Urine Routine Test has Potential Predictive Value in Premature Rupture of the Membranes

Zhuo Deng
Dalian Medical University
Dan Lu (ludan1968@126.com)
yangzhou university
Xuanqi Wang
Dalian Maritime University
Jingyi Wang
Dalian Medical University

Research article

Keywords: Routine urine test, premature rupture of membranes (PROM), preterm premature rupture of membranes (PPROM), vaginal microflora, bacteria

Posted Date: February 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-271808/v1

Abstract

Background: This study was conducted to discuss predictive value of a routine urine test for premature rupture of the membranes(PROM).

Methods: We carried out the retrospective research after collecting routine urine test data from 45 cases of full preterm premature rupture of membranes (PPROM) and 45 cases of full-term preterm premature rupture of membranes (fPROM). In addition 70 healthy pregnant women (Normal) and 70 non-pregnant adult healthy women were enrolled. Parametric and Non-parametric tests was performed respectively. The receiver operating characteristic (ROC) was established and we further calculated the area under the ROC curve (AUC). In this study multiple cutoffs were selected, afterwords the positive predictive value (PPV), the negative predictive value (NPV), the positive likelihood ratio (+LR) and negative likelihood ratio (-LR) were further calculated by sensitivity and specificity with the aim of finding the best cutoff point.

Results: The results indicated that S/G and COND were significantly different between PROM and Non-pregnant and Normal groups. Significant differences in pH, WBCs, RBCs, BAC and EC between the PPROM and Normal groups were observed. When the cutoff for bacteria was 89.15, it had the largest AUC of 0.744. We found that its PPV 70.6%, NPV was 74.1%, +LR was 3.79, and –LR was 0.55.

Conclusion: A routine urine test especially for bacterial counts can be used to predict the risk of PROM, which is expected to provide considerable predictive value for PROM.

1. Background

Premature rupture of membranes (PROM) refers to rupture of membranes before delivery, which is one of the common complications in obstetrics, with an incidence of 8%-10% [1]. Complications such as infection, trauma increased pressure of amniotic cavity and gestational diabetes may lead to rupture of membranes [2–3]. Preterm premature rupture of the membranes (PPROM) means the rupture of the membranes before labor starts prior to 37 weeks of gestation, which remains a significant obstetric problem that affects 3–4% of all pregnancies and precedes 40–50% of all preterm births [4]. The number of PPROM cases exceeds that of preelampsia and gestational diabetes. According to the report, neonatal death in newborns without chromosomal abnormality or congenital anomaly was mainly caused by prematurity [5–6]. In addition preterm births is also related to a series of long-term effects in survivors, including neurodevelopmental delay, cerebral palsy, blindness, hearing loss, and chronic lung disease [7, 8]. However, the empirical treatment that ignore the complexity and heterogeneity of PPROM pathopHysiology are not satisfactory, antibiotic therapy and antenatal corticosteroid treatment are typically administered to prolong pregnancy, prevent infection, and reduce gestational age dependent morbidities [9], and the result is futile because probably 90% of pregnant women give birth within one week [10–12].

PPROM results from complex, multifaceted pathways, and precise causes or risk factors of are unknown. Some research showed the etiology of PPROM was multifactorial, such as maternal reproductive tract

infections (e.g., bacterial vaginosis BV, trichomoniasis, gonorrhea, Chlamydia, and occult chorioamnionitis), behavioral factors (e.g., cigarette smoking, substance abuse, poor nutritional status, and coitus during pregnancy) and obstetric complications (e.g., multiple gestation, polyhydramnios, incompetent cervix, gestational bleeding, prior cervical surgery, and antenatal trauma) [13, 14]. Among of them, ascending bacterial invasion may lead to intrauterine infection that is the most common risk factor, which account for up to 60% of cases with PPROM [15, 16]. There are some additional risk factors for PPROM, history of PPROM in a previous pregnancy have been proposed [17, 18]. The pathogenesis are still unclear and recent studies have shown both disruption of fetal membrane integrity and activation of uterine contraction can be cauesd by inflammatory mediators. Current study showed inflammation–oxidative stress axis plays a major role in producing pathways that can lead to membrane weakening through a variety of processes. Bacterial products or/and pro-inflammatory cytokines can trigger that the membrane morpHology with PPROM altered. Activation of matrix metalloproteinases (MMP) have been implicated in the mechanism of PPROM [19]. The vaginal microflora of a healthy asymptomatic woman was consisted of a wide variety of anaerobic and aerobic bacterial genera and species dominated include the facultative, microaeropHilic, anaerobic genus Lactobacillus. The activity of Lactobacillus is essential to protect women from genital infections and to maintain the natural healthy balance of the vaginal flora. There is more and more evidence that abnormalities in vaginal flora during pregnancy is associated with preterm labor and delivery with potential neonatal sequelae due to prematurity and poor perinatal outcome pregnancy [20–23].

Early diagnosis of PPROM is necessary and important. It is possible to prevent PROM if treatment can be performed in the early stage of chorionic villous infection, but PROM is inescapable after amniotic layer occurs infection [24], with the reason that the chorion is thicker than amnion but has less tensile strength [25] Accurate diagnosis of PROM remains a frequent clinical problem in obstetrics. At present, there are only several tests to confirm a diagnosis of PPROM post-facto, including microfetal cell identification, amniotic fluid crystallization and intra-amniotic dye injection. The disadvantages of intra-amniotic injection are invasive, which increases the risk of infection and premature delivery. The inadequacy of microfetal cell identification or amniotic fluid crystallization is the long detection period and the high false positive rate, and not any method to reliably predict PPROM [26]. It is the lack of a non-invasive gold standard for the diagnosis of PROM that led to the appearance of several tests based on alternative biochemical markers [27]. The diagnostic performance of traditional indicators reflecting inflammation or infection includes leucocytes, IL-6, C-reactive protein (CRP), and procalcitonin (PCT), vaginal prolactin, alpHa-feto-protein (AFP), fetal fibronectin and insulin-like growth factor binding protein-1 (IGFBP-1), whcih need to be improved [28, 29] As a result, the biomolecular markers with high sensitivity and specifificity that can predict PPROM plays a very important role, which is the key of early clinical diagnosis [30].

Recent studies suggested that urine test is helpful for timely screening of high-risk pregnant women with PPROM. Urine test is a routine process for the hospitalized patients, which has good operability, low cost and non-invasiveness. It includes 20 important indicators, named leukocytes; (BLD): occult blood; (PRO): protein; (GLU): glucose; (KET): ketone bodies; (UBG): urobilinogen; (BIL): urobilirubin; pH; (SG): urine specific gravity; (NIT): nitrite; (WBCs): white blood cells; (RBCs): red blood cells; (EC): epithelial cell count;

Cast; (P.CAST): pathological cast; (BAC): bacteria; (SRC): small round cells; (BYST): yeast; Crystals; (Cond): electrical conductivity. The aim of this study was designed to investigate the value of urine test in diagnosis and prediction of PPROM.

2. Methods

2.1 Patients

This comparative prospective study was carried out over 1 year at Subei People's Hospital of Yangzhou University from February 2018 to February 2019. Patients with multiple pregnancies, antibiotic therapy in the past 2 weeks and urinary tract infection were excluded from this study. A total of 70 pregnant women with Normal gestational age > 37 weeks and < 42 weeks, 70 healthy Non-pregnant adult healthy women were included in this study. The 90 patients in premature rupture of membranes were divided into two groups according to gestation; gestational age > 37 weeks were included in PROM and < 37 weeks were included in PPROM. All patients received routine urine tests within 7 days before rupture of the fetal membranes. Urine routine specimens were collected within 24 hours before delivery for healthy pregnant women women. Clean midstream urine specimens in healthy women randomly collected. Diagnostic criteria for PROM are as follows: (a)patient's history of sudden gush of water, (b)pooling of amniotic fluid, (c)positive Ferning pattern, (d)positive Nitrazine test, (e)confirmed by visualization of fluid passing from the cervical canal during sterile speculum examination and (f)transabdominal ultrasound to measure the amniotic flfluid index (AFI ≤ 5 cm in PROM) [31, 32]. This experiment has no intervention measures and ensures the safety of personal privacy information, so informed consent and ethical approval are exempted.

2.2 Urine sample collection and processing

The women's clean mid-stream urine were collected by a disposable cup. The Arkray AX-4280 (Arkray Corp., Kyoto, Japan) was used to measure dry chemical analysis of urine that included eukocytes, occult blood, protein, glucose, ketone bodies (KET), urobilinogen, urobilirubin, pH values, urine specifific gravity (SG), and nitrite. Urinary components were analyzed by the Iris IQTM200 (Iris Corp., USA), which included white blood cells (WBCs), red blood cells (RBCs), epithelial cell count (EC), cast, bacterial counts (BAC), pathological cast, small round cells, yeast, crystals, and electrical conductivity (COND). A microscopic examination was used to confirm the numbers of WBC, RBC, EC and cast, because the samples could not be correctly detected by an instrument.

2.3 Data analysis

Data were collected, tabulated and analyzed by Statistical Package for Social Sciences (SPSS) computer software version 21. Before comparison of data, a general description of the data was performed. Firstly, the normality of distribution of continuous variables was tested by the KolmogorovSmirnov, continuous variables with a normal distribution are presented as the mean and standard deviation; non-normal variables were shown as median (interquartile range). Then the homogeneity of variance of two samples

was tested by the Levene method, the means groups of two groups continuous normally distributed variables were compared by independent sample Student's t-test. The Mann-Whitney U-test was used to compare the means of two groups of variables not normally distributed. $P < 0.05$ was considered to indicate a statistically significant difference.

2.4 Establishing of ROC curve

Sensitivity is the proportional detection of individuals with the disease of interest in the population. Specificity is the proportional detection of individuals without the disease of interest in the population. Both of them can be used to evaluate the authenticity of the model. The PPV is the proportion of all individuals with positive tests, who have the disease. The NPV is the proportion of all individuals with negative tests, who are non-diseased. The prediction ability of the model can be evaluated by PPV and NPV. Different cutoff point were used to calculate true positive rate (sensitivity) and false positive rate (1-specifificity) respectively, ROC curve was shown after the sensitivity and 1-specifificity were respectively plotted on the ordinate and the abscissa. The diagnostic values of the model was assessed via ROC curve and the AUC. AUC was calculated to determine which indicator had the largest AUC. When the two indicators need joint detection, the logistic regression analysis is used to generate the prediction probability and the ROC curve is performed to generate probability.

2.5 Diagnostic value assessment

The closer to the upper left corner of the ROC curve, the better the diagnosis of the model. In practice clinicians need a cutoff point to determine whether intervention is required after establishing the utility of a continuous indicator. The Youden index (J) can serve as an overall index of a indicator's accuracy, so cutoff point corresponding to the maximizing Youden index can be utilized for decision making [33]. J was expressed as J={ sensitivity + specificity−1} [34]. In this study multiple cutoffs were selected to calculate sensitivity and specificity, afterwords PPV, NPV, the positive likelihood ratio (+ LR) and negative likelihood ratio (-LR) were further calculated by sensitivity and specificity with the aim of finding the best cutoff point, which are meaningful indicators for the effectiveness.

3. Results

3.1 The basic situation of the research object

A total of 400 women were screened, of them 230 eligible met inclusion criteria and consented to study procedures. These numeration data, including occult blood, protein, glucose, KET, urobilinogen, urobilirubin, nitrite and crystal are not suitable for establishing an ROC curve, which were not selected and compared. As shown in Table 1, WBCs, RBCs, BAC, and EC do not satisfy the homogeneity of variance, $\alpha = 0.01$ as the test level. KolmogorovSmirnov method was used to test the normality, only COND are normal distributions in the four terms, we used the mean and standard deviation to describe the data distribution in Table 2. Similarly, $\alpha = 0.01$ is the test level.

Table 1
The results of normality and homogeneity of variance test

variable	Homogeneity		Normality test							
	of variance		PPROM		fPROM		Normal		Non-pregnant	
	Stat.	P	Stat.	P	Stat.	P	Stat.	P	Stat.	P
S/G	1.409	0.241	1.014	0.17	1.014	< 0.01	1.019	< 0.01	1.022	< 0.01
pH	2.868	0.037	6.8	< 0.01	6.8	< 0.01	6.3	< 0.01	6	< 0.01
WBC	13.891	< 0.01	147.3	< 0.01	105.8	< 0.01	47.4	< 0.01	17.6	< 0.01
RBC	14.465	< 0.01	202.1	< 0.01	432.6	< 0.01	34.9	< 0.01	26.3	< 0.01
BAC	9.964	< 0.01	305.2	< 0.01	285.6	< 0.01	809.7	< 0.01	273.5	< 0.01
EC	9.724	< 0.01	33.96	< 0.01	35.25	< 0.01	46.13	< 0.01	23.24	< 0.01
CAST	3.043	0.03	0.28	< 0.01	0.24	< 0.01	0.25	< 0.01	0.28	< 0.01
COND	1.609	0.188	15.4	0.018	14.6	0.2	17.8	0.2	18.7	0.2

SG: urine specific gravity; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; CAST: cast; BAC: bacterial counts; Cond.: electrical conductivity

Table 2
Distribution of each group

Variable	PPROM	fPROM	Normal	Non-pregnant
S/G	1.01 + 0.07	1.01 ± 0.10	1.02 ± 0.13	1.02 ± 0.10
pH	7.00 ± 1.50	6.50 ± 0.50	6.50 ± 1.00	6.00 ± 1.00
WBC	14.40 ± 55.50	21.00 ± 60.00	32.00 ± 66.70	7.70 ± 22.90
RBC	13.50 ± 182.90	33.20 ± 45.81	7.60 ± 28.80	12.40 ± 14.20
BAC	77.70 ± 314.60	117.70 ± 281.80	413.10 ± 1286.00	69.30 ± 217.00
EC	20.10 ± 33.45	25.50 ± 34.35	55.20 ± 74.70	14.60 ± 30.70
CAST	0.14 ± 0.34	0.13 ± 0.27	0.23 ± 0.41	0.13 ± 0.28
COND	15.44 ± 6.37	13.73 ± 5.25	16.97 ± 6.90	18.65 ± 1.00

Values are mean standard deviation. SG: urine specific gravity; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; CAST: cast; BAC: bacterial counts; Cond.:electrical conductivity.

3.2 Variable comparison

The pairwise comparisons among the PPROM, fPROM, Normal, and Non-pregnant groups were performed by the Mann-Whitney U-test. Table 3 indicated that pH was significantly lower in the Non-pregnant group compared with the other three groups (all $P < 0.05$). In addition, there was significant difference between fPROM and Non-pregnant and Normal groups regarding S/G and COND (all $P < 0.05$). CAST was significantly lower in the Normal group compared with Non-pregnant and fPROM groups (all $P < 0.05$). Statistical analysis showed that pH, WBCs, RBCs, BAC and EC were significantly different between the PPROM and Normal groups (all $P < 0.05$), RBCs, BAC and EC were significantly different between the fPROM and Normal groups (all $P < 0.05$). The next ROC curve was established by the parameters with significant difference.

Table 3
Mann–Whitney U test for each groups

Variable	fPROM vs Non-pregnant	Normal vs Non-pregnant	PPROM vs Non-pregnant	fPROM vs Normal	fPROM vs PPROM	Normal vs PPROM
S/G	< 0.01*	0.33	< 0.01*	< 0.01*	0.92	0.06
pH	< 0.01*	< 0.01*	< 0.01*	0.16	0.41	0.04*
WBC	< 0.01*	< 0.01*	0.08	0.16	0.26	0.02*
RBC	< 0.01*	0.12	0.15	< 0.01*	0.1	0.02*
BAC	0.2	< 0.01*	0.65	< 0.01*	0.53	< 0.01*
EC	0.03*	< 0.01*	0.21	< 0.01*	0.36	< 0.01*
CAST	0.71	< 0.01*	0.16	< 0.01*	0.05	0.49
COND	< 0.01*	0.18	< 0.01*	< 0.01*	0.23	0.16

*P < 0.05 was considered statistically significant. SG: urine specific gravity; WBCs: white blood cells; RBCs: red blood cells; EC: epithelial cell count; CAST: pathological cast; BAC: bacterial counts; Cond.: electrical conductivity.

3.3 ROC curve

In order to meet the requirement, the ROC curve was established between PPROM and Normal groups. According to the result of variable comparison, RBCs were excluded these inappropriate indicators, which are easily susceptible to vaginal bleeding. We selected three indicators to establish the ROC curve, including pH, BAC, pH + BAC (Fig. 1A,1B,1C). 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 (AUC), the higher the diagnostic accuracy. As shown in Fig. 1, the AUC of pH and BAC were respectively 0.608 and 0.744, the joint detection of pH + BAC had the AUC (0.735), we found the AUC for BAC was the largest.

3.4 Predicted value

The Youden index is a summary index for the overall performance of the ROC curve, best one of which is equivalent to maximizing the sum of sensitivity and specificity for all the possible values, corresponding to the cut-off point. Then the predictive value of each indicator was estimated by the sensitivity, specificity, PPV, NPV, +LR, and -LR. Table 4 indicated that When the variable bacteria had a cutoff of 81.95, the sensitivity was 53%, the specificity was 86%, the PPV was 70.6%, the NPV was 74.1%, +LR was 3.79, and –LR was 0.55.

Table 4
Comparison of the predictive value of different indicators.

variable	Youden index	Cut-off value	Sensitivity	Specificity	PPV	NPV	+LR	-LR
pH	0.390	81.95	53%	86%	70.6%	74.1%	3.79	0.55
BAC	0.207	6.75	58%	63%	50%	69.8%	1.57	0.67
pH + BAC	0.311	0.3207	91%	40%	53.2%	89.5%	1.52	0.23
[a]Predictive probability.								

4. Discussion

More and more studies confirmed multifactorial interactions induced the occurrence of PPROM. Vaginal infection was one of the most main risk factors for complications of pregnancy. As for women, the microecological of urethra and reproductive tract are easy to be influenced by exchange of bacteria. That is to say, the amount of bacteria in a routine urine test can reflect the status of the female vagina [35]. Previous research has examined lactobacilli predominate in normal circumstances, which is essential to protect women from genital infections and to maintain the natural healthy balance of the vaginal flora [36, 37]. In addition, the hormonal changes of pregnancy favored an increase in the concentration of lactobacilli [38]. However, in the patients with PPROM the normal healthy flora can be disturbed, and dominant bacteria can be replaced by pathogenic bacteria with the result of the decrease of lactobacilli.

By analyzing the 45 cases of PPROM, 45 cases of fPROM, 70 cases of Normal and 70 cases of Non-pregnant maternal, significant differences were observed among groups. pH was significantly higher in the PPROM group compared with Normal group. The pH of the amniotic fluid is normally 7.1–7.3, however the vaginal secretions usually has a pH of 4.5-6.0. The change of pH have confirmed occurrence PPROM [39, 40]. S/G and COND were significantly lower in the fPROM group compared with Non-pregnant and Normal groups, which is related to an increase in secreted aldosterone for pregnant women, further lead to the kidney reabsorb more sodium and chloride [41]. The WBCs and EC was lower in PPROM groups than Normal groups, which cannot be used to predict PPROM with the reason that mild or asymptomatic urethral infection may happen in pregnant women. The RBCs in the PPROM group were significantly higher than Normal group, which is probably due to the explanation that vaginal bleeding symptoms may occur in patients with PPROM. The results indicated that BAC in PPORM was

significantly less than Normal group, which indicated a decrease in the diversity of flora and increased the risk of PPORM [42].

In this study, non-parametric test between the PPROM and Normal groups was carried out, which screen out the different indicators. The better indicators with high sensitivity, specificity, PPV, NPV, +LR and -LR were selected by the establishment of ROC curve, the corresponding Youden index and cutoff point were further worked out. However there are some limitations, the main one is the AUC values of metrics were not high enough so that prediction value is limited. This also suggests that the urine routine only screen out the pregnant women with high-risk of PPROM, which must be combined with other indicators to predict PPROM.

5. Conclusions

An excellent indicator that can timely screen out pregnant women with PPROM is quietly important and necessary to diagnosis PPROM and and chorioamnionitis, which is helpful for preventing neonatal infection. To the best of the authors' knowledge, the research provide evidence that a routine urine examination has potential value in early prediction of PPROM. It needs to attach great importance that a decrease in the amount of bacteria in the urine sample is a high-risk factor, which indicates the loss of normal bacterial floral diversity. As a result the present study suggested that routine urine may be a novel potential indicator for early diagnosing of PPROM and the routine urine-based strip may be a helpful for preventing chorioamnionitis and reducing the maternal and perinatal morbidity.

Abbreviations

premature rupture of membranes (PROM); preterm premature rupture of membranes (PPROM); full-term preterm premature rupture of membranes (fPROM); the receiver operating characteristic (ROC); the area under the ROC curve (AUC); the positive predictive value (PPV); the negative predictive value (NPV); the positive likelihood ratio (+ LR); the negative likelihood ratio (-LR); occult blood (BLD); protein (PRO); glucose (GLU); ketone bodies (KET); urobilinogen (UBG); urobilirubin (BIL); pH; urine specific gravity (SG); nitrite (NIT); white blood cells (WBCs); red blood cells (RBCs); epithelial cell count (EC); pathological cast (P.CAST); bacteria (BAC); small round cells (SRC); yeast(BYST);electrical conductivity (Cond)

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Subei people's Hospital of Yangzhou University. All patients involved in the study signed informed consent forms.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing financial interest.

Funding

We gratefully acknowledge support from the National Natural Science Foundation of China (No. 82072088) of Dan Lu in interpretation of data and in writing, the Traditional Chinese Medicine Science and Technology Development Plan Project of Jiangsu Province (Project ID: YB201972) of Dan Lu in collection; Maternal and Child Health Research Project of Jiangsu Province (Project ID: F201809) of Dan Lu in interpretation of data.

Authors' contributions

D L Protocol/project development

Z D Manuscript writing/editing

Qq W Data analysis

Jy W Data collection or management

References

1. Yan YW, Hai BL, Guang LC., **et al**. **Placental protein 14 as a potential biomarker for diagnosis of preterm premature rupture of membranes**. Molecular Medicine Reports, 2018 (**18**):**113–122**.
2. Liu L, Wang L, Yang W. **et al**. Gestational hypertension and preclampsia and risk of spontaneous premature rupture of membranes: A population-based cohort study. International Journal of Gynecology Obstetrics. 2019;147(7):195–2010.
3. Caballero A, Dudley D, Ferguson J. **et al**. Maternal Human Papillomavirus and Preterm Premature Rupture of Membranes: A Retrospective Cohort Study. Journal of Womens Health. 2019;28(5):606–11.
4. Menon R, Richardson LS. **Preterm prelabor rupture of the membranes: A disease of the fetal membranes**. Seminars in Perinatology 2017: **S0146000517300848**.
5. Silverman RK, Wojtowycz M. Risk factors in premature rupture of membranes. Prim Care Update Ob Gyns. 1998;5(4):181.
6. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84.

7. Merenstein GB, Weisman LE. Premature rupture of the membranes: neonatal consequences. Semin Perinatol. 1996;20:375–80.
8. Waters TP, Mercer B. Preterm PROM: prediction, prevention, principles. Clinical Obstetrics Gynecology. 2011;54(2):307.
9. Menon R. Spontaneous preterm birth, a clinical dilemma: etiologic, pathopHysiologic and genetic heterogeneities and racial disparity. Acta Obstet Gynecol Scand. 2008;87(6):590–600.
10. Wolf MF, Miron D, Peleg D. **et al**. Reconsidering the current preterm premature rupture of membranes antibiotic propHylactic protocol. Am J Perinatol. 2015;32:1247–50.
11. Al Riyami N, Al-Ruheili I, Al-Shezaw F. **et al**. Extreme preterm premature rupture of membranes: risk factors and feto maternal outcomes. Oman Med J. 2013;28:108–11.
12. Macones GA, Parry S, Elkousy M, Clothier B, Ural SH. A polymorpHism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. Am J Obstet Gynecol. 2004;190(6):1504–8., Strauss JF **III**.
13. Burns DN, Landesman S, Muenz LR. **et al**. Cigarette smoking, premature rupture of membranes, and vertical transmission of HIV-1 among women with low CD4 + levels. J Acquir Immune Defific Syndr. 1994;7(7):718–26.
14. Silverman RK, Wojtowycz M. Risk factors in premature rupture of membranes. Prim Care Update Ob Gyns. 1998;5(4):181.
15. Waters TP, Mercer B. Preterm PROM. Prediction, Prevention, Principles[J]. Clin Obstet Gynecol. 2011;54(2):307–12.
16. Tülay Oludag, Gode F, Caglayan E. **et al**. Value of maternal procalcitonin levels for predicting subclinical intra-amniotic infection in preterm premature rupture of membranes[J]. Journal of Obstetrics Gynaecology Research. 2014;40(4):954–60.
17. Mercer BM, Goldenberg RL, Moawad AH. **et al**. The preterm prediction study: effect of gestational age and cause of preterm birth on subsequent obstetric outcome. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol. 1999;181(5 pt 1):1216–21.
18. Asrat T, Lewis DF, Garite TJ. **et al**. Rate of recurrence of preterm premature rupture of membranes in consecutive pregnancies. Am J Obstet Gynecol. 1991;165(4 pt 1):1111–5.
19. Tchirikov M, Maher J, Andreas S. **et al**. Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome[J]. Journal of Perinatal Medicine Official Journal of the Wapm. 2018;46(5):465–88.
20. Donati L, Vico AD, Nucci M. **et al**. Vaginal microbial flora and outcome of pregnancy[J]. Archives of Gynecology Obstetrics. 2010;281(4):589–600.
21. Witkin SS, Linhares IM, Giraldo P. Bacterial flflora of the female genital tract. function immune regulation. 2007;21(3):347–54. ().

22. Lidbeck A, Nord CE. Lactobacilli and the normal human anaerobic microflflora. Clin Infect Dis. 1993;16(Suppl 4):181–7. ().
23. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P. Hydrogen peroxide-producing Lactobacilli and acquisition of vaginal infections. J Infect Dis. 1996;174(5):1058–63. ().
24. Gratacos E, Sanin-Blair J, Lewi L. **et al**. A histological study of fetoscopic membrane defects to document membrane healing. Placenta. 2006;27:452–6.
25. Parry S, Strauss JF. Premature rupture of the fetal membranes. N Engl J Med. 1998;338:663–70.
26. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84.
27. Abdelazim IA. Insulin-like growth factor binding protein-1 (Actim PROM test) for detection of premature rupture of fetal membranes[J]. Journal of Obstetrics Gynaecology Research. 2014;40(4):7.
28. Jeurgens-Borst AJ, Bekkers RL, Sporken JM. Use of insulin like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes. Eur J Obstet Gynecol Reprod Biol. 2002;102:11–4., **van der** Berg PP.
29. Buyukbayrak EE, Turan C, Unal O, Dansuk R, Cengizoglu B. Diagnostic power of the vaginal washing-flfluid prolactin assay as an alternative method for the diagnosis of premature rupture of membranes. J Matern Fetal Neonatal Med. 2004;15:120–5.
30. Tsakiridis I. **Mamopoulos**, **et al**. Preterm Premature Rupture of Membranes: A Review of 3 National Guidelines. Obstet Gynecol Surv. 2018;73(6):368–75.
31. **Di** Renzo GC, Roura LC, Facchinetti F **et al**. Guidelines for the management of spontaneous preterm labor: Identifification of spontaneous preterm labor, diagnosis of preterm premature rupture of membranes and preventive tools for preterm birth. J Matern Fetal Neonatal Med 2011; 24: 659–67.
32. El-Messidi A, Cameron A. Diagnosis of premature rupture of membranes: Inspiration from the past and insights for the future. J Obstet Gynaecol Can. 2010;32:561–9.
33. Bantis LE, Nakas CT, Reiser B. Construction of confidence intervals for the maximum of the Youden index and the corresponding cutoff point of a continuous biomarker. Biom J. 2019;61:1–19.
34. Chen x, Jin y. **et al**. Partial Youden index and its inferences. Journal of biopHarmaceutical statistics. 2018;28(5):1–15.
35. Witkin SS, Linhares IM, Giraldo P. Bacterial flflora of the female genital tract. function immune regulation. 2007;21(3):347– 354.
36. Lidbeck A, Nord CE. Lactobacilli and the normal human anaerobic microflflora. Clin Infect Dis. 1993;16(Suppl 4):181–7.
37. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P. Hydrogen peroxide-producing Lactobacilli and acquisition of vaginal infections. J Infect Dis. 1996;174(5):1058–63.
38. Read JS, Klebanoff MA. Sexual intercourse during pregnancy and preterm delivery: effects of vaginal microorganism. The Vaginal infection and Prematurity Study Group. Am J Obstet Gynecol. 1993;168(2):514–9.

39. Alexander JM, Mercer BM, Miodovnik M. **et al**. The impact of digital cervical examination on expectantly managed preterm rupture of membranes. Am J Obstet Gynecol. 2000;183:1003–7.
40. Munson LA, Graham A, Koos BJ. **et al**. Is there a need for digital examination in patients with spontaneous rupture of the membranes? Am J Obstet Gynecol. 1985;153:562–3.
41. Harvey BJ, Thomas W. Aldosteroneinduced protein kinase signalling and the control of electrolyte balance. Steroids. 2018;133:67–74.
42. Liang H, Xie Z, Liu B. **et al**. **A routine urine test has partial predictive value in premature rupture of the membranes**. J INT MED RES 2019; 47(**6**).

Figures

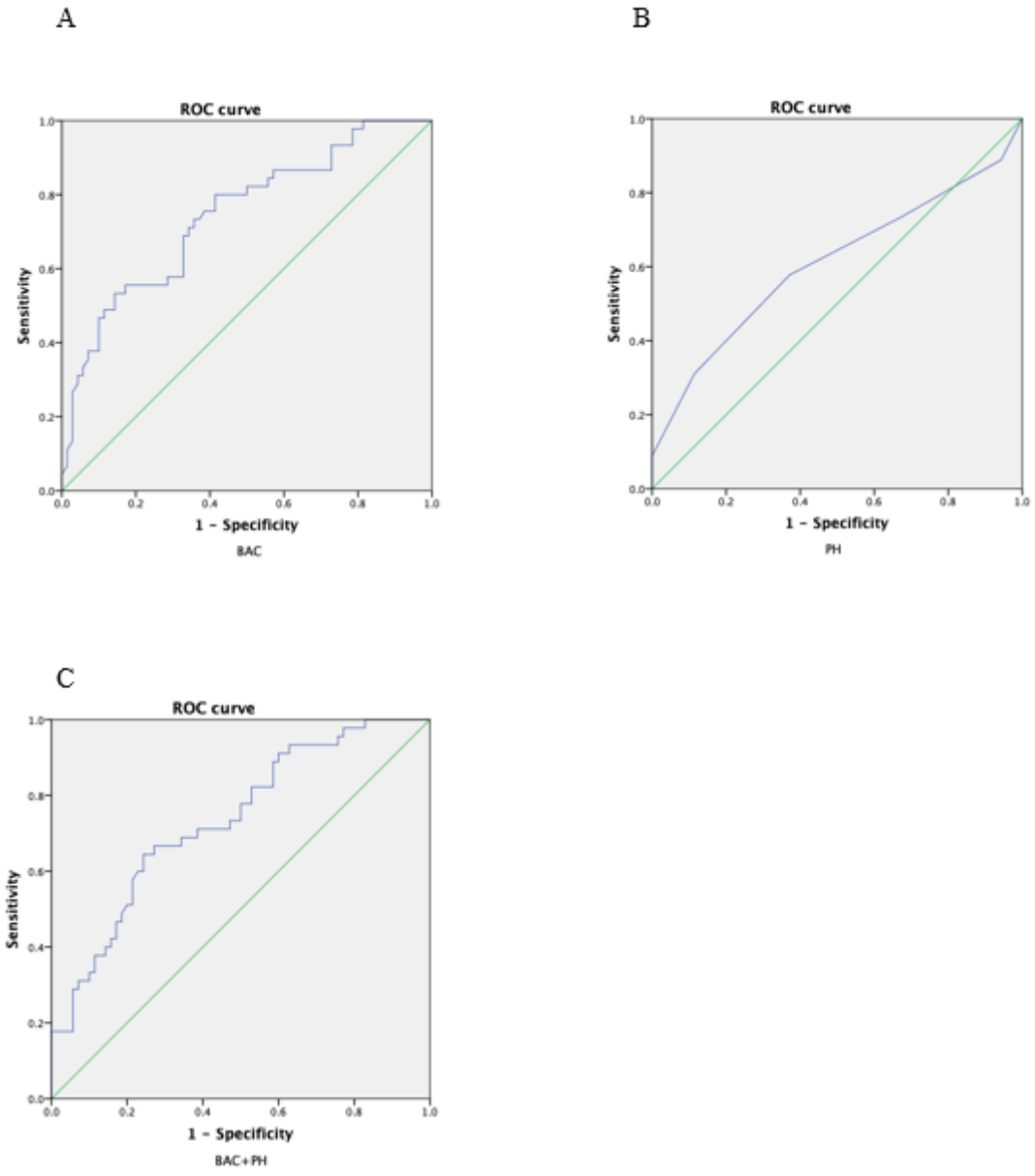

Figure 1

ROC curve of different indicators