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MATERNO-FETAL MEDICINE

Placental α-microglobulin-1 to detect uncertain rupture of membranes in a European cohort of pregnancies

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Abstract
Purpose We evaluated the performance of the placental alpha-microglobulin-1 immunoassay (AmniSure®, AT) in cervicovaginal secretions in patients with uncertain rupture of membranes (ROM) and investigated the influence of the examiners experience.
Methods This prospective cohort study was performed in pregnant women (17–42 weeks of gestation) with signs of possible ROM. Evaluation included clinical assessment, examination for cervical leakage, Nitrazine test and measurement of the amniotic fluid index by ultrasound and AT. ROM occurrence was based on review of the medical records after delivery.
Results 199 women were included. AT had a sensitivity of 94.4%; specificity of 98.6%; positive predictive value, 96.2%; negative predictive value, 98.0%. Clinical assessment showed a sensitivity of 72.2%; specificity of 97.8%; positive predictive value, 92.9%; negative predictive value, 90.6%. AT was more sensitive for diagnosing ROM ($p = 0.00596$) compared to clinical assessment, independent of the examiners experience. Furthermore, the sole use of AT reduced costs by 58.4% compared to clinical assessment.
Conclusions AT was more sensitive compared to clinical assessment, independent of the examiners experience and gestational age. Our data extend its use in patients with uncertain ROM. Moreover, AT seems to be a cost-effective approach in the assessment of these patients.

Keywords Amniotic fluid index (AFI) · Placental alpha-microglobulin-1 immunoassay · Pregnancy · Rupture of membranes (ROM) · Sensitivity · Specificity

Introduction

Preterm premature rupture of membranes (PPROM) before 37 weeks of gestation has an incidence of 2–3%, leads to 30–40% of preterm deliveries and is associated with an increased risk of perinatal morbidity and mortality [1–3]. Accurate diagnosis of PPROM would allow for gestational age-specific obstetric treatment but conversely, false-positive diagnosis may lead to unnecessary obstetric interventions such as hospitalisation or induction of labor.

The diagnosis of rupture of membranes (ROM) is largely a *clinical* diagnosis and is based on visual assessment when obvious leakage of amniotic fluid (AF) has occurred, but is more difficult when the leakage is subtle and intermittent, which occurs in up to 20% of all women with ROM [4]. The current conventional clinical assessment includes taking an accurate history, demonstration of visible AF in the vagina with a speculum examination, microscopic examination of the fluid for ferning of dried cervicovaginal discharge and the Nitrazine test. Unfortunately, Nitrazine testing of vaginal pH

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has an appreciable false-positive rate due to blood contamination, semen or bacterial vaginosis [5, 6]. The fern test also shows a considerable rate of false-positivity [7]. Both tests are time dependent and become progressively less accurate when 1 h has elapsed after ROM. Pregnancies complicated by ROM usually show a decreased amniotic fluid index (AFI) by transabdominal ultrasound, and ultrasound is therefore recommended as an additional diagnostic tool [8, 9]

The AmniSure® immunoassay (AT) is highly sensitive for placental Alpha-Microglobulin-1, (PAMG-1), a 34 kDa fetal glycoprotein, which was first isolated in 1975 from amniotic fluid by D. Petrunin [10]. PAMG-1 is present throughout all three trimesters of pregnancy in blood, amniotic fluid and cervicovaginal discharge of pregnant women. The concentration in the amniotic fluid (2,000–25,000 ng/ml), however, is 1,000- to 10,000-fold higher than that found in the background cervicovaginal discharge when the fetal membranes are intact (0.05–0.2 ng/ml) (AmniSure® test, package insert). Previous studies in patients with clear signs of PPROM reported a higher sensitivity and specificity with this assay compared to conventional clinical assessment which did not include AFI measurements [11, 12].

The aim of this prospective cohort study was to evaluate the reliability of the AT as a bedside test for uncertain ROM in a European collective compared with conventional clinical assessment including pooling of AF in the vagina, Nitrazine test evaluation and sonographic measurement of AFI, to evaluate the influence of examiner experience and to assess the cost-effectiveness of AT.

Materials and methods

In this prospective observational study, pregnant women in two Swiss centres [Department of Obstetrics and Gynaecology, University of Basel (approx. 2,000 deliveries/year) and Kantonsspital Liestal (approx. 600 deliveries/year)] in Switzerland were asked to participate with written informed consent. Approval for this study was obtained from the institutional review board of the University of Basel, Switzerland.

The inclusion criteria consisted of women with singleton pregnancies in cephalic position and *uncertain* signs or symptoms of ROM (patient complaint of leakage of fluid *without* complete, obvious rupture of membranes) in the second or third trimester. Patients with *clear* signs of ROM (large gush of clear vaginal fluid or a steady trickle) were excluded from the study. Also patients with active bleeding or vaginal examination in the last 12 h, severe pregnancy complications like preeclampsia, fetal malformation or death were excluded.

On admission of the patient, a conventional clinical assessment was performed, including the medical history, followed by a physical examination including a sterile speculum examination (without the use of antiseptic solution or lubricant) and Nitrazine test. In addition AT was performed according to the manufacturer's instructions (Pro Concepta AG, Zug, Switzerland). A sterile Dacron swab was placed in the posterior fornix of the vagina. After a period of 1 min, the swab was removed and agitated in a vial containing solvent for 1 min. The AT test strip was then placed in the solvent and the sample in the vial was allowed to migrate through the membrane strip by capillary action; the test result was available within 5 min.

Following this, all patients underwent a measurement of the AFI by transabdominal ultrasound according to Phelan et al. [13], using a 5-MHz transabdominal probe (Hitachi EUB-6000 plus, Hitachi Medical Systems, Germany and Toshiba Powervision 6000 ultrasound machine, Toshiba Medical Systems, Switzerland).

The diagnosis of ROM was made using the conventional clinical assessment if two of the following three criteria were positive: (1) visual leaking or pooling of AF from the cervix on the speculum examination, (2) positive Nitrazine test or (3) AFI <5 cm in the ultrasound examination. The experience of each examiner was recorded according to the years of training of the respective physician.

During the clinical assessments, physicians were not aware of the AT result, but were informed after the examination. In 16 cases with positive AT and a negative clinical assessment, the AT result was taken into account and the pregnant women were hospitalised and closely followed. Finally, 14 turned out to be correctly positive and 2 were false positive by AT.

Due to our limited experience with AT, the subsequent obstetrical management was based solely on the clinical assessment.

ROM was diagnosed definitively on review of the medical records when there was documented evidence of intact or ruptured membranes with consecutive loss of fluid during delivery. The sensitivity, specificity and positive/negative predictive value of the AT in diagnosing ROM was compared to the clinical assessment. The costs of both diagnostic approaches were calculated by using the ascertained costs of each examination based on the national reimbursement setting (TARMED, deduced from "tarif médical", http://www.tarmedsuisse.ch).

Statistical analysis

A power calculation was initially performed to compare sensitivity and specificity: with a sample size of 199 patients, there is a power of >80 to detect a minimal significant difference of 6% between the shift for AT positive

to clinical negative patients, compared to a shift from AT negative to clinical positive patients (McNemar test, alpha = 5%, two-sided). Sensitivities, specificities, negative predictive values and positive predictive values were estimated with a 95% confidence interval using the corresponding binomial distribution. To compare sensitivities and specificities, McNemar's Chi-square test was performed. Logistic regression was employed to detect influences on performance of AT and clinical assessment. A p value <0.05 was considered as significant (two-sided tests). All analyses were done using R version 2.7.1 (R: A language and environment for Statistical Computing).

Results

202 pregnant women were enrolled and of these, three had to be excluded retrospectively due to incomplete medical records. The median age in the collective of 199 women was 28.8 years (interquartile range, IQR 24.5–33.5 years); the median gestational age was 38 weeks [IQR 34–40 weeks, range 17–42 weeks (Fig. 1)]. 55% (109 of 199) were at term (37 + 0 or later) and 45% (90 of 199) were preterm.

AT was positive in 53/199 (26.6%) and clinical assessment led to the diagnosis of ROM in 42/199 (21.1%) patients. After review of the medical records, ROM was confirmed in 54/199 (27.1%) patients.

There were three false-negative cases tested by AT and 15 after clinical assessment. One of these cases was false negative in both diagnostic methods, while the remaining (two for AT and 14 for clinical assessment) were discordantly false negative in both diagnostic approaches. There were two false-positive cases for AT and three for clinical assessment in independent patients (Fig. 2).

In women with a positive AT and a false-negative clinical assessment (n = 14), the median (IQR) gestational age was 39 weeks (38–40). The gestational age in those cases was very similar to the remaining cases of positive AT and

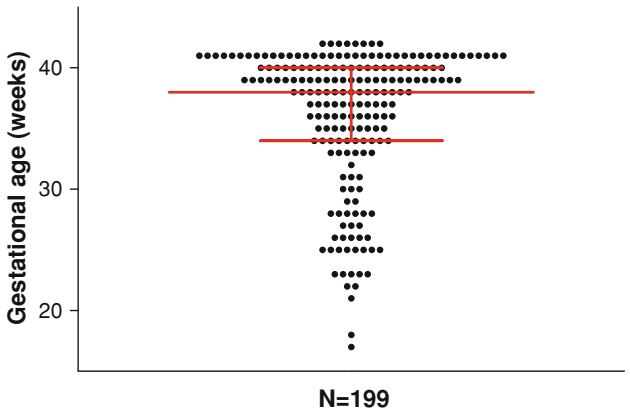

Fig. 1 Gestational age of the study collective (Median and interquartile range)

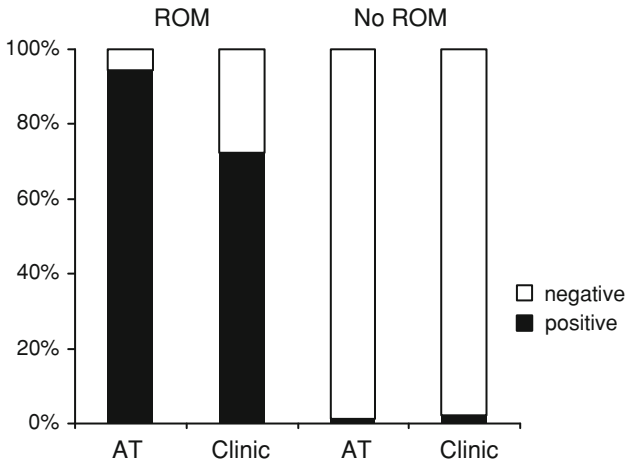

Fig. 2 Test results *ROM* rupture of membranes, *AT* AmniSure® immunoassay, *clinic* clinical assessment. Displayed are the proportions of positive/negative AT or clinical assessment in patients with confirmed ROM and in patients without ROM

correctly positive clinical assessment [n = 37, gestational age: 40 weeks (35.5–40.5), p = 0.5053]. In the mentioned 14 cases, clinical assessment was positive within the next 12–24 h and ROM confirmed during delivery.

Specificity [98.6% (CI 0.95–1.00)], positive predictive value [96.2% (CI 0.87–1.00)] and negative predictive value [98.0% (CI 0.94–1.00)] of AT did not differ from clinical assessment [specificity 97.9% (CI 0.94–1.00), positive predictive value 92.9% (CI 0.81–0.99), negative predictive value 90.5% (CI 0.85–0.99)]. At the same specificity (p = 1.0), AT was more sensitive [94.4% (CI 0.85–0.99)] in diagnosing ROM than clinical evaluation [72.2% (CI 0.58–0.84), p = 0.00596].

Gestational age had no influence on sensitivity and specificity of AT (p = 0.61 and 0.29) or the clinical assessment (p = 0.61 and 0.66). Clinical assessment relies on the ability to visualize pooling of AF during the speculum examination. Nevertheless, experience of the treating physician had no influence on the accuracy of the clinical diagnosis. Sensitivity and specificity were the same in experienced (three or more years of working experience) versus less experienced treating physicians (sensitivity, p = 0.50 and specificity, p = 1.0).

According to local calculations (based on TARMED), we evaluated the costs for each individual diagnostic test. Compared to the clinical evaluation (total costs 122.5 Euro) a cost reduction of 58.8% could be achieved by using only AT (total costs 50.5 Euro) (Table 1).

Discussion

The diagnosis of ROM remains difficult when there is no clear leakage of fluid. ROM is associated with a high

Table 1 Assessment of costs according to different combinations of examinations

	AT	Clinical assessment	Clinical assessment and AT
AT	15.9	0	15.9
Speculum examination	0	7.5	7.5
Nitrazine test	0	0.1	0.1
AFI	0	80.2	80.2
Expenditure of time (15 min)	29.6	29.6	29.6
Bacterial Swap test	5.0	5.0	5.0
Total costs (Euro)	50.5	122.4	138.3

Calculation is based on "TARMED", a national taxpoints system according to medical and technical work (in Euro, as of May 8, 2010)

AT Amnisure test alone

perinatal mortality and maternal morbidity [1–3]; therefore, a specific non-invasive test or biochemical marker would be highly helpful to diagnose uncertain rupture of membranes. These limitations of conventional methods stimulated the search for non-invasive tests based on the detection of biochemical markers in cervicovaginal discharge such as alpha-fetoprotein [14, 15], vaginal prolactin [16], fetal fibronectin [17, 18], beta-subunit of human chorionic gonadotropin (β-hCG) [19], creatinine [20], urea [21], lactate [22], and insulin-like growth factor binding protein-1 (IGFBP-1) [23–27]. However, none of them turned out to be the optimal marker for ROM, as they are gestational-age dependent, such as fetal fibronectin, or require a speculum examination, as with IGFBP-1. A recent study by Tagore et al. [28] demonstrated that AT performed equal or even superior in detecting ROM than the IGFBP-1 rapid strip test (Actim PROM®) with a sensitivity of 92.7 versus 87.5% and a specificity of 100 versus 94.4%.

Physician qualification has an impact on the traditional clinical assessment with speculum examination, visualization of cervical leakage, performance and evaluation of the Nitrazine test or ferning, and the measurement of the AFI by transabdominal ultrasound. In contrast, our study demonstrated that the AT is not training-dependent and is more sensitive at same specificity compared to clinical assessment. We included patients with gestational ages ranging from 17 to 42 weeks and demonstrated that the duration of pregnancy had no influence on sensitivity and specificity of AT.

Our data confirm and extend the excellent sensitivity and specificity of AT reported previously [11, 12, 23]. Lee et al. and Cousin et al. [11, 12] both found AT to be highly sensitive (98.7 and 98.9% compared to 94.4% in our study) and specific (87.5 and 100% compared to 98.6% in our study). However, both studies used pooling, Nitrazine test and ferning for clinical evaluation of ROM which is rarely used in Europe. In clear contrast to the previous studies, all of our participants presented with uncertain ROM, and clinical assessment included more comprehensive diagnostic measures such as ultrasound.

At the current time further reduction of costs does not seem feasible, as ultrasound is recommended in international and national guidelines in cases with ROM; nevertheless, the savings could be 58.8% using AT alone instead of the traditional approach.

In conclusion, AT is a simple, reliable, highly sensitive and cost-effective test with superior performance compared to clinical assessment in the diagnosis of ROM in the second and third trimesters. Early diagnosis clearly helps to avoid long- and short-term sequelae of ROM and minimizes serious adverse outcomes, such as chorioamnionitis and neonatal sepsis. Further studies should evaluate the performance of AT in an outpatient midwifery-based setting or in countries where technical equipment is less available.

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Conflict of interest None.

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