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Clinical evaluation of soluble intercellular adhesion molecule-1 and insulin like growth factor-binding protein-1-based rapid immunoassays for the diagnosis of prelabor rupture of membranes*

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Abstract

Aims: To evaluate the clinical value of two rapid tests, based on soluble intercellular adhesion molecule-1 (Leakection) and insulinlike growth factor-binding protein-1 (Amnioquick), for the diagnosis of prelabor rupture of membranes.
Methods: A total of 200 pregnant women were recruited in this study: 100 pregnant women with membrane rupture and 100 healthy pregnant women as controls. Patients and controls were randomly divided into Leakection and Amnioquick groups, respectively. Sensitivity and specificity were calculated on the basis of the detection results.
Results: For the 100 women tested with Leakection, the sensitivity and specificity was 94% and 96%, respectively; the total accuracy was 95%. For the 100 women tested with Amnioquick, the sensitivity and specificity was 80% and 100%, respectively; the total accuracy was 90%.
Conclusions: Both Leakection and Amnioquick are non-invasive and inexpensive rapid tests for the diagnosis of premature or prelabor rupture of membranes with high sensitivity and specificity. These tests could greatly help the timely diagnosis of premature or prelabor rupture of membranes in clinical practice.

Keywords: Amnioquick; insulinlike growth factor-binding protein 1; Leakection; prelabor rupture of membrane; soluble intercellular adhesion molecule-1.

Introduction

Premature or prelabor rupture of membranes (PROM) is the rupture of the fetal membranes before the onset of labor. Preterm PROM is defined as PROM before 37 weeks' gestation. Preterm PROM occurs in approximately 2–3% of pregnancies and leads to 30–40% of preterm births. It increases the risk of prematurity, perinatal and neonatal complications, and perinatal morbidity and mortality [12]. Early and accurate diagnosis of PROM/preterm-PROM is critical for improving the outcome and minimizing the complications [9].

Dye injection test with instillation of indigo carmine dye into the amniotic cavity has been the gold standard for PROM diagnosis [6, 8, 13]. Nonetheless, it is an invasive procedure and cannot be routinely used in clinical practice. Conventional clinical diagnosis of PROM depends on a thorough history, physical examination for visible pooling of amniotic fluid (AF) in the posterior fornix, and selected laboratory studies, mainly nitrazine test and crystallization test with ferning pattern [1]. These laboratory methods have been criticized because of high false-positive and false-negative rates [7, 10, 15, 16]. Therefore, a rapid test with high accuracy for PROM diagnosis will greatly help clinicians for the proper management of PROM.

We previously described soluble intercellular adhesion molecule-1 (sICAM-1) leaked out with AF as an excellent biomarker for the diagnosis of PROM [18]. sICAM-1 has now been developed into a lateral flow immunoassay-based rapid strip test and named as Leakection. In this study, Leakection was clinically evaluated and compared with a commercial product Amnioquick that detected insulin-like growth factor-binding protein-1 (IGFBP-1). The results showed that Leakection had a high sensitivity and specificity for the diagnosis of PROM.

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Materials and methods

Subjects

The research protocol was approved by the Institutional Review Board of West China Second University Hospital, Sichuan University, and all patients provided informed consent. Between September 2011 and January 2012, 200 pregnant women were enrolled in this study. All participants were Chinese, predominantly of the Han race. Of them, 100 pregnant women were diagnosed as PROM or preterm-PROM; 100 pregnant women (91 were in labor and nine not in labor) with normal pregnancy and intact membranes before delivery were recruited as healthy controls. The mean age and the range of the age of PROM/preterm-PROM patients were comparable to those of the control pregnant women (Table 1). PROM/preterm-PROM was confirmed according to the following criteria: (1) leaking of AF was observed before the onset of labor, (2) positive result of cervical-vaginal fluid (CVF) sample with nitrazine/pH strip test, and (3) positive result of microscopic fern testing (AF crystallization test). Patients with vaginal bleeding were excluded because plasma contained sICAM-1 and IGFBP-1 that would give a false-positive result for the tests. Controls with intact membrane were randomly recruited according to the following criteria: (1) no leaking of AF was observed before the onset of labor, (2) negative result of CVF sample with nitrazine/pH strip test, and (3) negative result of microscopic fern testing.

The SAS procedure PLAN was used to generate randomization codes for the randomization allocation of the participants. Eligible PROM patients and healthy controls were numbered and randomly assigned to either the Leakection test group or the Amnioquick test group for evaluation. Consequently, Leakection and Amnioquick groups each had 100 individuals: 50 PROM and 50 controls.

Sample collection and Leakection test

CVF samples were collected. A sterile cotton-tipped swab was placed underneath the posterior cervical lip and rotated five times for approximately 10–15 s. The swab was then dipped in a test tube containing 0.8 mL of sterile phosphate-buffered saline and rotated five times for approximately 10 s. Following the collection, the sample was added to the sample hole of the Leakection test card (Origissay Diagnostic, Ltd., Chengdu, Sichuan, China). One or two lines could be seen in the control and test window within 3–5 min (Figure 1). The presence of the orange-purple test line and the orange-purple control line was determined as positive for PROM. The absence of the test line and the presence of the control line was determined as negative for PROM (Figure 1). In the absence of the control line, the test was considered as invalid and a new test strip would be used to run the test one more time.

Sample collection and Amnioquick rapid immunoassay

The sterile Dacron swab provided in the kit was used for the collection of a sample from the surface of the vagina. The Dacron tip of the swab was inserted into the vagina until the fingers contacted the skin (no more than 5–7 cm deep). The swab was then withdrawn from the vagina after 1 min. Alternatively, a speculum was used, and the cervical excretion was collected by leaving the swab in the cervix for 15 s. The Dacron tip of the swab was placed into the diluent vial and rinsed by rotating for approximately 10 s. Following the collection, an Amnioquick test strip was dipped directly into the diluent vial. One or two lines could be seen as the control and test lines after approximately 10 min. The presence of an orange test line and

Table 1 Clinical characteristic of the study subjects.

	Leakection rapid immunoassay				Amnioquick rapid immunoassay			
	PROM/preterm-PROM (n=50)	95% CIs*	Normal pregnancies (n=50)	95% CIs	PROM/preterm-PROM (n=50)	95% CIs	Normal pregnancies (n=50)	95% CIs
Maternal age (year)								
Mean±SD	29.38±3.99	29.38±1.11	29.08±4.85	29.08±1.34	28.76±4.99	28.76±1.38	29.94±5.21	29.94±1.44
Range	21–38		19–43		18–38		22–40	
[a]Gestational age at sample collection ($week^{+day}$)								
Mean±SD	38.20±4.02	38.20±1.11	38.85±1.51	38.85±0.42	37.67±4.29	37.67±1.19	38.97±1.73	38.97±0.48
Range	$19–41^{+2}$		$34^{+5}–41^{+3}$		$17^{+2}–41^{+1}$		$30^{+3}–41^{+1}$	

[a]Gestational age when PRO/pre-PROM occurred for patients.
*CIs=confidence intervals; PROM=premature or prelabor rupture of membranes.

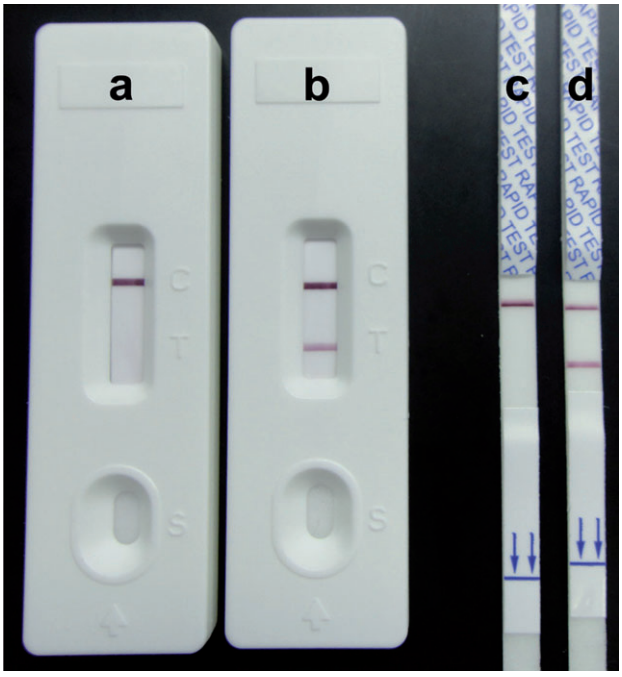

Figure 1 Test results of Leakection (a and b) or Amnioquick (c and d).
(a) Presence of the orange-purple test line and the orange-purple control line was determined as positive for PROM. (b) Absence of the test line and presence of the control line was determined as negative for PROM. (c) Presence of the orange test line and the orange control line was determined as positive for PROM. (d) Absence of the orange test line and presence of the orange control line was determined as negative for PROM. PROM=premature or prelabor rupture of membranes.

an orange control line was determined as a positive result. The absence of the test line and the presence of the control line was determined as a negative result (Figure 1). In the absence of a control line, the test was considered as invalid and it was repeated with a new strip.

Statistical analysis

The number of subjects (PROM/preterm-PROM patients or controls) with positive or negative results was counted. Sensitivity, specificity, positive predictive value, negative predictive value, validity, mistake diagnostic rate, and omission diagnostic rate of Leakection and Amnioquick rapid tests were calculated as follows: sensitivity=number of true-positive specimens (TP)/[TP+number of false-negative specimens (FN)]; specificity=number of true-negative specimens (TN)/[TN+number of false-positive specimens (FP)]; positive predictive value=TP/(TP+FP); negative predictive value=TN/(FN+TN); validity=(TP+TN)/(TP+FP+TN+FN); mistake diagnostic rate=FP/(FP+TN); and omission diagnostic rate=FN/(TP+FN).

Results

A total of 200 pregnant women were recruited for this study, 100 PROM and 100 controls. They were randomly assigned to either the Leakection group or the Amnioquick group for the strip tests. The results are presented in Table 2. Of the 50 PROM cases, 47 were detected positive and three negative by Leakection. For the 50 PROM cases tested by Amnioquick, 40 cases were determined to be positive and 10 cases were determined to be negative. Meanwhile, of the 50 controls, 48 cases showed negative results and two cases showed positive results for Leakection. For the 50 controls examined by using Amnioquick, all were negative.

Table 2 Test results of Leakection and Amnioquick.

	Leakection		Amnioquick	
	Positive	Negative	Positive	Negative
PROM/preterm-PROM	47	3	40	10
Normal pregnancies	2	48	0	50

PROM=premature or prelabor rupture of membranes.

Diagnostic values of Leakection and Amnioquick tests were calculated and are summarized in Table 3. Leakection had a better sensitivity (94%) than that of Amnioquick (80%), while both Leakection and Amnioquick were shown to have a very high specificity, at 96% and 100%, respectively. The total accuracy was confirmed at 95% for Leakection and at 90% for Amnioquick.

Among the 100 patients, 15 were diagnosed as pre-PROM and 85 as PROM. No significant difference was observed in terms of sensitivity or specificity for the diagnosis of PROM or pre-PROM (Supplementary Figure 1 and Tables 1–4) by using Leakection or Amnioquick. In addition, both Leakection and Amnioquick each had one case of invalid result (no visible signal was present), and these cases were immediately retested with acceptable results.

Discussion

At the time of study design we initially aimed to conduct the tests on the same patients and controls for both Leakection and Amnioquick. However, it was practically not doable, because the sample diluent was not the same for the two tests. If one of the diluents was used for both tests, it would compromise the other test and would not generate comparable results. If samples were collected one after another from the same patient, the sample collected after the first one would not be of optimal quality and would compromise the results for the second test. Because of the invasive nature of the indigo carmine dye test, it would be very hard to get permission from

Table 3 Diagnostic value of Leakection and Amnioquick rapid immunoassay.

	Leakection (%)	Amnioquick (%)
Sensitivity	94	80
Specificity	96	100
Positive predictive value	95.9	100
Negative predictive value	94.12	83.3
Validity	95	90
Mistake diagnostic rate	4	0
Omission diagnostic rate	6	20

patients and approval from the institutional review board. Therefore, the gold standard for the diagnosis of PROM was not used for the present study. Instead, the clinical working diagnosis criteria were used. Furthermore, though ultrasound examination is routinely used to diagnose oligohydramnios due to rupture of membranes in many other hospitals, it was not routinely conducted for the diagnosis of PROM at our hospital, partly because of the cost and also the low sensitivity for small leakages.

We previously described sICAM as a highly sensitive and specific biomarker for the diagnosis of PROM by using enzyme-linked immunosorbent assay [18]. However, enzyme-linked immunosorbent assay is a test that takes hours to generate results in a lab equipped with a colorimetric plate reader. Because PROM is a disease that needs quick diagnosis and treatment, a point-of-care strip test will facilitate a quick reading in cases suspected of PROM. As we previously described, 2 ng/mL of sICAM in CVF was used as the cutoff value for the diagnosis of PROM, which is coincidently the detection limit for lateral flow-based immunoassay using colloid gold as the signal indicator [11, 19–22]. Thus, Leakection was naturally designed and manufactured by using this assay platform.

In the current study, Leakection proved to yield results in 3–5 min after applying a diluted CVF sample onto the test card. Meanwhile, sensitivity and specificity were shown to be 94% and 96%, respectively. These results were very similar to what we previously described at 96.4% and 92.7%, respectively, when sICAM was quantified by enzyme-linked immunosorbent assay [18]. Therefore, Leakection seems to be a very useful rapid test for helping the diagnosis of PROM, especially in hospitals where expertise and sophisticated equipments are not readily available.

In our previous quantitative study, we showed that IGFBP-1, as a biomarker for the diagnosis of PROM, had a sensitivity of 93.3% and a specificity of 86.8% [18]. Nevertheless, using Amnioquick as a point-of-care test, we were not able to show a similar high sensitivity (80% in the present study), but the specificity was greatly improved (at 100%). Based on our results, there seemed to have been a trade-off for the IGFBP-1 rapid test, increased specificity with a declined sensitivity. In several studies by other investigators of IGFBP-1 for the diagnosis of PROM, used either as a rapid test or as an enzymatic quantitative test, the sensitivity was reported to be in the range of 86–100% and the specificity was reported to be in the range of 74–98.2% [2, 4, 17]. Similar results were obtained in our studies. Furthermore, Leakection, the rapid test detecting sICAM-1, has a capacity comparable to that of Amnioquick, if not better, for the diagnosis of PROM.

For Leakection test, a couple of cases presented false-positive results. This was because a small percentage of healthy pregnancies had slightly higher sICAM-1 in the vaginal fluid [18]. Moreover, in the case of vaginal bleeding, both tests will not produce reliable results, as blood contains sICAM-1 and IGFBP-1 [18].

Another rapid test for the diagnosis of PROM is based on the detection of placental α-microglobulin-1 (marketed as AmniSure). In clinical studies using Amnisure for the diagnosis of PROM, the sensitivity was reported at 94.4–97.2% and the specificity at 69.0–98.6%. It was concluded that the AmniSure test for the diagnosis of PROM was better than both the conventional clinical assessment and the nitrazine test with a higher sensitivity while the specificity was controversial [2–5, 14, 17].

In conclusion, both Leakection and Amnioquick are non-invasive and inexpensive rapid tests for the diagnosis of PROM with high sensitivity and specificity. These tests could greatly help the timely diagnosis of PROM in clinical practice.

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Prelabour rupture of membranes: overview of diagnostic methods

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Purpose of review
To evaluate diagnostic accuracy studies for rupture of the fetal membranes (ROM).

Recent findings
Sample sizes of recent studies are small and studies used different 'silver standard' definitions for ROM. Therefore, reported results should be interpreted with caution. Over the review period the focus of diagnostic studies has been on two bedside test strips: insulin-like growth factor-binding protein-1 (IGFBP-1) and placental α microglobulin-1 (PAMG-1). Bedside tests improve the confidence of the clinician about their diagnosis. Compared to nitrazine or ferning test alone, IGFBP-1 and PAMG-1 are more accurate. However, compared to the conventional testing (combination of history, ferning, nitrazine, speculum and ultrasound) no statistical difference in accuracy was found. In-vitro PAMG-1 is shown to be superior to IGFPB-1. Furthermore, soluble intercellular adhesion molecule-1 and Axl receptor tyrosine kinase (Axl) seem to be promising new specific biomarkers for diagnosing ROM.

Summary
IGFBP-1 and PAMG-1 are the most commonly used bedside tests for diagnosing ROM. Both tests seem to be sensitive and specific, however, evidence is lacking especially in equivocal cases and comparative studies against the real gold standard (amnio-dye) have still not been published. Further effectiveness research is needed before tests can be applied in practice.

Keywords
diagnosis, diagnostic method, PROM, rupture of membranes

INTRODUCTION

Prelabour rupture of the fetal membranes (PROM) complicates 5–10% of all pregnancies [1,2] and it is associated with an increased incidence of chorioamnionitis, prematurity and with increased perinatal and maternal morbidity and mortality [3]. In the majority of women, the diagnosis of ruptured fetal membranes can be based on a history of PROM with speculum examination. This clinical approach has, however, a 12% false-negative rate [4]. In approximately 10% of all cases, the diagnosis of rupture of membranes is difficult to establish [5,6].

In order to improve the accuracy to diagnose PROM a wide variety of tests have been introduced, the first one to be alkaline testing introduced in the 1930s [7]. For decades, a combination of visual pooling of amniotic fluid during speculum examination, alkaline pH determination and microscopic evidence of ferning and decreased amniotic fluid by ultrasound has been widely used to determine rupture of membranes. This combination has been referred to as 'conventional testing'. These tests, however, are prone to false-positive results secondary to vaginal contamination with blood, urine, or semen [5,8–10].

Besides the inaccuracy of the conventional test, many women find the use of a speculum examination intrusive [11]. In order to improve the accuracy of diagnostic test and simplify test procedures without the use of a speculum, dozens of

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KEY POINTS

- Studies on diagnostic methods for rupture of membranes are small and all lacking a gold standard.
- The used second best 'silver standard' varies among studies, which makes results hard to compare and results should be interpreted with caution.
- PAMG-1 seems to be superior to IGFBP-1, but new, possibly more accurate tests (sICAM and Axl) are being developed.
- In order to find the real accuracy of current and newly developed diagnostic methods for ROM, these methods should be tested preferably in large randomized controlled trial(s) against the gold standard (amnio-dye infusion).

tests have been developed over the last decades. In this review, we highlight and report the diagnostic accuracy studies on diagnostic tests for PROM that have been published since a systematic review on diagnostic methods for ROM in equivocal cases [12▪].

SYSTEMATIC REVIEW

We performed a systematic review to assess the accuracy of several tests for the diagnosis of ROM in equivocal cases [12▪]. Over a review period from 1960 to September 2010 we identified and obtained 146 full manuscripts, 133 were excluded due to multiple reasons. The remaining 13 studies were scored by an expert panel. Only three studies [13–15] with a total of 155 patients fulfilled all criteria for diagnostic test accuracy studies [16–19]. These articles tested three different methods, pH measurement (64 patients) [13], insulin-like growth factor binding protein-1 (IGFBP-1, 83 patients) [14] and α fetoprotein (AFP, eight patients) [15]. Sensitivity varied from 88% (pH) to 100% (AFP), specificity varied from 56% (IGFBP-1) to 100% (AFP). Based on the limited evidence on the accuracy of tests to diagnose ruptured membranes, we concluded that the use of a particular test cannot be recommended [12▪].

For the present review, we repeated our search strategy for the period September 2010 until May 2012 and found seven new articles, which will be described in more detail.

THE LACK OF A GOLD STANDARD

Amniocentesis with infusion of a dye is widely considered as the gold standard for the diagnosis of rupture of membranes. However, this procedure is invasive, costly and may itself cause rupture of membranes and other complications, such as infections [13,20–22]. Because of this, many researchers and medical ethical committees find it unethical to expose women to amnio-dye infusion. However, over the last decades due to better ultrasound technology, success rate of amniocentesis improved and complication rate seems nowadays to be low [23]. Studies reporting adverse outcome were published between 1976 and 1983 [20–22]. The last published study, which used amnion infusion with a dye as a gold standard was performed more than 15 years ago. In that study, it took researchers 12 years to include 64 women [13]. Recently, preliminary results have been published in which placental α-microglobulin 1 (PAMG-1) was compared to amnio-dye test [24]. The final results have, however, not been published yet.

Meanwhile, the lack of a noninvasive gold standard test for PROM is a severe limitation to study (new) diagnostic tests [25]. The recently published studies that are discussed in this review all lack the use of a gold standard and still do not meet all the criteria for the methodological assessment and reporting of diagnostic accuracy studies as suggested in previously reported guidelines [16–19].

RATIONALE OF CURRENT DIAGNOSTIC TESTS

Because of a lack of a gold standard for the majority of the clinical studies and the limitations and inaccuracy of 'conventional testing' as well as the need for a less invasive, less intrusive method, researchers have been searching for the identification biochemical markers which are present in high quantities in case of ROM, and absent in cervicovaginal discharge when membranes are intact. Many of these markers have shown to be less valuable because they are also present in other physiological fluid such as blood, vaginal secretion of seminal fluid [25]. Other markers such as fetal fibronectin seem to indicate the mechanical or inflammatory-mediated detachment of the membranes from the decidua and are nowadays merely used as a predictor for preterm delivery and are no longer considered to indicate ruptured membrane [26–28]. IGFBP-1 and PAMG-1 meet the criteria of high concentration in amniotic fluid and low concentration in other physiological fluids [29,30]. Therefore, in the past year the focus of the research has been on these two tests [31–34,35▪,36]. Other proteins, such as soluble intercellular adhesion molecule-1 (sICAM-1) and Axl receptor thyrosine kinase (Axl) might be used as biomarkers in the future [37▪].

A BEDSIDE TEST TO IMPROVE A DOCTOR'S CONFIDENCE

Neil and Wallace [34] studied the clinical utility of PAMG-1 testing in daily practice. They questioned how often and in whom a bedside test might enhance the clinical diagnosis of PROM and change clinical management. In a prospective observational study they included 184 women (100 term pregnancies and 84 preterm pregnancies) in a 12-month period. Based on history and clinical examination (speculum examination) the attending obstetrician was certain with the diagnosis in 53% of the women and uncertain in 47%. Obstetricians were more confident with preterm women than with term women ($P = 0.02$). The confidence in the initial diagnosis (ROM or intact membranes) increased significantly when the obstetrician knew the result of the PAMG-1 test. Post test result the obstetrician was certain in 92% of the women with his/her diagnosis ($P < 0.0001$). Diagnosis and management was changed after PAMG-1 test especially in the proposed intact membranes group (toward proposed ruptured membranes, 14 out of 82 cases, 17%). The study did not test the accuracy of the PAMG-1 test, nor did it follow the women until delivery, not giving any insight in the effect on outcome. Results on the accuracy of PAMG-1 in this study should, therefore, be interpreted with caution. This study, however, does show the need for clinicians to increase their confidence by using a bedside test [34].

DIAGNOSTIC ACCURACY STUDIES

Pollet-Villard *et al.* [35▪] studied *in vitro* the sensitivity of IGFBP-1 and PAMG-1 using different detection limits after dilution of amniotic fluid in a comparative study. They recruited 41 women over 37 weeks of gestational age who were scheduled for a caesarean section. During the caesarean section 0.5 ml samples of amniotic fluid were collected with a syringe before rupture of the membranes and fetal extraction. The samples were diluted with physiological saline solution (NaCl 0.9%) in a 1 : 10, 1 : 20, 1 : 40, 1 : 80, 1 : 160, 1 : 320 and 1 : 640 dilution series. For each dilution both IGFBP-1 and PAMG-1 tests were performed. Up to a dilution of 1 : 40 PAMG-1 showed a sensitivity of 100%, whereas the sensitivity for IGFBP-1 dropped from 100 to 97.5 to 88% for 1 : 10, 1 : 20 and 1 : 40 dilution, respectively. For the dilution of 1 : 40, this difference was significant ($P < 0.05$). This study tried to mimic the vaginal dilution of amniotic fluid in the vagina after PROM. However, they only took samples for the term population and it might be questionable whether dilution with NaCl 0.9% will actually mimic the clinical condition. Nevertheless, in this *in vitro* dilution study PAMG-1 has a higher sensitivity and better reproducibility than IGFBP-1 [35▪].

Two recent articles studied PAMG-1 for the detection of rupture of membranes [31,33]. The first study was a prospective observational study in 199 women (gestational age 17–42 weeks) with uncertain signs or symptoms of ROM [33]. Rupture of membranes was first diagnosed using a conventional method with two out of three of the following criteria: positive visual leaking or pooling, positive nitrizine test, amniotic fluid index (AFI) less than 5 cm. PAMG-1 testing was performed after initial diagnosis was made and the investigator was not blinded. Final diagnosis of ROM was made on medical records after delivery. PAMG-1 test was found to be more sensitive (94.4 vs. 72.2%, $P = 0.006$) but had the same specificity (98.6 vs. 97.9%) compared to conventional testing. Due to the costs of ultrasound examination, PAMG-1 testing alone was significantly less expensive than conventional testing [33]. The second study was an unblinded comparative prospective study in 150 term women (<37 weeks), 75 of whom had definite ROM, based on history (sudden gush), pooling, positive nitrazine and ferning and visual fluid passing the cervical canal during speculum examination, the remaining 75 women had no signs of ROM and were scheduled for induction of labour [31]. PAMG-1 testing in women with certain ROM had a sensitivity of 97 vs. 84% for ferning and 87% for nitrazine test, specificity was 99, 79 and 81%, respectively [31].

Two other studies compared IGFBP-1 and PAMG-1 testing for diagnosis of ROM [32,36].

In the first prospective observational study 179 women between 16 and 41 weeks of gestation were included [32]. ROM was primarily diagnosed using a conventional method (pooling, positive ferning, positive nitrazine testing and AFI measurement). The definite diagnosis was made afterwards by two of the researchers, unaware of the IGFPB-1 and PAMG-1 test result, based on duration of latency, results of (repeated) speculum examination, (repeated) ferning, nitrazine and decreased AFI by follow-up as well as clinical signs of fetal distress or chorioamnionitis. The presence of at least two of the above was needed for diagnosis of ROM [32]. The investigators found that the sensitivity (94, 90, 87%, for PAMG-1, IGFBP-1 and conventional testing, respectively) and specificity (98, 98 and 95%, respectively) were high and not statistically different. Related to ferning alone IGFBP-1 and PAMG-1 were significantly more accurate. However, as the researchers commented, ferning or nitrazine testing alone have been shown to be less accurate and are only used in a combined conventional

method [32]. The second study compared IGFBP-1, PAMG-1 and nitrazine testing for diagnosing PROM. In a prospective observational study 100 consecutive women between 17 and 37 weeks of gestation with signs and symptoms of ROM were included [36]. ROM was diagnosed if three of the following were present: definite pooling, oligohydramion at ultrasound, signs and symptoms of chorioamnionitis, preterm delivery within a week of presentation along with a convincing history of leaking as judged by the attending clinician. Medical records were reviewed after delivery [36]. PAMG-1 had a sensitivity of 93% and specificity of 100%; IGFPB-1 had a sensitivity of 88% and specificity of 94%, the difference between both tests was not statistically significant. Compared to nitrazine testing alone, PAMG-1 and IGFPB-1 were significantly more accurate [36].

In another small observational study vaginal creatine was studied for diagnosing ROM in definite suspected and absent ROM. It was concluded that vaginal creatine might be useful in diagnosing ROM but material and methods of the study were poorly described, therefore, no results are mentioned in this review [38].

FUTURE TESTING METHODS

To date, PAMG-1 and IGFBP-1 are the most commonly used bedside test strips for diagnosing ROM. There is, however, some evidence that PAMG-1 might also be a marker for short time to delivery [39]. Fragmented and phosphorylated forms of IGFBP-1 are associated to predict preterm labour [40,41]. Like other diagnostic tests in the past, it might be possible that the sensitivity and specificity of PAMG-1 and IGFBP-1 will turn out to be not as high as reported to date.

Obviously, researchers are working on new tests, which might be more accurate than currently available ones [37▪]. Wang *et al.* [37▪] used a cytokine/chemokine antibody array in order to identify proteins which are high in amniotic fluid and low in cervical–vaginal fluid (CVF) and tested these proteins in 110 patients with unequivocal ROM and 110 controls [37▪]. From the 174 cytokines that were studied in the kit, sICAM1, Axl, IGFBP-1, MCP-1, MIP-1δ, TIMP-1 and CD14 were found most interesting. The authors decided to focus on sICAM-1, Axl (Axl receptor tyrosine kinase) and IGFBP-1. sICAM, Axl and IGFBP-1 were respectively 85-fold, 482-fold and 72-fold higher in amniotic fluid than in CVF. sICAM and Axl maybe useful as diagnostics for ROM in which sICAM seems to be a better candidate for the development of a bedside test because the technology to manufacture this test is widely accepted, reliable and inexpensive [37▪].

IS THERE A NEED FOR A BEDSIDE TEST?

There is growing evidence that there is less need for immediate induction of labor when membranes rupture late prematurely [42,43]. This makes it questionable if for the near-term population a bedside test is needed for a small minority of patients in which the clinician cannot certainly make a diagnosis based on conventional testing. However, in contrast early PROM is associated with high perinatal morbidity and mortality. Preterm delivery is a frequent sequel of this complication and it is estimated that approximately 25–40% of preterm deliveries are preceded by PROM. Although early preterm delivery (<32 weeks' gestation) occurs in only 1–2% of total births, it is estimated to account for nearly 50% of all long-term neurological morbidity and about 60% of perinatal mortality [44].

In this perspective, correct diagnosis in equivocal cases is mandatory because a correct diagnosis would bring down the unnecessary burden for the health system. False-positive test results could lead to overtreatment (hospital admittance, corticosteroids and antibiotics), whereas a missed diagnosis of PROM could delay administration of corticosteroids. In equivocal cases, diagnosis of early PROM is often hindered by vaginal bleeding. In a patient with mild vaginal bleeding in the second trimester the perspective in case of (masked) PROM is significantly different, and a validated test in this case would be valuable.

CONCLUSION

Since we published a systematic review on the diagnostic tests for rupture of membranes [12▪] several new studies have been published [31,32, 34,35▪,36,37▪,38]. Due to the lack of a noninvasive gold standard, the use of a second best 'silver standard' varies among different studies as well as the included population (preterm, term) and complaints (equivocal or unequivocal ROM). There were no randomized controlled trials; all studies were prospective and observational. Studies had small sample sizes (maximum 199 participants, [33]) and, therefore, it is difficult to compare different tests with each other. PAMG-1 seems to be the most sensitive and specific test, and is the subject of the only study with a design including the real gold standard dye-infusion results still being underway. New tests are being developed [37▪]. But as long as current and newly developed tests are not tested against the gold standard, results of new studies will always be an issue of debate. Furthermore, in our opinion studies should be more focused on the early PROM group.

Acknowledgements

None.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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RESEARCH ARTICLE

Proteins in leaked amniotic fluid as biomarkers diagnostic for prelabor rupture of membranes

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Purpose: Early diagnosis of prelabor rupture of membranes (PROM) is essential to protect mother and fetus from intra-uterus infection and preterm birth. A simple and rapid bedside test would help clinicians confirm the diagnosis for early treatment.
Experimental design: A protein array was used to screen cervical–vaginal fluid (CVF) and amniotic fluid (AF) samples collected from normal and PROM pregnant women. Enzyme-linked immunosorbent assay was used to quantify two novel and potentially useful analytes, soluble intercellular adhesion molecule-1 (sICAM-1) and Axl receptor tyrosine kinase (Axl).
Results: The mean concentration of sICAM-1 and Axl was 85 and 482 times higher separately in 30 healthy AF samples than in 110 CVF samples of normal pregnancies. Comparing 110 CVF samples of PROM/Preterm PROM with 110 CVF samples of normal pregnancies, the diagnostic value for PROM was demonstrated by their high sensitivity and specificity (96.4 and 92.7%, respectively, for sICAM-1, and 92.4 and 90.4%, respectively, for Axl).
Conclusions and clinical relevance: The results indicate that sICAM-1 and Axl in AF leaked to vagina are sensitive and specific biomarkers for the diagnosis of PROM. Furthermore, sICAM-1 or Axl can be developed into a rapid strip test for bedside use.

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Amniotic fluid / Axl / IGFBP-1 / Prelabor rupture of membrane / sICAM-1

1 Introduction

Prelabor rupture of membranes (PROM) is the rupture of the fetal membranes before the onset of labor. In most cases, it occurs near term. When membrane rupture occurs before 37 wk' gestation, it is known as Preterm PROM. Preterm PROM complicates approximately 3% of pregnancies and leads to one-third of preterm births. It increases the risk of prematurity and leads to a number of other perinatal and neonatal complications, including a 1–2% risk of fetal death [1]. Because appropriate management can result in improved outcomes, a rapid diagnosis is very important. However, accurate and timely diagnosis of PROM remains a frequent clinical challenge [2].

The gold standard for PROM diagnosis requires instilling 10 mL of indigo carmine dye into the amniotic cavity. If the membranes are ruptured, the blue dye will pass into a vaginal tampon within 30 min post-instillation [1, 3–7]. Nonetheless, this is an invasive procedure and not routinely used in the clinical practice. The clinical diagnosis of PROM depends on a thorough history, physical examination, and

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Abbreviations: AF, amniotic fluid; **Axl,** Axl receptor tyrosine kinase; **CVF,** cervical-vaginal fluid; **IGFBP-1,** insulin-like growth factor-binding protein 1; **PAMG-1,** placental α-microglobulin-1; **PROM,** prelabor rupture of membranes; **ROC,** receiver-operating characteristics; **RT,** room temperature; **sICAM,** soluble intercellular adhesion molecule-1

*These authors contributed equally to this work.

selected laboratory studies [1, 8]. Patients often report a sudden gush of fluid with continued leakage. Evidence of fluid pooling in the vagina, or leaking from the external orifice of the uterus when the patient coughs or when fundal pressure is applied, will help determine PROM [1, 8]. Crystallization test with ferning pattern and nitrazine test, a dye test for alkaline pH, has been criticized due to high false-positive and false-negative rates in both cases [9–12]. Therefore, a rapid and simple test that surpasses the sensitivity and specificity of the current methods for the diagnosis of PROM will lend a great help for proper treatment of pregnant women suspected of PROM. To this end, clinicians and scientists have been searching for such tests, and one example is placental α-microglobulin-1 (PAMG-1) immunoassay test, which is marketed as AmniSure currently available in Europe, and has recently been approved by the Food and Drug Administration for use in the United States [2].

Proteins in amniotic fluid (AF) could be very different quantitatively from those in the cervical–vaginal fluid (CVF) [13]. We assumed that there were proteins of much higher concentrations in the AF than those in the CVF. Such proteins, if leaked along with AF to the vagina, can be specifically and rapidly detected with antibody-based immunoassays and will provide accurate diagnostic results in minutes. Indeed, this novel noninvasive approach uses a mechanism similar to instilling of indigo carmine dye. Instead of visualizing an injected external dye, internal proteins of high concentration in AF will be detected. Though diluted after leaking into CVF, the proteins would still be much higher quantitatively than those in the uncontaminated CVF. The examination of such proteins should provide a sensitivity and specificity for the diagnosis of PROM similar to the dye method, greatly surpassing the accuracy of the current clinical observation and lab tests. We used an antibody array as a screening tool and have found proteins that are of very high concentration in AF compared with those in the CVF. Based on these findings, we have proven these proteins being excellent biomarkers diagnostic for PROM.

2 Materials and methods

2.1 Subjects

The research protocol was approved by the Institutional Review Board of West China Second University Hospital, Sichuan University, and all patients provided informed consent. Between September 2009 and June 2010, 250 pregnant women were enrolled in this study. Of them, 110 pregnant women were diagnosed as PROM (80 patients) or Preterm PROM (30 patients); 110 pregnant women with normal pregnancy and intact membranes before delivery were recruited as healthy controls; and AF samples were collected from 30 patients with term delivery that had elective cesarean section. The mean age and the range of the age of PROM/Preterm PROM patients were comparable to those of the healthy control pregnant women (Supporting Information Table 1). PROM/Preterm PROM was confirmed according to the following criteria: (i) leaking of AF was observed before the onset of labor; (ii) positive result of CVF sample with nitrazine/pH strip test; and (iii) positive result of microscopic fern testing (AF crystallization test). Controls with intact membrane were recruited according to the following criteria: (i) no leaking of AF was observed before the onset of labor; (ii) negative result of CVF sample with nitrazine/pH strip test; and (iii) negative result of microscopic fern testing [8].

2.2 Sample collection

CVF samples were collected by avoiding visible blood contamination. A sterile cotton-tipped swab was placed underneath the posterior cervical lip and rotated for approximately 10–15 s [14, 15]. The swab was then dipped into a test tube containing 1 mL of sterile PBS, and rotated 10 times during approximately 30 s. Following the collection, samples were centrifuged at $400 \times g$ and 4°C for 10 min, and the supernatant was then aliquoted and stored at −80°C until use.

AF samples were collected from 30 normal pregnancies during cesarean section. Approximately 10 mL of AF was collected free of visible blood contamination from each pregnant woman during delivery. Samples were then centrifuged at $400 \times g$ and 4°C for 10 min, and the supernatant was aliquoted and stored at −80°C until use.

2.3 Screening assay using a cytokine antibody array

Two PROM CVF samples, two control CVF samples, and two control AF samples were randomly selected for screening assay by using RayBio Human Cytokine/Chemokine Antibody Array C Series 2000 (RayBiotec, Norcross, GA, USA). This array could simultaneously detect 174 human cytokines and related proteins. The experiments were conducted by following the manufacturer's suggested procedures. Briefly, array membranes were incubated in 2 mL of blocking buffer at room temperature (RT) for 6 h. After removing the blocking buffer, samples were respectively diluted with blocking buffer and added, and incubated at 4°C overnight. After the incubation, the sample fluid was discarded, and the membranes were washed twice with 2 mL of washing buffer. Following incubation with biotin-conjugated detection antibodies for 1–2 h at RT, membranes were washed three times. Two milliliters of HRP-conjugated streptavidin were then added and incubated at RT for 2 h. After washing three times each with 2 mL of washing buffer, signals were detected with the addition of the detection mixture by using a chemiluminescence imaging system (Molecular Imager ChemiDoc XRS+System, Bio-Rad Laboratories, Hercules, CA, USA).

2.4 ELISA

Concentrations of the soluble intercellular adhesion molecule-1 (sICAM-1), Axl receptor tyrosine kinase (Axl), and insulin-like growth factor-binding protein 1 (IGFBP-1) in CVF and AF samples were determined in duplicate by ELISA using commercial kits purchased from R&D Systems (Minneapolis, MN, USA), Bender MedSystems (Vienna, Austria), and RayBiotec. Samples with the levels higher than the kit detection limits were diluted and measured again. In our laboratory, the intra-assay and inter-assay coefficients of variation were both $<10\%$ for all three assays. The experiments were conducted by following the manufacturer's suggested procedures. Briefly, diluted AF and CVF samples were each added into duplicate wells (100 μL in each well) together with 50 μL of HRP conjugate per well in a 96-well plate. After incubation at RT for 2 h on a microplate shaker rotating at 100 rpm, the plate was then washed. Color development was conducted by the addition of 100 μL of tetramethylbenzidine (TMB) substrate solution to each well and incubation at RT for approximately 10 min. After stopping the reaction, the optical absorbance of each microwell was immediately read on a spectro-photometer (Infinite M200, Tecan Trading, Switzerland) using 450 nm as the primary wavelength. The concentration of each analyte was determined by referring to a standard curve generated simultaneously on the same plate.

2.5 Statistical analysis

The levels of sICAM-1, Axl, and IGFBP-1 in CVF and AF were expressed as mean value ± standard deviation (SD). The statistical significance of the findings was assessed by Student' *t*-test using a computer software Prism 5 from GraphPad Software (San Diego, CA, USA). *p*-Value ≤ 0.05 was considered significant. The CVF sICAM-1, Axl, and IGFBP-1 threshold that gave the maximal sensitivity and specificity for the diagnosis of PROM was determined by using receiver-operating characteristics (ROC) curves. Sensitivity, specificity, and the likelihood ratio of the CVF tests were calculated as follows: sensitivity = number of true-positive specimens (TP)/[TP + number of false-negative specimens (FN)]; specificity = number of true-negative specimens (TN)/[TN + number of false-positive specimens (FP)]; and likelihood ratio = sensitivity/(1.0–specificity).

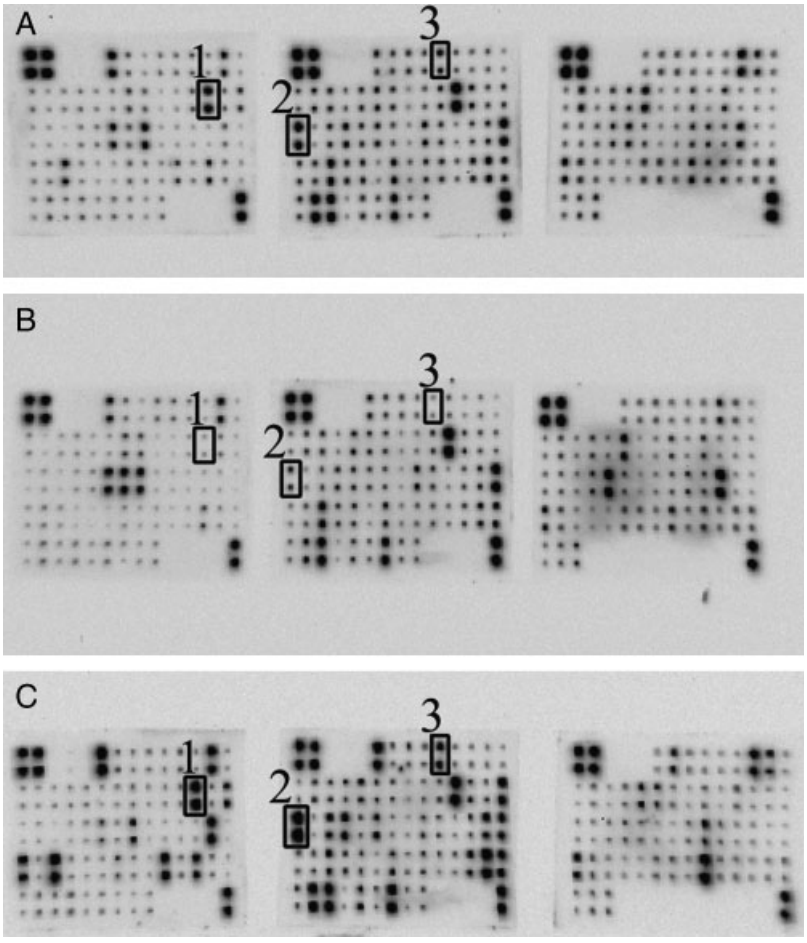

Figure 1. An antibody array was used to screen a CVF sample of PROM (A), a control CVF sample (B) and an AF sample of a normal pregnancy (C). Among the positive signals, IGFBP-1 (1), sICAM-1 (2) and Axl (3) were found strongest in the AF sample, and in reduced intensity in the CVF sample of PROM, and at the background level in the control CVF sample.

3 Results

3.1 Screening of CVF and AF samples

The cytokine/chemokine antibody array used in this study could simultaneously detect 174 cytokines and related proteins in a given sample. The results were semi-quantitative when assessed by the density of each analyte spot. As represented for one set of the samples in Fig. 1, multiple analytes were detected positively in each one of the three samples (Supporting Information Fig. 1). Among them, many more analytes were revealed positively and strongly in the AF sample. While the control CVF showed relatively fewer positive analytes, the CVF sample from PROM exhibited a pattern between those of the AF and the control CVF samples.

Among the positive analytes, sICAM-1, Axl, and IGFBP-1, MCP-1, MIP-1δ, TIMP-1, and CD14 signals were found most interesting (Supporting Information Table 2 and Supporting Information Fig 2). While their signals were strongly positive in both the AF and CVF samples from PROM, they were at a much lower level in the control CVF sample. This constitutes an essential feature of excellent diagnostic biomarkers: being strikingly different between diseased individuals and healthy people.

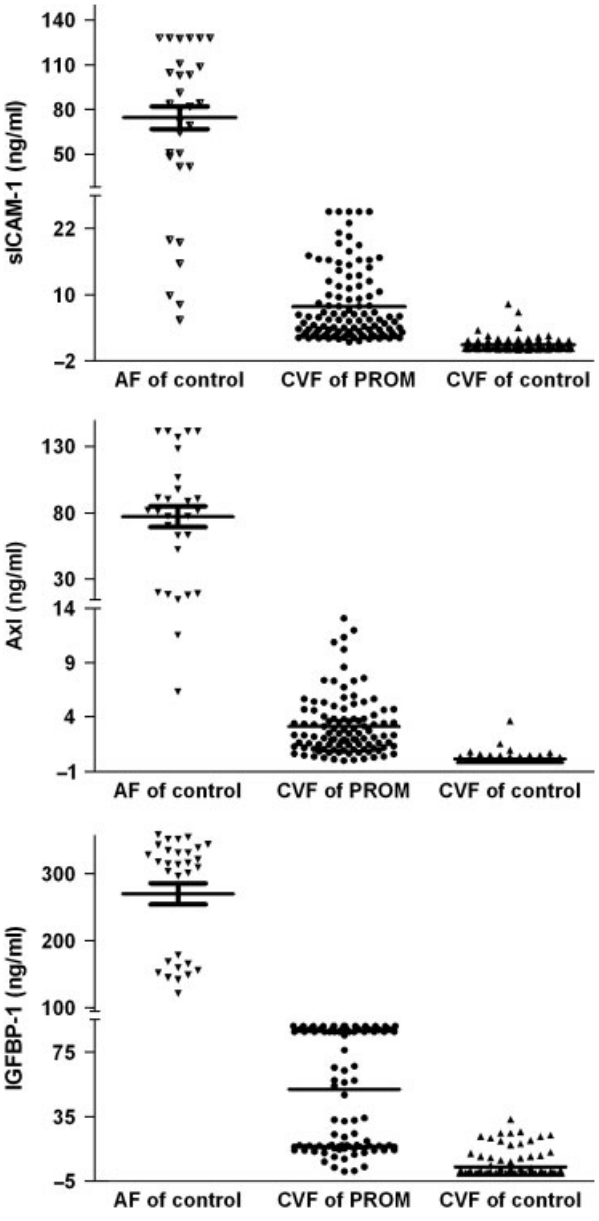

Figure 2. Concentration difference of sICAM-1, Axl, and IGFBP. Normal AF, PROM CVF, and control CVF samples were evaluated using ELISA, and the concentration in 30 AF samples was 85, 482, and 72 folds, respectively, for sICAM-1, Axl, and IGFBP-1, of that in 110 control CVF samples. All three analytes were significantly higher in AF samples than in PROM CVF samples ($p<0.01$), and in PROM CVF samples than in control CVF samples ($p<0.01$).

3.2 sICAM-1, Axl, and IGFBP-1 levels in AF and control CVF samples

After further screening using ELISA, we focused only on sICAM-1, Axl, and IGFBP-1, because they looked much more promising than the other four analytes (Supporting Information Table 3). As given in Fig. 2, the contrast of sICAM-1, Axl, and IGFBP-1 levels between AF and control CVF was further revealed quantitatively in multiple samples. sICAM-1, Axl, and IGFBP-1 were determined by ELISA in 30 AF samples and 110 control CVF samples. The mean values of sICAM-1, Axl, and IGFBP-1 in AF samples were, respectively, at 74.53 ± 41.36, 77.08 ± 42.73, and 269.47 ± 85.05 ng/mL, and in control CVF samples were, respectively, at 0.88 ± 1.18, 0.16 ± 0.42, and 3.75 ± 7.54 ng/mL. Consequently, sICAM-1, Axl, and IGFBP-1 were 85, 482, and 72 folds higher in the AF samples than in the control CVF samples. This huge difference led us to assume that when AF leaked from the amniotic cavity into the vagina in PROM, even if it was diluted, the sICAM-1, Axl, and IGFBP-1 levels in CVF samples obtained from PROM would still be much higher than in those of the healthy controls.

Table 1. Concentrations of sICAM-1, Axl, and IGFBP in CVF samples of Preterm PROM and PROM

Group	Proteins (ng/mL)		
	sICAM-1	Axl	IGFBP-1
Preterm PROM ($n=30$)	8.75 ± 7.72	3.47 ± 2.98	36.17 ± 33.54[a)]
PROM ($n=80$)	7.57 ± 5.98	3.02 ± 2.60	56.68 ± 3.86

a) $p<0.05$ compared with PROM.

Clinical Relevance

Early diagnosis of PROM is essential to protect mother and fetus from intra-uterus infection and preterm birth. A simple and rapid bedside test would help clinicians confirm the diagnosis. Using a sensitive protein array, a couple of novel biomarkers, sICAM-1 and Axl, diagnostic for prelabor rupture of membranes have been discovered. Both of them are highly sensitive and specific based on the quantitative assay. Moreover, a bedside rapid test can be developed using sICAM-1 or Axl for clinical use.

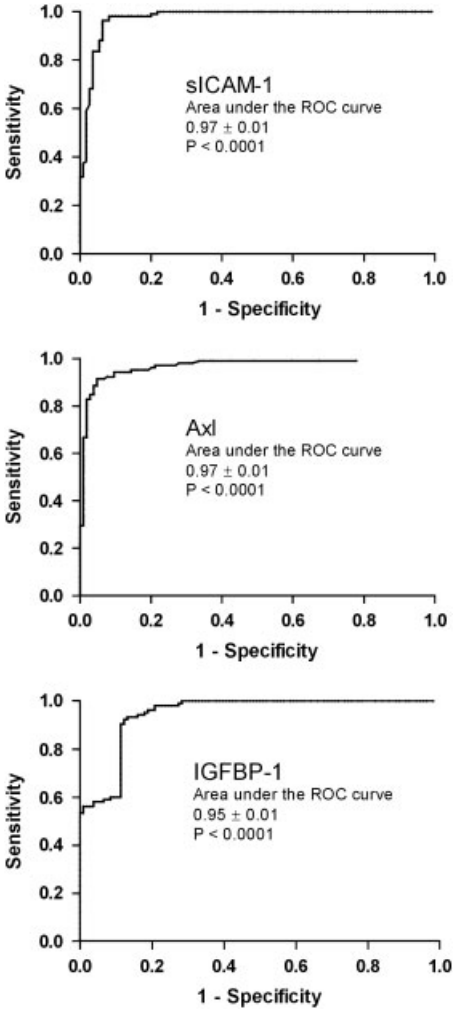

Figure 3. Diagnostic value of sICAM-1, Axl, and IGFBP-1 for PROM. Receiver-operating characteristics (ROC) curves were plotted for each of the three analytes, and all exhibited high sensitivity and specificity for the diagnosis of PROM.

3.3 sICAM-1, Axl, and IGFBP-1 levels in PROM and control CVF samples

We tested the above-mentioned hypothesis by recruiting 110 PROM patients and evaluated the levels of sICAM-1, Axl, and IGFBP-1 in CVF samples taken from these individuals. As depicted in Fig. 2, the concentrations of sICAM-1, Axl, and IGFBP-1 in the CVF samples of PROM patients were at 7.83 ± 6.48, 3.14 ± 2.70, and 51.21 ± 34.83 ng/mL, respectively, significantly higher than those in the CVF samples of the healthy controls at 0.88 ± 1.18, 0.16 ± 0.42, and 3.75 ± 7.54 ng/mL ($p < 0.01$), respectively. The concentration difference of the three analytes in the two groups remained huge, separately at 8.9, 19.6, and 13.7 folds, though not as many as that found between the AF and CVF samples from the healthy controls. These analytes were indeed expected to be diluted after leaking from amniotic cavity into the vagina. Furthermore, as presented in Table 1, the concentrations of sICAM-1 and Axl did not exhibit significant difference between PROM and preterm PROM patients, while the mean concentration of IGFBP-1 in the CVF samples of preterm PROM patients was significantly lower than that in the CVF samples of the PROM patients.

3.4 Diagnostic value of sICAM-1, Axl, and IGFBP-1 for PROM

Based on the individual values of the CVF samples from PROM/preterm PROM and the healthy controls, the diagnostic value of these three analytes was then evaluated using ROC analysis. The threshold that gave the maximal sensitivity and specificity for the diagnosis of PROM was chosen as the cut-off values. The results are shown in Fig. 3 and Table 2. Using a cut-off value at 2.0, 0.4, and 11.2 ng/mL, respectively, for sICAM-1, Axl, and IGFBP-1, the sensitivity and specificity were all impressively high, with sICAM being the highest at 96.4 and 92.7%.

4 Discussion

In the present study, we assumed that proteins of a high concentration in AF could be used as biomarkers for the diagnosis of PROM after they leaked into the vagina. This hypothesis was proven to be correct by using a screening assay and a subsequent quantification of selected proteins, sICAM-1, Axl, and IGFBP-1, in CVF samples. As previously discussed, the diagnosis of PROM remains a clinical challenge, given that the current diagnosis of PROM depends on the observation of AF pooling and the positive results of nitrazine test and ferning. The absence of one of the above findings is an indication for further testing,

Table 2. Diagnostic value of sICAM-1, Axl, and IGFBP-1 for prelabor rupture of membranes

	Cut-off (ng/mL)	Sensitivity (%)	95% CI[a)]	Specificity (%)	95% CI	Likelihood ratio
sICAM-1	2.0	96.4	91.0–99.0	92.7	86.2–96.8	13.25
Axl	0.4	92.4	85.5–96.7	90.4	83.0–95.3	9.61
IGFBP-1	11.2	93.3	86.8–97.3	86.8	78.8–92.6	7.07

a) Confidence interval.

because other factors can contribute to false-positive or false-negative results. Alkaline pH in nitrazine can be caused by vaginal infections or the presence of blood or semen in the sample [12, 16]. Cervical mucus can cause ferning as well [16–18].

Similar to indigo carmine dye injection, the AF proteins sICAM-1, Axl, and IGFBP-1 remained at much higher levels after AF leaking into vagina in PROM compared with those in the control individuals. Based on the findings in the present study, sICAM-1, Axl, and IGFBP-1 in leaked AF may become excellent diagnostic biomarkers for PROM. Considering the high sensitivity and specificity, these tests may easily surpass nitrazine test that has a sensitivity and specificity reported at 90.7 and 77.2% [19]. In previous studies, some AF proteins were examined as biomarkers for the diagnosis of PROM, such as prolactin, α-fetoprotein, β-subunit of human chorionic gonadotropin, fetal fibronectin, diamine oxidase, lactate, creatinine, urea, PAMG-1, and IGFBP-1 [2]. In a previous clinical study, Lee et al. reported using PAMG-1 test for the diagnosis of PROM at initial presentation with a sensitivity of 98.7% (157 of 159), specificity of 87.5% (21 of 24), positive predictive value of 98.1% (157 of 160), and negative predictive value of 91.3% (21 of 23). They concluded that PAMG-1 test was better than both the conventional clinical assessment and the nitrazine test alone in confirming the diagnosis of PROM [20]. IGFBP-1 was investigated using a rapid test or an enzymatic quantitation test. The sensitivity was reported in the range of 95.3–100%, and the specificity in the range of 93.1–98.2% in several studies each consisting of approximately 50 PROM patients [19, 21–23]. In the present single center study with 110 PROM patients and 110 healthy controls, we confirmed the huge difference of IGFBP-1 concentration between the AF and the CVF samples (72 folds), and also its high sensitivity (93.3% in the present study) as a biomarker to diagnose PROM but failed to reveal a specificity (86.8% in the present study) as high as previously reported. Instead, sICAM-1 and Axl had a comparable sensitivity (96.4 and 92.4%) and a higher specificity (92.7 and 90.4%).

Comparing sICAM-1, Axl, and IGFBP-1, sICAM-1 seems to be the best candidate for developing an immunoassay-based bedside rapid test, for example, a strip test using colloidal gold as the positive indicator. This technology is widely accepted because it is reliable, easy to use, and inexpensive [24–27]. A good example is the pregnant strip test that detects human chorionic gonadotrophin in urine samples. This technology has a detection sensitivity around 2 ng/mL, matching the sICAM-1 cut-off level determined in the present study. Besides, sICAM did exhibit the highest sensitivity and specificity for the diagnosis of PROM. We believe a rapid and inexpensive strip test using sICAM as the analyte could greatly help the diagnosis of PROM in the clinical practice, especially in hospitals located in developing countries.

In conclusion, we have discovered a couple of novel biomarkers, sICAM-1 and Axl, that are diagnostic for PROM. While both of them are highly sensitive and specific, sICAM seems to be a better candidate for the development of a bedside rapid test for clinical use.

The authors have declared no conflicts of interest.

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可溶性细胞间黏附分子-1快速检测方法在胎膜早破诊断中的价值

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【摘要】 **目的**:探讨阴道液中可溶性细胞间黏附分子-1(sICAM-1)快速检测方法在胎膜早破诊断中的临床应用价值。**方法**:采用胶体金免疫层析法对106例健康妊娠妇女和76例胎膜早破妇女阴道液中的sICAM-1进行定性检测;并将sICAM-1检测数据分别与本研究入选者分组诊断标准、羊水积液检测、阴道液pH值检测和羊齿植物叶状结晶检测数据相比较,进行一致性检验。**结果**:sICAM-1对胎膜早破诊断的灵敏度为100%,特异度为97.17%,阳性预测值为96.20%,阴性预测值为100%,准确度为98.35%;sICAM-1检测结果与常规诊断方法、羊水积液检测、阴道液pH值检测和羊齿植物叶状结晶检测结果的一致性较好(*kappa*>0.75,*P*=0.000)。**结论**:sICAM-1是一个具有高灵敏度和高特异性的胎膜早破诊断生物标志物,具有较强的临床应用价值。

【关键词】 可溶性细胞间黏附分子-1;胎膜早破;诊断

中图分类号:R714.43^{+}3 文献标志码:A

Rapid Diagnostic Method of Soluble Intercellular Adhesion Molecules-1 for Premature Rupture of Membranes

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【**Abstract**】 **AIM**: To investigate the rapid diagnostic method of soluble intercellular adhesion molecules-1 in vaginal fluid for premature rupture of membranes. **Methods**: The soluble intercellular adhesion molecules-1 in vaginal fluid of 106 healthy pregnant women and 76 pregnant women with premature rupture of membranes was detected qualitatively by colloidal- gold immunochromatographic assay. Consistency test was performed between soluble intercellular adhesion molecules-1 and pooling of amniotic fluid, pH test and amniotic fluid crystallization test. **Results**: The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of soluble intercellular adhesion molecules-1 for diagnostic test of premature rupture of membranes were 100%, 97.17%, 96.20%, 100% and 98.35%. Consistency test showed that the result of soluble intercellular adhesion molecules-1 was highly consistent with the result of current diagnostic method, pooling of amniotic fluid, pH test and amniotic fluid crystallization test(*kappa*>0.75, *P*=0.000). **Conclusions**: Soluble intercellular adhesion molecules-1 was a good biomarker with high sensitivity and specificity for diagnosing premature rupture of membranes.

【**Key words**】 Soluble intercellular adhesion molecules-1; Premature rupture of membranes; Diagnosis

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胎膜早破是产科常见疾病之一,其发病率最高可达 17%[1],如不能及时诊断将引起早产、母婴感染甚至围生儿的死亡等,严重威胁母婴的健康。临床上对胎膜早破的诊断主要依靠妊娠期妇女自觉阴道流液的病史联合阴道液 pH 值检测、阴道液羊齿植物叶状结晶检测、超声检查、羊膜镜检查等物理检查方法进行综合判断,而这些方法存在准确度不高、费时或无法常规临床使用等缺点[2,3]。随着蛋白质组学和免疫学技术的发展,寻找羊水中特异的生物标志物,并将其开发为体外快速检测系统成为目前胎膜早破诊断的研究热点。本研究以胶体金免疫层析法定性检测妊娠期妇女阴道液中的可溶性细胞间黏附分子-1(soluble intercellular adhesion molecules-1, sICAM-1),探讨 sICAM-1 在临床胎膜早破诊断中的价值。

1　对象与方法

1.1　对象　选择 2011 年 8 ~ 11 月在本院就诊的妊娠期妇女,孕 15 ~ 41 周,入选者均自愿加入本研究并签署知情同意书。研究对象分为两组,即胎膜早破组和健康对照组。胎膜早破的诊断标准[4]:阴道窥器检查可见阴道后穹隆处有羊水积液;pH 试纸检查阳性;羊齿植物叶状结晶试验阳性。满足以上 3 种检查项目中的任意两项即诊断为胎膜早破。健康对照组为临床确诊胎膜完整的健康妊娠妇女。排除标准:胎盘前置状态或前置胎盘、阴道流血、阴道内使用药物未超过6 小时、临产者。最终入选者共 182 例,其中胎膜早破组 76 例(足月前胎膜早破 9 例,足月胎膜早破 67 例),健康对照组 106 名。两组的年龄、孕周、孕次和产次比较,差异均无统计学意义($P > 0.05$)。

表 1　两组一般资料的比较

Tab 1　Comparision of Clinical Parameter Between Two Groups

	胎膜早破组 ($n = 76$)	健康对照组 ($n = 106$)	t	P
年龄范围(岁)	28.43 ± 0.26	28.29 ± 0.22	0.40	0.80
孕周(周)	37.76 ± 0.22	37.04 ± 0.31	1.64	0.10
孕次(次)	2.10 ± 0.09	1.99 ± 0.06	1.04	0.30
产次(次)	0.14 ± 0.03	0.13 ± 0.03	0.32	0.75

1.2　方法

1.2.1　检测原理　sICAM-1 快速检测试剂盒由成都创宜生物科技有限公司提供。该试剂盒利用胶体金颗粒作为阳性指示剂,使用一对高特异性的抗人 sICMA-1 抗体,采用免疫层析原理定性检测妊娠期妇女阴道液中的 sICAM-1。其中一种抗体与呈紫红色的 40 nm 胶体金颗粒偶联,用于结合检测样本中的 sICAM-1;另一种抗体则包被于检测试纸条硝酸纤维素膜上的检测线位置,用于捕获已与第 1 种抗体结合的 sICAM-1。当检测样本中存在 sICAM-1 时,sICAM-1 会与偶联了胶体金颗粒的抗体相结合,该结合物由于层析原理流动至第 2 种抗体的包被位置,与第 2 种抗体结合而呈现一条紫红色的检测线。同时为了验证检测的有效性,硝酸纤维素膜上还设置了质控线,若检测结果有效,则在质控线位置也会出现一条紫红色的线条。

1.2.2　样本采集及检测方法　样本采集及检测方法按照试剂盒说明书操作进行。首先将一次性无菌棉签伸入阴道后穹隆处旋转 5 圈取样,之后将棉签头插入样本稀释液管内的液体中旋转 5 次,接着将棉签紧贴管壁旋转挤压,尽量挤干棉签上液体。用试剂盒内的滴管吸取样本液滴入检测卡加样孔内,大约 3 ~ 6 分钟后观察结果。若出现两条紫红色的线条则判定为阳性,仅质控线位置出现线条则判定为阴性,若质控线位置无线条出现则该次检测无效。

1.2.3　研究方法　采用胶体金免疫层析法对 106 例健康妊娠妇女和 76 例胎膜早破妇女阴道液中的 sICAM-1 进行定性检测;并将 sICAM-1 检测数据分别与本研究入选者分组诊断标准、羊水积液检测、阴道液 pH 值检测和羊齿植物叶状结晶检测数据相比较,进行一致性检验。整个研究采用“同步盲法”:临床医生对研究对象编号,完成羊水积液检测、阴道液 pH 值检测以及样本的采集。采集到的样本送交实验室,由实验室人员对采集到的样本进行羊齿植物叶状结晶检测和 sICAM-1 检测试剂盒检测。临床医生不知道采集样本的羊齿植物叶状结晶检测结果及试剂盒检测结果,而实验室人员也不知道检测样本属于胎膜早破组还是健康对照组。整个研究的最终结果由第三方人员收集、整理并进行统计分析。

1.2.4　统计学处理　诊断性能各项指标计算方法如下:灵敏度 = 真阳性/(真阳性 + 假阴性) × 100%;特异度 = 真阴性/(真阴性 + 假阳性) × 100%;阳性预测值 = 真阳性/总阳性 × 100%;阴性预测值 = 真阴性/总阴性 × 100%;假阴性率 = 假阴性/(真阳性 + 假阴性) × 100%;假阳性率 = 假阳性/(真阴性 + 假阳性) × 100%;准确度 = (真阳性 + 假阴性)/总例数 × 100%。运用 *kappa* 统计量评估 sICAM-1 检测结果的一致性,若 *kappa* > 0.75,说明一致性较好。

2　结　果

采用胶体金免疫层析法对 182 例研究对象阴道液中 sICAM-1 的定性检测,胎膜早破组 76 例中 sICAM-1 检测均阳性,健康对照组 106 例中 sICAM-1 阳性 3 例。采用该方法诊断胎膜早破的灵敏度为 100%(76/76),特异度为 97.17%(103/106),阳性预测值为 96.20%(76/79),阴性预测值为 100%(103/103),

假阳性率为 2.83%(3/106),假阴性率为 0,准确度为 98.35%(179/182)。sICAM-1 检测胎膜早破的准确度与孕周大小无关,诊断足月前胎膜早破准确度 100%(9/9),诊断足月胎膜早破准确度 100%(67/67)。sICAM-1 检测结果与常规诊断方法、羊水积液检测、阴道液 pH 值检测和羊齿植物叶状结晶检测结果的一致性较好(*kappa* >0.75, *P* =0.000)。见表 2。

表 2 sICAM-1 检测结果与各种诊断方法的一致性评价

Tab 2 Evaluation The Consistency Between sICAM-1 and Other Diagnostic Methods

	sICAM-1		*Kappa*	*z*	*P*
	阴性	阳性			
常规诊断方法					
阴性	103	3	0.966	13.044	0.000
阳性	0	76			
羊水积液检测					
阴性	102	4	0.955	12.899	0.000
阳性	0	76			
阴道液 pH 检测					
阴性	102	4	0.944	12.740	0.000
阳性	1	75			
羊齿植物叶状结晶检测					
阴性	97	36	0.509	7.327	0.000
阳性	6	43			

3 讨 论

国内外对胎膜早破诊断的研究始于 1929 年,诊断方法从胎儿细胞染色法、阴道液 pH 值检测法、羊水羊齿植物叶状结晶检查法、超声波检查法、羊膜腔染料注射法、糖类检测法发展到目前的羊水特异性生物标志物检查法。诊断方法从有创、准确度不高、操作复杂发展到了目前的无创无害,方便快捷且准确度极高的体外诊断方法。诊断时间也从过去的一天发展到仅需几分钟的快速诊断系统[3]。基于以特异性羊水生物标志物为检测目标发展而成的快速体外诊断方法在临床应用方面的实用性,因此特异性的羊水生物标志物正在逐步被研究人员发现,目前已发现的可以用于胎膜早破诊断的生物标志物有甲胎蛋白、人胎盘催乳激素、胎儿纤连蛋白、胰岛素样生长因子结合蛋白-1、胎盘 α 微球蛋白-1 等,其中胰岛素样生长因子结合蛋白-1、胎盘 α 微球蛋白-1 已分别被芬兰和美国的公司开发为胎膜早破快速诊断系统“Actim PROM”和“AmniSure”[5~8]。

最近 Wang 等[9]通过蛋白质芯片技术筛选出一个新的胎膜早破诊断生物标志物—sICAM-1。该团队的研究结果显示,sICAM-1 在羊水中的浓度为 74.53 ±41.36 ng/ml,在健康妊娠妇女阴道液中的浓度为 0.88 ± 1.18 ng/ml,而在胎膜早破妇女阴道液中的浓度则为 7.83 ± 6.48 ng/ml,sICAM-1 在胎膜早破妇女和健康妊娠妇女阴道液样本中浓度的差异也达 8.9 倍。同时他们评估了 sICAM-1 的诊断价值,发现在最佳临界值为 2.0 ng/ml 时,sICAM-1 的灵敏度和特异度分别为 96.4% 和 92.7%,其诊断性能明显高于目前常用的胰岛素样生长因子结合蛋白-1,由此证明 sICAM-1 是一个极为优秀的胎膜早破诊断生物标志物。

本研究采用胶体金免疫层析法定性检测妊娠妇女阴道液中的 sICAM-1,结果显示 sICAM-1 对胎膜早破诊断的灵敏度为 100%,特异度为 97.17%,准确度为 98.35%;并且 sICAM-1 检测结果与常规诊断方法、羊水积液检测、阴道液 pH 值检测和羊齿植物叶状结晶检测结果的一致性较好(*kappa* >0.75, *P* =0.000),再次验证了 sICAM-1 在胎膜早破诊断方面的重要价值。再者,本研究采用胶体金免疫层析法进行检测具有直观、方便、快捷等优点,提示该方法可作为床旁快速检测方法;且使用该方法时无须进行特殊的培训,不需要专业的知识和技术,因此也非常适用于基层医院的医生使用。

综上所述,利用胶体金免疫层析法定性检测妊娠妇女阴道液中的 sICAM-1 是一个具有较高临床应用价值的方法,可常规使用以辅助临床胎膜早破的诊断。

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可溶性细胞间粘附分子-1 检测试剂盒诊断胎膜早破的临床试验

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[关键词] 胎膜早破; 细胞间粘附分子-1; 妊娠并发症; 临床试验

[摘要] 目的 评价可溶性细胞间粘附分子-1 (sICAM-1) 检测试剂盒用于诊断胎膜早破的敏感度及特异性。方法 选择妊娠 15 至 42 周到医院就诊的妊娠妇女, 进行阴道液 pH 值测定、阴道后穹窿积液检查、阴道液涂片羊齿状结晶检查, 其中两项阳性者诊断为胎膜早破, 两项阴性者诊断为胎膜未破。所有妊娠妇女均进行 sICAM-1 试剂盒检测。运用 kappa 统计量评估检测结果的一致性和相关性。结果 1 047 例妊娠妇女进入该临床试验研究, 经阴道液 pH 值、阴道后穹窿积液和阴道液涂片羊齿状结晶三项检查, 诊断胎膜早破 423 例, 胎膜未破 624 例; sICAM-1 检测试剂盒检测出胎膜早破 446 例, 胎膜未破 601 例。sICAM-1 检测试剂盒诊断胎膜早破的敏感度为 99.53%, 特异性为 95.99%, 假阳性率为 4.01%, 假阴性率为 0.47%, 阳性预测值为 94.39%, 阴性预测值为 99.67%, 准确度为 97.42%。sICAM-1 检测试剂盒诊断胎膜早破与阴道液 pH 值测定、阴道后穹窿积液检查具有极强一致性 (kappa = 0.919 3、0.919 2), 与阴道液涂片羊齿状结晶检查具有中度一致性 (kappa = 0.493 1)。整个试验无不良事件发生。结论 sICAM-1 检测试剂盒在诊断胎膜早破中具有较高的敏感度、特异性、准确度和安全性。

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Clinical trial of soluble intercellular adhesion molecule - 1 detection kit in diagnosis of premature rupture of membrane

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[**KEY WORDS**] premature rupture of fetal membranes; intercellular adhesion molecule-1; pregnancy complications; clinical trials

[**ABSTRACT**] AIM To evaluate the sensitivity and specificity of soluble intercellular adhesion molecule-1 (sICAM-1) detection kit in the diagnosis of premature rupture of membrane (PROM). METHODS Pregnant women with gestational week from 15 to 42 weeks were detected by pH strip detection, vaginal posterior fornix effusion detection and fern-like crystals detection. If two of the three results were positive, the patient was enrolled in PROM group. If two of the three results were negative, the patient was enrolled in non-PROM group. All of the pregnant women were also detected by sICAM-1 detection kit. Kappa statistics were used to evaluate the consistency and correlation of different results. RESULTS Totally 1 047 pregnant women were enrolled in this study, 423 with PROM and 624 with non-PROM detected by pH strip detection, vaginal posterior fornix effusion detection and fern-like crystals detection. There were 466 patients detected as PROM and 601 patients detected as non-PROM by sICAM-1 detection kit. The sensitivity of sICAM-1 detection kit in the diagnosis of PROM was 99.53%, specificity was 95.99%, false positive rate was 4.01%, false negative rate was 0.47%, positive predictive value was 94.39%, negative predictive value was 99.67%, and accuracy was 97.42%. There was very high consistency in diagnosis of PROM between pH strip detection, vaginal posterior fornix effusion detection and sICAM-1 detection kit (kappa = 0.919 3, 0.919 2). There was medium consistency between fern-like crystals detection and sICAM-1 detection kit (kappa = 0.493 1). There was no adverse event during the whole process. CONCLUSION sICAM-1 detection kit has high sensitivity, specificity, accuracy and safety in the diagnosis of PROM.

胎膜早破 (premature rupture of membranes) 是产科常见并发症，其妊娠结局与破膜时孕周有关。孕周越小，围生儿预后越差，是导致早产和母婴感染的主要原因[1,2]。目前临床上还没有检测胎膜破裂的金标准。常用的方法[3]包括阴道液pH值测定、阴道后穹窿积液检查和阴道液涂片羊齿状结晶检查。有些病例在胎膜破口小或高位破裂、羊水流出少、破裂时间长或有阴道分泌物污染等情况下，使用常用的检测方法难以诊断胎膜早破。本研究旨在评价可溶性细胞间粘附分子-1 (soluble intercellular adhesion molecule-1, sICAM-1) 检测试剂盒用于诊断胎膜早破的敏感度及特异性，以为临床早期微量检测胎膜早破提供新的方法。

对象与方法

病例选择和分组 采用大样本、多中心、单盲对照的临床试验，试验方案经重庆医科大学附属第二医院伦理委员会批准。受试人群为2011年8月至2011年11月到6家参加本试验医院产科就诊的孕妇。试验中将临床常用检测胎膜早破的三种方法——阴道液pH值测定、阴道窥器检查羊水流出和阴道液涂片检查羊齿状结晶结合使用来判断胎膜早破。由于临床缺乏诊断胎膜早破的金标准，经专家讨论达成共识，将本研究的金标准定义为：上述三项检查中任两项结果为阳性者诊断为胎膜早破，任两项结果为阴性者诊断为胎膜未破。孕妇根据就诊时间经上述三种方法检测后，进入胎膜早破组和胎膜未破组。所有孕妇均使用sICAM-1检测试剂盒检测 (取样与检测人员不同)。入选标准：孕周在15～42周之间胎膜早破和未破的孕妇；自愿参加并签署知情同意书者。排除标准：胎盘前置状态或前置胎盘；阴道流血；阴道内使用药物未超过6 h；分娩活跃期者；阴道炎症。剔除标准：样本污染；无法统计的入组病例应予剔除，剔除的病例应说明原因，其病例报告表应保

留备查。

检测试剂 sICAM-1 检测试剂盒（胶体金法），成都创宜生物科技有限公司生产，包装规格：每盒 1 人份，批号：20110601，有效期：18 个月，保存条件：2 ℃ ~ 30 ℃储存。pH 试纸，生产厂家：杭州特种纸业有限公司，包装规格：每本 80 条，pH 值测定范围：1 ~ 14，批号：20100817，有效期：3 年，保存条件：避光干燥储存，勿使受潮。

检测方法及结果判断 sICAM-1 试剂盒检测：孕妇取膀胱截石位，窥开阴道后，用一次性无菌棉签伸入阴道后穹窿处，旋转 5 圈取样，将棉签头插入样本稀释液中，旋转 5 次紧贴管壁挤压旋转 2 次，于检测卡加样孔内滴入 3 滴样本液，3 ~ 10 min 内观察结果。检测结果质控线（C 线）与检测线（T 线）同时显色，判定为阳性；仅质控线（C 线）显色，判定为阴性；质控线（C 线）不显色（无论 T 线是否显色），判定为无效，需重新检查。pH 试纸检测：将试纸放在阴道后穹窿处，约 10 s 内观察结果。与试纸标准比色卡对照，pH ≥ 7.0 判为阳性，pH < 7.0 判为阴性。阴道后穹窿积液检查：窥阴器暴露阴道后穹窿，直接观察是否有积液，如果有判断为阳性，没有判断为阴性。阴道液羊齿状结晶检测：将采集的阴道液制成涂片后在光学显微镜下观察，如查见羊齿状结晶判为阳性，未查见羊齿状结晶判为阴性。所有检查均有专人负责。根据 sICAM-1 检测试剂盒和本研究所设金标准的检测结果，计算前者的灵敏度、特异性、准确度、假阳性率、假阴性率、阳性预测值、阴性预测值等指标。

安全性评价 所有进入试验的孕妇均观察有无不良事件（外阴阴道疼痛、不适和出血等）及严重不良事件的发生并记录。

统计学分析 研究方案、表格、病例观察表和随机表统一设计，资料统一收集进行数据处理。运用 kappa 统计量来评估检测结果的一致性和相关性。设 sICAM-1 检测试剂盒和本研究金标准的检测结果如表 1，则灵敏度（真阳性率）/% = A/(A + C) × 100%，特异性（真阴性率）/% = D/(B + D) × 100%，假阳性率/% = B/(B + D) × 100%，假阴性率/% = C/(A + C) × 100%，阳性预测值/% = A/(A + B) × 100%，阴性预测值/% = D/(C + D) × 100%，准确度/% = (A + D) /(A + B + C + D) × 100%。

表 1 可溶性细胞间粘附分子-1（sICAM-1）检测试剂盒和本研究金标准的假设检测结果

sICAM-1 检测试剂盒	本研究金标准	
	阳性/例	阴性/例
阳性/例	A	B
阴性/例	C	D

结 果

一般资料 研究入选受试者 1 047 人，完成 1 047 人，经本研究金标准检查，其中胎膜早破 423 人，胎膜未破 624 人。研究中无剔除病例。胎膜早破组平均年龄为（28.3 ± 4.2）岁、平均孕周为（35.2 ± 6.1）周，胎膜未破组平均年龄为（29.1 ± 4.5）岁、平均孕周为（34.5 ± 5.6）周。两组病例一般临床资料比较，无显著差异（$P > 0.05$），提示两组间具有可比性。

临床评价结果 本研究金标准检测出胎膜早破 423 例，sICAM-1 检测试剂盒检测出 446 例；金标准检测胎膜未破 624 例，sICAM-1 检测试剂盒检测出 601 例，具体评价结果见表 2。按表 1 统计，A = 421，B = 25，C = 2，D = 599；sICAM-1 检测试剂盒灵敏度为 99.53%，特异性为 95.99%，假阳性率为 4.01%，假阴性率为 0.47%，阳性预测值为 94.39%，阴性预测值为 99.67%，准确度为 97.42%。

一致性评价 经 kappa 一致性检验，sICAM-1 检测试剂盒与 pH 试纸检测、阴道后穹窿积液检测具有极强一致性，kappa 统计值分别为 0.919 3 和 0.919 2；与阴道液羊齿状结晶检测具有中度一致性，kappa 统计值为 0.493 1。

安全性评价 试验中无不良事件发生。

讨 论

阴道后穹窿积液检查、阴道液 pH 测定以及阴

表 2 可溶性细胞间粘附分子-1（sICAM-1）检测试剂盒与 pH 试纸检测、后穹窿积液检测及阴道液羊齿状结晶的检测结果

例（%）

sICAM-1 检测试剂盒	pH 试纸检测		后穹窿积液检测		羊齿状结晶检测	
	阴性	阳性	阴性	阳性	阴性	阳性
阴性	593 (56.63)	8 (0.76)	597 (57.02)	4 (0.38)	516 (49.28)	84 (8.02)
阳性	33 (3.15)	413 (39.44)	37 (3.53)	409 (39.06)	169 (16.14)	277 (26.46)

道液涂片羊齿状结晶检查是目前临床上常规检测胎膜早破的三种方法。但由于存在易污染、检查繁琐以及假阳性、假阴性率高等不足，其应用受到一定限制[2]。此外当发生少量或高位破膜时，仅依靠上述方法很难得出准确的诊断结果。sICAM 由蛋白酶裂解细胞外膜型成分脱落而来，sICAM-1 在羊水中浓度极高，胎膜破裂时羊水将外漏至阴道内，导致阴道液中 sICAM-1 浓度显著增加；而胎膜完好时 sICAM-1 在阴道液中的背景浓度非常低[4,5]。sICAM-1 检测试剂盒利用胶体金免疫层析原理，检测人 sICAM-1。金标鼠抗人 ICAM-1 单克隆抗体在样品垫区捕获待测样本中的 sICAM-1，形成 ICAM-1/单抗结合体并从吸附区流送至检测区，与预先固化在检测卡测试区的羊抗人ICAM-1 多克隆抗体结合后固定在不溶的载体中，在检测线（T 线）形成一条肉眼可见的红色检测线条，说明样本中含有高浓度 sICAM-1，提示羊水外漏。质控线（C 线）用来显示检测试剂盒功能是否正常，当羊抗鼠 IgG 多克隆抗体捕获到金标鼠抗人 ICAM-1 单克隆抗体时，质控线显示红色。为了将错误结果的发生率降到最低，该试剂盒还特别采用一对特异性结合的抗体将检测试剂盒的敏感阈值降到了最理想的水平。这个非常低的阈值水平可以检测到阴道分泌物中极少量的羊水。因此 sICAM-1 检测试剂盒能够快速、微量检测胎膜早破，满足整个孕期不同时间、不同程度的胎膜早破诊断需求，有利于临床医生及时发现胎膜早破，早期采取有效措施，防止母婴并发症发生，确保母婴安全。

本研究结果显示，sICAM-1 检测试剂盒与研究所设金标准具有较好的一致性，具有较高的灵敏度、特异性及准确度，假阳性率及假阴性率均较低。由于在前期的预实验中发现血液可能会对 sICAM-1 检测试剂盒造成污染，因此在本研究中没有纳入有阴道出血的病例，以后可增加阴道出血病例进一步做干扰试验。安全性分析显示，sICAM-1 检测试剂盒在检测过程中无不良事件发生。sICAM-1 检测试剂由于不直接接触人体，因此对受试对象没有风险。

综上所述，sICAM-1 检测试剂盒检测胎膜早破有较高的敏感度、特异性、准确度和安全性，值得在临床推广使用。

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·临床经验交流·

可溶性细胞间黏附分子-1 金标试纸条在胎膜早破诊断中的临床价值*

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【关键词】 绒毛膜羊膜炎; 细胞间黏附分子 1; 绒毛膜绒毛; 血清; 孕妇; 对照组
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胎膜早破是指临产前胎膜破裂, 常引起早产、母婴感染和围生儿死亡等严重并发症。研究表明, 破膜超过 48h 新生儿感染率达 100%[1], 及时、快速、准确地诊断并给予适当的治疗可明显改善胎膜早破的妊娠结局、降低并发症的发病风险和严重程度[2,3]。目前临床上主要根据孕妇自觉阴道流液病史、观察羊水积液、阴道液 pH 值和阴道液羊齿植物叶状结晶检测等判断, 这些方法易受破膜时间、阴道炎症、宫颈粘液、阴道内药物和临床医生的经验等因素的干扰而影响其准确度。最近发现, 可溶性细胞间黏附分子-1(soluble intercellular adhesion molecules-1, sICAM-1)可作为胎膜早破诊断的生物标记物且诊断性能优异[4]。因此本文通过研究sICAM-1金标试纸条在胎膜早破诊断方面的性能, 初步探讨 sICAM-1 快速检测方法在临床中的使用价值。

1 资料与方法

1.1 研究对象 选取 2011 年 8 月至 2011 年 11 月就诊我院的胎膜早破 45 例孕妇, 其中足月前胎膜早破 22 例, 足月胎膜早破 23 例。选取同期临床确诊胎膜完整的 50 例孕妇为健康对照组。入选者均知情同意并签署知情同意书。胎膜早破诊断标准: (1) 孕妇有自觉阴道流液的病史; (2) 阴道窥器检查可见阴道后穹隆处有羊水积液; (3) pH 试纸检查阳性; (4) 羊齿植物叶状结晶试验阳性。满足以上中的任意 3 项即诊断为胎膜早破。胎盘前置状态或前置胎盘、阴道流血、阴道内使用药物未超过 6h、临产者未纳入本研究。两组一般资料比较, 均无统计学差异($P>0.05$), 见表 1。

表 1 两组孕妇的一般资料

组别	n	年龄(岁)	孕周(周)	孕次(次)	产次(次)
胎膜早破组	45	20 ~ 43	25 ~ 40	1 ~ 7	0 ~ 4
健康对照组	50	18 ~ 40	21 ~ 41	1 ~ 9	0 ~ 3

1.2 样本采集及检测方法 采用“同步单盲法”, 由临床医生对研究对象进行编号, 询问病史、检测羊水积液及阴道液 pH 值。用 sICAM-1 金标试纸条所配备的一次性无菌棉签在阴道后穹窿处旋转 5 圈, 将棉签置于样本稀释液管的液体中旋转 5 次, 将棉签紧贴管壁旋转挤压, 尽量挤干棉签上的液体。将含样本的液体送至实验室, 由实验室人员进行羊齿植物叶状结晶和金标试纸条检测。整个研究过程中, 实验室人员不知道他们检测的样本属于胎膜早破组还是健康对照组, 临床医生也不知道所采集样本的检测结果。最终结果由第三方人员收集、整理和统计分析。

1.3 结果判断 用试剂盒内的滴管吸取样本液滴入金标试纸条卡壳的加样孔内, 3 ~ 6min 后观察结果。若出现两条紫红色线条则判定为阳性, 仅质控线位置出现线条则判定为阴性, 若质控线位置无线条出现则该次检测无效。整理数据后, 评估 sICAM-1 金标试纸条的临床诊断性能, 并分别与羊水积液检查、阴道液 pH 值和羊齿植物叶状结晶检查数据进行比较。

1.4 统计学处理 用 kappa 评估检测结果的一致性。灵敏度 = 真阳性例数/(真阳性例数 + 假阴性例数) × 100%, 特异度 = 真阴性例数/(真阴性例数 + 假阳性例数) × 100%, 准确度 = (真阳性例数 + 真阴性例数)/总例数 × 100%, 阳性预测值 = 真阳性例数/总阳性例数 × 100%, 阴性预测值 = 真阴性例数/总阴性例数 × 100%, 假阴性率 = 1 - 灵敏度; 假阳性率 = 1 - 特异度。

2 结 果

采用 sICAM-1 金标试纸条对 95 例孕妇阴道液中 sICAM-1 进行定性检测结果显示, 诊断胎膜早破的灵敏度 100%, 特异度 98%, 准确度 98.95%, 假阳性率 2%, 假阴性率 0, 阳性预测值 97.83%, 阴性预测值 100%。sICAM-1 金标试纸条检测结果与羊水积液检测、阴道液 pH 值及羊齿植物叶状结晶检测结果一致性评估显示, sICAM-1 金标试纸条检测与这三种检测方法的一致性较好(kappa 系数 > 0.75)。见表 2、3。

表 2 sICAM-1 金标试纸条检测结果(n)

组别	sICAM-1 金标试纸条		合计
	阳性	阴性	
胎膜早破组	45	0	45
健康对照组	1	49	50

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表 3　sICAM-1 金标试纸条检测与常规检测方法的一致性评价

常规检测方法		sICAM-1 阳性	sICAM-1 阴性	Kappa 系数	95% CI	Z	P
羊水积液检测	阴性	49	0	0.9367	0.8663～1.0000	9.1477	0.0000
	阳性	3	43				
阴道液 pH 检测	阴性	46	3	0.9158	0.8351～0.9965	8.9342	0.0000
	阳性	1	45				
羊齿植物叶状结晶检测	阴性	48	1	0.8944	0.8045～0.9843	8.7353	0.0000
	阳性	4	42				

3　讨　论

随着新的胎膜早破诊断生物标记物的不断被发掘，以此为基础发展而成的快速诊断系统也成为目前胎膜早破诊断的研究热点。sICAM-1 金标试纸条是利用免疫层析原理制备而成的快速检测产品，它以 40nm 的胶体金颗粒作为阳性指示剂，配合一对高特异性的抗人 sICMA-1 抗体，定性检测妊娠期妇女阴道液中的 sICAM-1，其具有准确度高、重复性好、直观、操作简单和快捷等优点。

目前文献报道的胎膜早破诊断方法有：肉眼直接观察法、胎儿细胞染色法、阴道液 pH 值检测法、羊齿植物叶状结晶检查法、超声波检查法、羊膜腔染料注射法、糖类检测法、尿素及肌酐检测法以及用放射免疫分析法、电化学发光法、免疫层析法检测胎膜早破特异性生物标记物等方法[5]。免疫层析法具有准确、直观、快速和方便等优点，被广大临床医生所接受。目前采用免疫层析法的产品主要有芬兰的“Actim PROM”（以胰岛素样生长因子结合蛋白-1 为检测指标）和美国的“AmniSure”（以胎盘 α 微球蛋白-1 为检测指标）[6,7]。

Wang 等[4] 通过蛋白质芯片技术和酶联免疫吸附法筛选出新的胎膜早破诊断生物标记物 sICAM-1。该研究定量检测 110 例胎膜早破和 110 例胎膜完整孕妇阴道液中 sICAM-1 的浓度发现，sICAM-1 在胎膜早破和健康孕妇浓度差异高达 8.9 倍，且在最佳临界值为 2.0ng/ml 时，其诊断灵敏度为 96.4%，特异度为 92.7%，似然比为 13.25。结果还显示，sICAM-1 诊断性能明显优于胰岛素样生长因子结合蛋白-1。本研究结果显示，sICAM-1 对胎膜早破诊断的灵敏度为 100%，特异度为 98%，可有效辅助临床胎膜早破的诊断。sICAM-1 金标试纸条操作步骤简单，且采用卡壳式设计，并在卡壳上清晰标记了检测线和质控线的位置，避免了对结果的错误判读，同时避免检测试剂与操作人员和孕妇及检测样本与周围环境的接触，无创无害，防止对环境造成污染，让操作人员放心使用。此外该金标试纸条检测，最短仅需数十秒，最长不超过 6min 即可判读结果，可明显提高医疗效率。

综上所述，利用金标试纸条快速检测妊娠期妇女阴道液中的 sICAM-1 辅助临床胎膜早破诊断是一种具有较高临床应用价值的方法，适合常规使用。

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