the two indicators need joint detection, the first use of logistic regression analysis is to generate the prediction probability and then the probability of ROC curve.

3 | RESULTS

3.1 | The basic situation of the research object

A total of 270 eligible patients were screened in this study. Using the Levene method, the variance homogeneity test was performed on the two samples. After testing, Age, Days, WBC, NE.%, LY.%, EO.%, BA.%, MO.%, NE.#, LY.#, MO.#, NLR, and PLR do not satisfy the homogeneity of variance, $\alpha = 0.1$ as the test level. Using the

TABLE 1 The results of normality and homogeneity of variance test

			Normality test					
	Variance Homogeneity		pPROM		fPROM		Normal	
Variable	Statistics	*P*	Statistics	*P*	Statistics	*P*	Statistics	*P*
Age	8.42	0.00	0.00	0.00	0.16	0.00	0.15	0.00
Days	70.8	0.00	0.20	0.00	0.11	0.28*	0.16	0.00
WBC	9.41	0.00	0.12	0.00	0.07	0.20*	0.11	0.01
NE.%	5.44	0.00	0.06	0.20*	0.11	0.03	0.07	0.20*
LY.%	4.37	0.01	0.06	0.20*	0.12	0.02	0.09	0.03
EO.%	2.51	0.08	0.18	0.00	0.17	0.00	0.19	0.00
BA.%	8.66	0.00	0.26	0.00	0.18	0.00	0.19	0.00
MO.%	3.42	0.03	0.08	0.10*	0.06	0.20*	0.09	0.05
NE.#	12.54	0.00	0.12	0.00	0.07	0.20*	0.05	0.20*
LY.#	9.01	0.00	0.12	0.00	0.07	0.20*	0.09	0.03
EO.#	2.15	0.12*	0.21	0.00	0.20	0.00	0.20	0.00
BA.#	1.73	0.18*	0.26	0.00	0.22	0.00	0.25	0.00
MO.#	8.35	0.00	0.06	0.20*	0.07	0.20*	0.06	0.20*
RBC	0.11	0.89*	0.06	0.20*	0.06	0.20*	0.06	0.20*
HGB	1.40	0.25*	0.06	0.20*	0.08	0.20*	0.08	0.07
HCT	0.54	0.58*	0.07	0.20*	0.08	0.20*	0.12	0.00
MCV	0.65	0.52*	0.09	0.06	0.09	0.20*	0.06	0.20*
MCH	0.62	0.54*	0.09	0.04	0.14	0.00	0.06	0.20*
MCHC	0.11	0.89*	0.10	0.02	0.16	0.00	0.07	0.20*
RDW-CV	1.64	0.20*	0.20	0.00	0.08	0.20*	0.14	0.00
RDW-SD	0.77	0.47*	0.13	0.00	0.06	0.20*	0.07	0.20*
PLT	1.26	0.29*	0.08	0.18*	0.10	0.07	0.05	0.20*
PCT	0.72	0.49*	0.11	0.01	0.13	0.01	0.09	0.03
PLCR	1.50	0.22*	0.05	0.20*	0.12	0.02	0.05	0.20*
MPV	1.61	0.20*	0.07	0.20*	0.14	0.00	0.08	0.07
PDW	1.73	0.18*	0.08	0.08	0.14	0.00	0.07	0.2*
NLR	29.96	0.00	0.19	0.00	0.11	0.04	0.07	0.2*
PLR	8.37	0.00	0.13	0.00	0.13	0.00	0.08	0.15*

*$P > 0.1$

Kolmogorov-Smirnov method, the normality test was performed on the two samples. After testing, only the NE.%, LY.%, MO.%, MO.#, RBC, HGB, HCT, PLT, PLCR, and MPV in the pPROM group meet normal distribution. In the PROM group, only Days, WBC, MO.%, NE.#, LY.#, MO.#, RBC, HGB, HCT, MCV, RDW-CV, and RDW-SD meet the normal distribution; NE.%, NE.#, MO.#, RBC, MCV, MCH, MCHC, RDW-SD, PLT, PLCR, PDW, NLR, and PLR are normal distributions in the Normal group. Similarly, $\alpha = 0.1$ is the test level.

To sum up, when $\alpha = 0.1$, the variables do not satisfy the standard deviation and normality of parameter test, so the variables are compared by nonparametric rank sum test. The results are shown in Table 1.

3.2 | Variable comparison

We used $\bar{x} \pm s$ to describe the data distribution of case and control variables. The K-S Z-rank test was used for the nonparametric test in pPROM group and fPROM group, pPROM group and Normal group, and fPROM group and Normal group. Statistical analysis showed that Days and WBC have significant differences in the pairwise comparisons of the three groups. There were significant differences in WBC, NE.%, LY.%, NE.#, RBC, HGB, HCT, NLR between the pPROM group and other two groups. The results are shown in Table 2, and the above parameters were selected for the next ROC curve established.

3.3 | ROC curve

Each cutoff point represents a sensitivity and a specificity, sensitivity as the ordinate, 1 minus the specificity as the abscissa, and the ROC curve is composed of different cutoff points. The area under the ROC curve is usually used to reflect the accuracy of the diagnostic system. The more curve to the left, the greater the area under the curve, the higher the diagnostic accuracy. Since the establishment of a routine

TABLE 2 Case group compared with the control

	$\bar{x} \pm s$					
Variable	pPROM	fPROM	Normal	pPROM vs fPROM	pPROM vs Normal	fPROM vs Normal
Age	31.79 ± 0.51	31.17 ± 0.45	29.96 ± 0.39	0.48	0.00*	0.15
Days	228.71 ± 3.46	265.91 ± 0.52	282.57 ± 0.40	0.00*	0.00*	0.00*
WBC	10.61 ± 2.94	8.99 ± 1.98	8.46 ± 1.54	0.01*	0.00*	0.04*
NE.%	74.92 ± 8.01	71.52 ± 5.54	70.21 ± 5.86	0.00*	0.00*	0.20
LY.%	17.72 ± 6.7	20.39 ± 4.93	21.39 ± 5.03	0.00*	0.00*	0.18
EO.%	0.66 ± 0.66	0.80 ± 0.55	0.90 ± 0.76	0.02*	0.11	0.58
BA.%	0.25 ± 0.13	0.31 ± 0.13	0.33 ± 0.18	0.02*	0.05	0.73
MO.%	6.45 ± 1.92	6.98 ± 1.45	7.17 ± 1.44	0.22	0.05	0.87
NE.#	8.07 ± 2.9	6.47 ± 1.68	5.98 ± 1.39	0.00*	0.00*	0.24
LY.#	1.78 ± 0.63	1.80 ± 0.46	1.77 ± 0.4	0.25	0.21	0.41
EO.#	0.07 ± 0.08	0.07 ± 0.05	0.08 ± 0.07	0.02*	0.28	0.55
BA.#	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.92	0.70	1.00
MO.#	0.67 ± 0.22	0.63 ± 0.17	0.60 ± 0.14	0.31	0.02*	0.49
RBC	3.92 ± 0.37	4.05 ± 0.36	4.06 ± 0.33	0.03*	0.00*	0.86
HGB	116.88 ± 11.78	121.61 ± 12.61	121.88 ± 10.24	0.01*	0.00*	0.61
HCT	35.31 ± 3.07	36.76 ± 3.24	37.00 ± 2.71	0.00*	0.00*	0.29
MCV	90.39 ± 5.46	90.98 ± 5.09	91.25 ± 4.58	0.52	0.70	0.69
MCH	29.92 ± 2.38	30.10 ± 2.36	30.07 ± 2.08	0.78	0.91	0.49
MCHC	330.64 ± 11.17	330.44 ± 11.26	329.25 ± 10.12	0.98	0.37	0.22
RDW-CV	13.38 ± 1.57	13.33 ± 1.07	13.41 ± 0.94	0.30	0.28	0.66
RDW-SD	43.71 ± 4.36	43.95 ± 3.33	44.44 ± 3.06	0.33	0.04	0.58
PLT	213.94 ± 55.75	214.84 ± 53.6	208.94 ± 47.23	0.69	0.81	0.70
PCT	0.22 ± 0.05	0.22 ± 0.05	0.22 ± 0.05	1.00	0.47	0.72
PLCR	29.14 ± 7.91	28.82 ± 7.33	28.09 ± 6.87	0.46	0.53	0.64
MPV	10.54 ± 0.98	10.49 ± 0.9	10.39 ± 0.86	0.72	0.47	0.73
PDW	12.24 ± 2.33	12.22 ± 2.37	11.95 ± 1.92	0.67	0.58	0.52
NLR	5.25 ± 3.21	3.77 ± 1.2	3.53 ± 1.11	0.00*	0.00*	0.21
PLR	136.56 ± 52.46	125.51 ± 40.86	121.79 ± 33.29	0.30	0.08	0.70

*$P < 0.05$.

ROC curve was required between the two groups, we chose the pPROM group and the Normal group. Finally, the ROC curve established by six indicators was selected, including WBC, NE.%, NE.#, NLR, WBC+ NE.%, and WBC+NE.#. The results are shown in Figure 1A-F.

3.4 | Predicted value

A diagnostic cutoff point was obtained by using ROC curve. The sensitivity, specificity, positive predictive value, negative predictive value, and Youden index were used to comprehensively analyze the predictive value of each index. The joint detection of WBC and NE.# is an optimal combination. When the prediction probability is 0.536, the sensitivity is 73%, the specificity is 81%, the positive predictive value is 79.3%, and the negative predictive value is 75%. The results are shown in Table 3.

4 | DISCUSSION

So far, the pathogenesis of PROM has been unclear, often as a result of multifactorial interactions. In addition to the fetal membranes

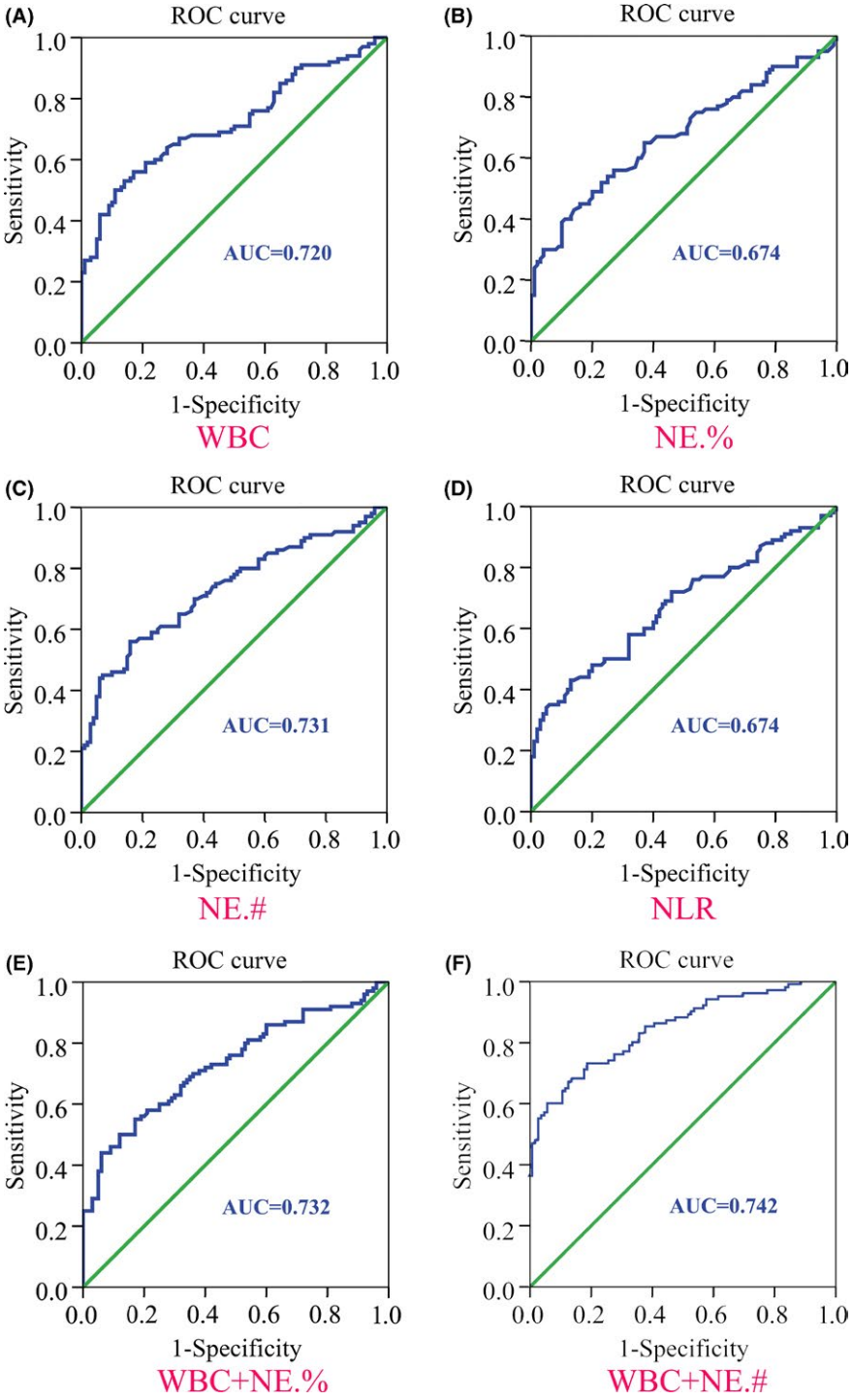

FIGURE 1 ROC curve of different indicators. A: WBC; B: NEU%; C: NEU#; D: NLR; E: WBC+NEU%; F: WBC + NEU#

TABLE 3 Comparison of the predictive value of different indicators

Variable	Youden index	Cutoff value	Sensitivity	Specificity	PPV	NPV	+LR	−LR
WBC	0.41	9.63	58%	83%	77.3%	66.4%	3.41	0.51
NE.%	0.3	73.9	57%	73%	67.9%	62.9%	2.11	0.59
NE.#	0.42	7.12	58%	84%	78.4%	66.7%	3.63	0.50
NLR	0.3	4.59	43%	87%	75.4%	60.1%	3.31	0.66
WBC + NE.%	0.38[a]	0.544[a]	52%	88%	80%	64.4%	4.33	0.55
WBC + NE.#	0.38[a]	0.536[a]	73%	81%	79.3%	75%	3.84	0.33

[a]Predictive probability.

itself, there is dysplasia, and retrograde infection of pathogens in the reproductive tract has been the most important factor. More than 60% of PROM are associated with infection and subsequent inflammatory response,[12] which mainly refers to chorioamnionitis. When chorioamnionitis occurs, pregnant women may have fever, tachycardia, uterine tenderness, leucorrhea odor, and other clinical symptoms.[13] Unfortunately, most pregnant women have no obvious clinical symptoms, often manifested as subclinical chorioamnionitis or chorioamnionitis. Until the occurrence of chorioamnionitis, not only pregnancy was forced to terminate, but it is also a serious threat to maternal life safety.

By analyzing the 100 cases of pPROM, 70 cases of fPROM, and 100 cases of Normal maternal, multiple indicators showed significant differences among groups, of which Age is the first one. There was a significant difference in the age between the PROM group and the Normal group, and the results suggest that the older the patient, the more they prone to PROM. Compared with the Normal group, the values of WBC, NE.%, NE.#, and NLR of PROM group are increased and the values of LY.%, RBC, HGB, and HCT decreased. Patients with pPROM showed a more pronounced inflammatory response and a more severe anemia. The difference between the fPROM group and the Normal group was smaller. This may be related to normal maternal before childbirth may also start a molecular mechanism similar to rupture of membranes in order to facilitate childbirth.

ROC analysis not only plays an important role in clinical trials, clinical diagnosis, and evidence-based medicine, but also has very important application value in evaluating the validity of the model. The area under the ROC curve has been generally considered as an indicator to evaluate the accuracy of the model. In theory, if the area is between 0.50 and 0.70, it indicates that the diagnostic value of the model is low, if the area is between 0.70 and 0.90, it represents a moderate diagnostic value, and the value above 0.90 indicates the higher diagnostic value.[14] Of course, this study also has certain limitations. The biggest regret is that all indicators do not show a sufficiently high AUC value. This also means that the use of these indicators alone to predict the occurrence of PROM is limited but must be combined with other indicators. In addition, due to individual differences, pregnant women's blood routine data can exhibit considerable diversity. Normal pregnant women may also be accompanied by a significant or weak inflammatory reaction at the time of delivery, which has caused some difficulties in the analysis of the data.

In this study, we first selected the nonparametric test method to screen out the differences between the patients with premature rupture of membranes and the normal population, and then through the establishment of ROC curve to further screen out the indicators with a better sensitivity, specificity, positive predictive value, and negative predictive value. Blood RT is a regular pregnancy test project, the predictive model can theoretically screen high-risk pregnant women with premature rupture of membranes and give timely attention, and close monitoring and early prevention will help reduce the incidence of premature rupture of membranes.

5 | CONCLUSIONS

To find a method that has good specificity, accuracy, and practicability to diagnose subclinical chorioamnionitis in time and to choose the most appropriate timing to timely treat or terminate the pregnancy, it is important to improve the prognosis of mother and child. In this study, we used the blood index to evaluate the risk of premature rupture of membranes, screening high-risk patients and providing a new reference for the early diagnosis of premature rupture of membranes. Among them, WBC-combined NE.# is the best indicator, with a sensitivity of 73%, a specificity of 81%, a positive predictive value of 79.3%, and a negative predictive value of 75%.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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