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A routine urine test has partial predictive value in premature rupture of the membranes

Haifeng Liang[1], Zhiqiang Xie[2], Baohong Liu[1], Xiaojie Song[3] and Guanghui Zhao[4]

Abstract

Objective: This study aimed to examine the predictive value of a routine urine test for premature rupture of the fetal membranes.

Methods: Routine urine test data of 100 patients with preterm premature rupture of the membranes (PPROM) and 100 patients with full-term premature rupture of the membranes (PROM) were collected by the case-based method. Additionally, 100 healthy pregnant women and 100 nonpregnant adult healthy women were selected as the negative control group and blank control group, respectively. A receiver operating characteristic curve was established after identifying the different parameters.

Results: We found that occult blood, glucose, ketone bodies, urine specific gravity, red blood cell count, epithelial cell count, bacteria, yeast, crystals, and electrical conductivity were significantly different between the PPROM and PROM groups. There were significant differences in occult blood, protein, glucose, ketone bodies, pH, red blood cell count, bacteria, urine specific gravity, crystals, and electrical conductivity between the PPROM and full-term groups. Receiver operating characteristic curve analysis showed that when the cut-off for bacteria was 130.15, it had the largest area under the curve value of 0.696.

Conclusion: A routine urine test, especially for bacterial counts, has certain predictive value for PROM.

[1]Hospital Infection Management, Qilu Hospital of Shandong University, Qingdao, Shandong Province, China
[2]Department of Stomatology, Qilu Hospital of Shandong University, Qingdao, Shandong Province, China
[3]Department of Respiration, Qilu Hospital of Shandong University, Qingdao, Shandong Province, China
[4]Clinical Laboratory Medicine Center, Qilu Hospital of Shandong University, Jinan, Shandong Province, China

Corresponding author:
Guanghui Zhao, Clinical Laboratory Medicine Center, Qilu Hospital of Shandong University, Jinan, Shandong Province 250012, China.
Email: zgh2009100@163.com

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Introduction

The prevalence of premature rupture of the membranes (PROM) in China is 2.7% to 17%.[1] Preterm premature rupture of the membranes (PPROM) at <37 weeks of gestation is the most harmful, with an incidence of 2.0% to 3.5%.[2] A total of 30% to 40% of preterm births are related to PPROM,[3] and preterm birth is associated with 75% of perinatal deaths.[4] At present, treatment of PPROM often involves traditional single conservative treatment, including suppression of contractions, antibiotics to prevent infection, and glucocorticoids to promote lung maturation.[5] This therapeutic effect is not ideal, and approximately 90% of pregnant women with PPROM will deliver in 1 week.[6]

The pathogenesis of PPROM is unclear, but it usually results from interaction of many factors. Studies have shown that direct invasion of pathogens, damage of the inflammatory response, excessive degradation of matrix metalloproteinases in the extracellular matrix of fetal membranes, apoptosis, the oxidative stress response, tissue acetylation, and microelement deficiency are involved.[7,8] Reproductive tract infection is a major concern of researchers. More than 60% of PPROM is related to infection and subsequent cascade amplification of the inflammatory response.[9] Invasion of pathogens is the beginning of occurrence of PPROM, including Group B *Streptococcus*, Candida, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, human herpes simplex virus, and certain anaerobes.[10–14]

Proliferation of chorionic cells is stronger than that of amnion cells. If intervention can be performed in the early stage of chorionic villous infection, there is a possibility of preventing PROM. Once infection occurs in the amniotic layer, PROM is inevitable.[15] Traditional inflammatory markers, such as the white blood cell (WBC) count, C-reactive protein, and procalcitonin, are commonly used by clinicians to diagnose chorionic amnionitis.[16,17] Sensitivity and specificity need to be improved for these indicators. Amniotic fluid culture requires a long time and the positive rate is not high. Vaginal microecology is an extremely complex system. The resident flora and opportunistic pathogens constantly change and maintain a dynamic balance. Genovese et al.[18] found that up to 54.2% of pregnant women had a vaginal infection or imbalance of vaginal microflora at the 28th week of gestation. Paramel et al.[19] further found that during pregnancy, vaginal microflora widely varied among individuals. *Lactobacillus vaginalis* is still dominant in only 13/70 of pregnant women.

A urine test is routine for pregnant women and it is a noninvasive procedure. This test also has the advantage of low cost and is easily accepted. This study aimed to examine the value of the urine test in diagnosis and prognosis of PPROM.

Materials and methods

Patients

We included pregnant women who were admitted to the Maternity Department of Qilu Hospital of Shandong University from February 2017 to October 2018. All selected patients met the following criteria: (a) no urinary tract infection; (b) singleton pregnancy; and (c) no antibacterials were used in the past 2 weeks. Patients with the following conditions were excluded: (a) patients who did not meet the inclusion criteria; and (b) the specimen collection time did not meet the prescribed time limit. All participants used disposable sterile urine cups to collect specimens and prevent cross-infection.

A total of 100 patients with PPROM whose gestation was <37 weeks were selected and they received routine urine tests within 7 days before rupture of the fetal membranes. A total of 100 patients with PROM with a gestational age >37 weeks were selected and a routine urine routine examination was conducted 7 days before rupture of the membranes. Additionally, 100 women with full-term gestational age >37 weeks and <42 weeks (full-term group) were randomly selected and urine routine specimens were collected within 24 hours before delivery. Finally, 100 non-pregnant adult healthy women (normal group) were randomly selected and clean midstream urine specimens were collected. All of these selected subjects did not have a history of urinary tract infection. This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. All patients involved in the study signed informed consent forms.

Urine analysis method

A disposable cup was used to collect the patient's clean mid-stream urine. Dry chemical analysis of urine was performed using the Arkray AX-4030 (Arkray Corp., Kyoto, Japan). Measurements included leukocytes, occult blood, protein, glucose, ketone bodies (KET), urobilinogen, urobilirubin, pH values, urine specific gravity (SG), and nitrite. Urinary components were analyzed using the Sysmex UF1000i (Sysmex Corp., Kobe, Japan). These components mainly included WBC count, red blood cell (RBC) count, epithelial cell count (EC), cast, pathological cast, bacteria, small round cells, yeast, crystals, and electrical conductivity. In specimens that could not be correctly detected by an instrument, the numbers of WBCs, RBCs, ECs, cast, and crystals were confirmed by a microscopic examination.

Data analysis

In statistical analysis, the patient's personal information was hidden and replaced with a digital code. Before comparison of data, a general description of the data was performed. First, the Kolmogorov–Smirnov test was used to test the normality of two samples. The mean and standard deviation are shown for data that conformed to a normal distribution. For data that were not normally distributed, the median and interquartile range are shown. The Levene method was used to test the homogeneity of variance of two samples. A parametric test was used if variables in the two groups satisfied homogeneity of variance. If homogeneity of variance was not present, a nonparametric test, such as the Kolmogorov–Smirnov Z-rank test, was used. IBM SPSS Statistics 21.0 (SPSS Corporation, Chicago, IL, USA) was used for data analysis. Values of $P < 0.05$ were considered statistically significant.

Establishing the receiver operating characteristic curve

The receiver operating characteristic (ROC) curve is also referred to as a sensitivity curve. The true positive rate (sensitivity) was plotted on the ordinate and the false positive rate (1-specificity) was plotted on the abscissa. We calculated the area under the ROC curve (AUC) for each test separately to determine which test had the largest AUC. This method has the advantages of simplicity and intuitiveness. When two indicators needed to be detected jointly, logistic regression analysis was used to generate the prediction probability, and then the ROC curve was generated with the probability.

Cut-off determination and diagnostic value assessment

The closer to the upper left corner of the ROC curve, the higher the accuracy of this test. The best cut-off value was determined by the Youden index, and then the corresponding sensitivity and specificity were calculated. Sensitivity has the ability to recognize patients. Specificity has the ability to recognize non-patients. Both of these measures can be used to assess the authenticity of the model. The positive predictive value (PPV) and the negative predictive value (NPV) were further calculated by the cut-off value. The PPV was used to evaluate the possibility of the target disease in the positive population in the screening test, and the NPV was the probability that there was no target disease in the negative population. The positive likelihood ratio (+LR) and negative likelihood ratio (−LR) were further calculated by sensitivity and specificity. +LR and −LR combine the advantages of sensitivity, specificity, PPV and NPV. +LR and −LR are relatively independent, and they are a clinically meaningful indicator of the effectiveness of diagnostic tests.

Results

Homogeneity of variance and normal distribution

We investigated urine sample data of 628 pregnant women. After screening, a total of 300 eligible patients were included in this study. The homogeneity of variance test showed that the variables of age, days of gestation, leukocytes, occult blood, protein, glucose, KET, urobilirubin, RBC count, yeast, and electrical conductivity did not satisfy the homogeneity of variance test, with $\alpha = 0.5$ as the test level. Only age in the PPROM group and electrical conductivity in each group showed a normal distribution, with $\alpha = 0.5$ as the test level (Table 1). Normally distributed data are shown in Table 2.

Comparison of variables

Pairwise comparisons among the PPROM, PROM, full-term, and normal groups were performed using the Kolmogorov–Smirnov Z-rank test (Table 3). We found that age was significantly younger in the full-term group compared with the other three groups (all $P < 0.05$). Occult blood, KET, and the RBC count were significantly higher, and electrical conductivity was significantly lower in the PPROM group compared with the other three groups (all $P < 0.05$). Bacteria and crystals were significantly different between the PPROM group and the PROM and full-term groups (all $P \leq 0.05$). SG, EC, and yeast were significantly different between the PPROM and PROM groups (all $P < 0.05$), while protein, pH, and small round cells were significantly different between the PPROM and full-term groups (all $P < 0.05$).

Table 1. Results of normality and homogeneity of variance tests.

	Homogeneity of variance		Normality test							
			PPROM		PROM		Full-term		Nonpregnant	
Variable	Stat.	*P*	Stat.	*P*	Stat.	*P*	Stat.	*P*	Stat.	*P*
Age	6.11	<0.01	0.99	0.43	0.96	0.01	0.88	<0.01	0.10	0.02
Days	75.43	<0.01	0.77	<0.01	0.94	<0.01	0.92	<0.01	–	–
LEU	2.48	0.09	0.69	<0.01	0.79	<0.01	0.80	<0.01	–	–
BLD	34.02	<0.01	0.70	<0.01	0.44	<0.01	0.43	<0.01	–	–
PRO	16.18	<0.01	0.51	<0.01	0.44	<0.01	0.27	<0.01	–	–
GLU	38.67	<0.01	0.60	<0.01	0.21	<0.01	0.31	<0.01	–	–
KET	37.43	<0.01	0.70	<0.01	0.45	<0.01	0.29	<0.01	–	–
UBG	1.15	0.32	0.08	<0.01	0.12	<0.01	0.12	<0.01	–	–
BIL	4.08	0.02	–	–	0.08	<0.01	–	–	–	–
pH	1.57	0.21	0.94	<0.01	0.94	<0.01	0.92	<0.01	0.19	<0.01
SG	1.46	0.23	0.96	<0.01	0.94	<0.01	0.97	0.02	0.08	0.10
NIT	0.19	0.83	0.08	<0.01	0.08	<0.01	0.08	<0.01	–	–
WBCs	1.98	0.14	0.53	<0.01	0.42	<0.01	0.34	<0.01	0.17	<0.01
RBCs	17.71	<0.01	0.29	<0.01	0.27	<0.01	0.12	<0.01	0.18	<0.01
EC	1.19	0.31	0.37	<0.01	0.80	<0.01	0.65	<0.01	0.14	<0.01
Cast	0.73	0.48	0.22	<0.01	0.24	<0.01	0.88	<0.01	0.17	<0.01
P.CAST	1.89	0.15	0.89	<0.01	0.89	<0.01	0.25	<0.01	0.25	<0.01
BAC	0.04	0.96	0.28	<0.01	0.25	<0.01	0.35	<0.01	0.23	<0.01
SRC	2.59	0.08	0.27	<0.01	0.23	<0.01	0.85	<0.01	0.17	<0.01
BYST	4.05	0.02	0.15	<0.01	–	–	0.08	<0.01	–	–
Crystals	1.33	0.26	0.14	<0.01	0.08	<0.01	0.08	<0.01	0.40	<0.01
Cond.	3.79	0.02	0.97	0.05	0.99	0.50	0.99	0.45	0.05	0.20

PPROM: preterm premature rupture of the membranes; PROM: premature rupture of the membranes; Stat: statistic; days: days of gestation; LEU: leukocytes; BLD: occult blood; PRO: protein; GLU: glucose; KET: ketone bodies; UBG: urobilinogen; BIL: urobilirubin; SG: urine specific gravity; NIT: nitrite; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; P.CAST: pathological cast; BAC: bacteria; SRC: small round cells; BYST: yeast; Cond.: electrical conductivity.

ROC curve

Urinary dry chemical indicators, including leukocytes, occult blood, protein, glucose, KET, urobilinogen, urobilirubin, and nitrite are numeration data, and RBCs are easily altered by vaginal bleeding. Therefore, these indicators are not suitable for establishing an ROC curve. The AUC was calculated by establishing the ROC curve. We found that bacteria had the largest AUC (0.696). The AUC for electrical conductivity was 0.596 and that for crystals was 0.577. The ROC curve was established by combining these three indicators in pairs and we found that the AUC did not increase (Figure 1a–f).

Predicted value of indicators

After determining the cut-off value of the ROC curve, the sensitivity, specificity, PPV, NPV, +LR, and −LR were calculated to estimate the predictive value of each indicator. When the variable bacteria had a cut-off of 130.15, the sensitivity was 58%, the specificity was 79%, the PPV was 73.4%, the NPV was 65.3%, +LR was 2.76, and –LR was 0.53 (Table 4).

Table 2. Distribution of data for each group.

Variable	PPROM	PROM	Full-term	Nonpregnant
Age	31.48 ± 0.55	31.75 ± 0.44	30.11 ± 0.40	31.63 ± 5.06
Days	228.12 ± 3.60	267.06 ± 0.44	282.49 ± 0.41	–
LEU	2.13 ± 0.17	2.27 ± 0.15	2.19 ± 0.14	1.00 ± 0.00
BLD	1.96 ± 0.14	1.33 ± 0.09	1.26 ± 0.07	1.00 ± 0.00
PRO	1.36 ± 0.08	1.28 ± 0.07	1.11 ± 0.04	1.00 ± 0.00
GLU	1.84 ± 0.16	1.14 ± 0.07	1.22 ± 0.08	1.00 ± 0.00
KET	1.97 ± 0.15	1.42 ± 0.11	1.16 ± 0.06	1.00 ± 0.00
UBG	1.02 ± 0.02	1.05 ± 0.04	1.04 ± 0.03	1.00 ± 0.00
BIL	–	1.02 ± 0.02	–	1.00 ± 0.00
pH	6.43 ± 0.06	6.56 ± 0.06	6.63 ± 0.05	6.04 ± 0.59
SG	1.01 ± 0.00	1.01 ± 0.00	1.01 ± 0.00	1.02 ± 0.01
NIT	1.02 ± 0.02	1.03 ± 0.03	1.03 ± 0.03	1.00 ± 0.00
WBC	80.76 ± 16.83	64.30 ± 14.55	56.58 ± 14.86	6.16 ± 5.15
RBC	191.96 ± 73.38	19.70 ± 5.56	22.45 ± 12.19	11.06 ± 8.19
EC	25.17 ± 5.81	24.90 ± 2.37	26.21 ± 3.25	9.73 ± 8.47
Cast	1.15 ± 0.33	1.14 ± 0.29	0.77 ± 0.07	0.44 ± 0.41
P.CAST	0.38 ± 0.04	0.40 ± 0.04	0.50 ± 0.14	0.21 ± 0.21
BAC	870.87 ± 326.98	1207.75 ± 374.58	1296.69 ± 317.22	300.77 ± 394.63
SRC	6.89 ± 2.05	9.92 ± 3.37	5.47 ± 0.51	1.44 ± 1.06
BYST	0.91 ± 0.61	–	0.46 ± 0.43	0.00 ± 0.00
Crystals	4.10 ± 2.82	6.02 ± 5.79	12.05 ± 11.52	0.48 ± 1.83
Cond.	13.28 ± 0.84	18.69 ± 0.62	15.91 ± 0.72	20.46 ± 5.99

Values are mean ± standard deviation. PPROM: preterm premature rupture of the membranes; PROM: premature rupture of the membranes; days: days of gestation; LEU: leukocytes; BLD: occult blood; PRO: protein; GLU: glucose; KET: ketone bodies; UBG: urobilinogen; BIL: urobilirubin; SG: urine specific gravity; NIT: nitrite; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; P.CAST: pathological cast; BAC: bacteria; SRC: small round cells; BYST: yeast; Cond.: electrical conductivity.

Discussion

The occurrence of PPROM is often the result of multifactorial interactions. Among them, retrograde infection of reproductive tract pathogens is the most important cause. In women, the urethra and reproductive tract are susceptible to exchange of bacteria. To some extent, the amount of bacteria in a routine urine test can reflect the microecological status of the female perineum.[20] When PPROM occurs, dominant bacteria, such as *Lactobacillus* in the vagina, are replaced by pathogenic bacteria, and the diversity of the flora is significantly reduced.

By analyzing routine urine data in the PPROM, PROM, full-term, and normal groups, we found significant differences in various variable among the groups. We found that PROM was more likely to occur with age. Occult blood and the RBC count were significantly higher in the PPROM group compared with the other groups. This finding is probably because patients with PPROM were more likely to have vaginal bleeding symptoms. The WBC count was lower in the normal (nonpregnant) group than in the other three groups, but no differences were observed among the pregnant groups. These results suggest that women may have mild or asymptomatic urethral infection during pregnancy, but WBCs cannot be used as a diagnostic indicator for PROM. The

Table 3. Pairwise comparisons for each group.

	Mann–Whitney U					
Variable	PPROM versus PROM	PPROM versus Full-term	PPROM versus Normal	PROM versus Full-term	PROM versus Normal	Full-term versus Normal
Age	0.65	0.03*	0.65	<0.01*	0.90	<0.01*
Days	<0.01*	<0.01*	–	<0.01*	–	–
LEU	0.39	0.53	<0.01*	0.84	<0.01*	<0.01*
BLD	<0.01*	<0.01*	<0.01*	0.90	<0.01*	<0.01*
PRO	0.48	0.01*	<0.01*	0.03*	<0.01*	0.01*
GLU	<0.01*	<0.01*	<0.01*	0.30	0.01*	<0.01*
KET	<0.01*	<0.01*	<0.01*	0.04*	<0.01*	<0.01*
UBG	0.56	0.56	0.32	0.99	0.16	0.16
BIL	0.32	1.00	1.00	0.32	0.32	1.00
pH	0.12	0.01*	<0.01*	0.41	<0.01*	<0.01*
SG	0.02*	0.75	<0.01*	<0.01*	<0.01*	<0.01*
NIT	0.99	0.99	0.32	1.00	0.32	0.32
WBC	0.59	0.93	<0.01*	0.56	<0.01*	<0.01*
RBC	0.01*	<0.01*	0.02*	0.38	0.82	0.21
EC	0.04*	0.10	<0.01*	0.64	<0.01*	<0.01*
CAST	0.93	0.16	<0.01*	0.25	<0.01*	0.01*
P.CAST	0.80	0.93	<0.01*	0.90	<0.01*	<0.01*
BAC	<0.01*	<0.01*	0.15	0.64	<0.01*	<0.01*
SRC	0.06	0.04*	<0.01*	0.84	<0.01*	<0.01*
BYST	0.02*	0.25	0.02*	0.16	1.00	0.16
Crystal	0.03*	0.05*	0.10	0.85	0.63	0.78
Cond.	<0.01*	0.02*	<0.01*	0.01*	0.07	<0.01*

*$P < 0.5$ was considered statistically significant. PPROM: preterm premature rupture of the membranes; PROM: premature rupture of the membranes; days: days of gestation; LEU: leukocytes; BLD: occult blood; PRO: protein; GLU: glucose; KET: ketone bodies; UBG: urobilinogen; BIL: urobilirubin; SG: urine specific gravity; NIT: nitrite; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; P.CAST: pathological cast; BAC: bacteria; SRC: small round cells; BYST: yeast; Cond.: electrical conductivity.

amount of bacteria was significantly lower in the PPROM group than in the PROM and full-term groups, but there was no difference between the PPROM and normal groups. This finding suggested that the amount of bacteria in the urinary tract in PPORM was significantly reduced, although it was still higher than that in the normal group. The decrease in bacteria is a risk factor, indicating a decrease in the diversity of flora. Crystals and electrical conductivity were significantly reduced in the PPROM group. This low electrical conductivity in the PPROM group may be related to maternal endocrine function and an increase in secreted aldosterone, which could lead to an increase in sodium and chloride reabsorption in the kidney.[21]

We first used non-parametric test methods to screen differential indicators between the PPROM and normal groups. ROC curves were then used to further select indicators with good sensitivity, specificity, PPV, NPV, +LR, and −LR. However, this study has some limitations. The main limitation is that none of the metrics showed high enough AUC values. This also indicates that use of these indicators

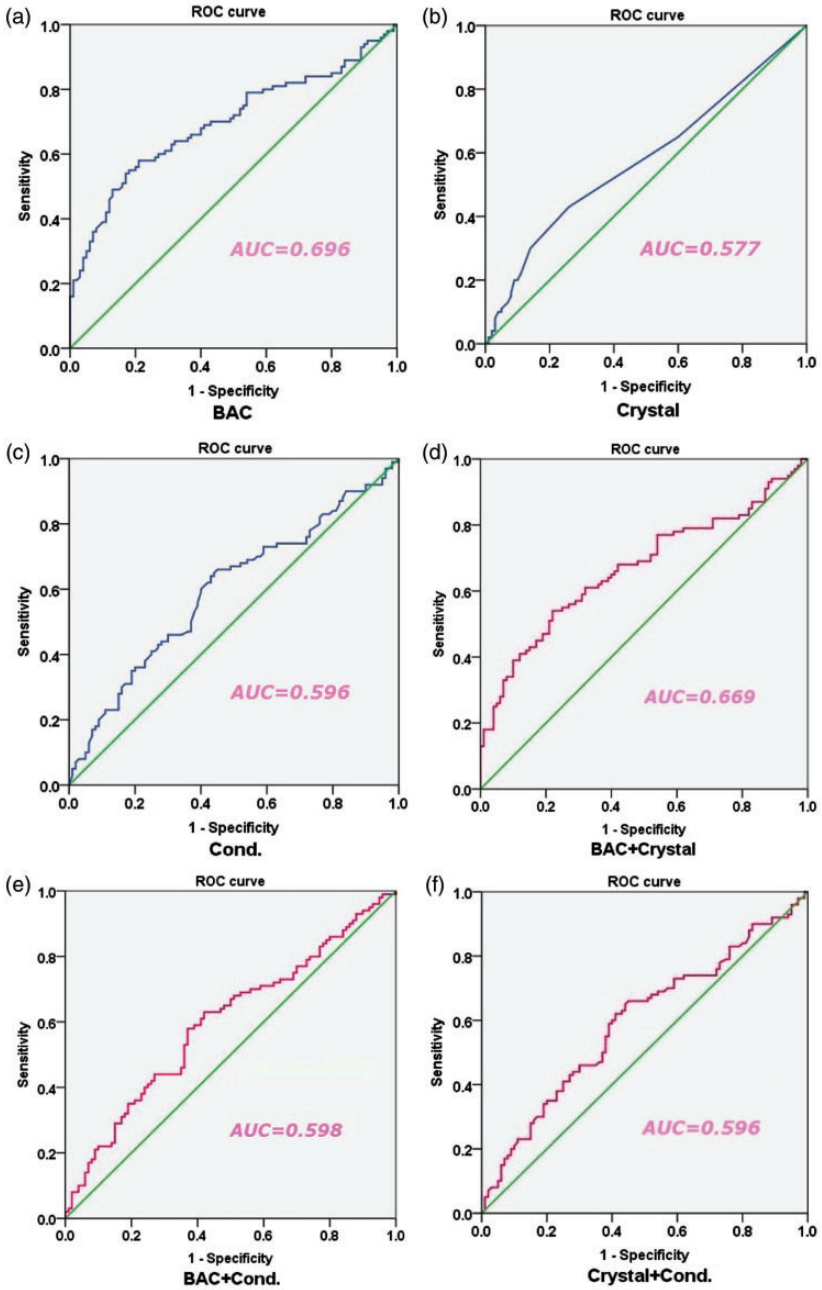

Figure 1. ROC curve of different indicators. (a) BAC; (b) crystals; (c) Cond.; (d) BAC+crystals; (e) BAC+Cond.; (f) crystals+Cond. AUC: area under the curve; ROC: receiver operating characteristic; BAC: bacteria; Cond.: electrical conductivity.

Table 4. Comparison of the predictive value of different indicators.

Variable	Youden index	Cut-off value	Sensitivity	Specificity	PPV	NPV	+LR	−LR
BAC	0.37	130.15	58%	79%	73.4%	65.3%	2.76	0.53
Crystal	0.3	73.9	57%	73%	67.9%	62.9%	2.11	0.59
Cond.	0.21	15.15	64%	57%	58.9%	61.4%	1.49	0.63
BAC+crystals	0.32	0.5128	54%	78%	69.2%	62.3%	2.45	0.59
BAC+Cond.	0.21	0.5097	58%	63%	61.1%	60%	1.57	0.67
Crystals+Cond.	0.21	0.4948	65%	56%	59.6%	61.5%	1.48	0.63

PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; −LR: negative likelihood ratio; BAC: bacteria; Cond.: electrical conductivity.

to predict occurrence of PROM is of limited value and must be combined with other indicators. Additionally, qualified urine specimens are precursors to authenticity of the data, although all of the urine specimens used in this study underwent preliminary conformance testing. However, because of the special circumstances of pregnant women, especially pregnant women with PPROM, obtaining uncontaminated urine samples again in a short time is difficult.

Conclusions

Identifying an indicator with better specificity, accuracy, and practicality in diagnosing subclinical chorioamnionitis and predicting the occurrence of PPROM is still a large challenge. This study shows that a routine urine examination is of certain value in early diagnosis of PPROM. In particular, a reduction in the amount of bacteria in the urine sample is a high-risk factor, indicating the loss of normal bacterial floral diversity. The results of this study are helpful for timely screening of high-risk pregnant women with PPROM. Our findings could also be helpful for selecting the most appropriate time for treatment or termination of pregnancy, which has important significance for improving the prognosis of mothers and children.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Guanghui Zhao http://orcid.org/0000-0001-9417-8421

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