Diagnosis of Premature Rupture of Foetal Membranes

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Abstract

Premature rupture of foetal membranes (PROM) complicates 10 % of pregnancies and is the most important identifiable cause of pre-term delivery. The diagnosis of PROM is based on the detection of amniotic fluid in the vagina. This can be achieved by chemical, clinical or biochemical methods. An ideal test should be specific for amniotic fluid and should not be affected by gestational age or interfering factors present in vaginal samples. Several studies have demonstrated that the rapid test based on insulin-like growth factor-binding protein-1 (IGFBP-1) fulfils these requirements, making it an ideal tool for PROM diagnosis.

Keywords

Premature rupture of foetal membranes, IGFBP-1, markers, pre-term delivery

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Premature rupture of membranes (PROM) is a potentially serious pregnancy complication that is defined as rupture of the foetal membranes before the onset of labour. It complicates approximately 10 % of all pregnancies and the majority of cases occur after the 37th week of pregnancy. Pre-term PROM occurs earlier in the course of pregnancy and is observed in 2–4 % of singleton and 7–20 % of twin pregnancies; it is the cause of approximately one-third of all pre-term deliveries.[1,2] In addition to increasing the risk of pre-term delivery, ruptured membranes also increase the risk of infection for both the mother and foetus.

Diagnosing Premature Rupture of Membranes

Diagnosing PROM is easy in cases with obvious rupture and clinical confirmation is possible in almost 90 % of such cases.[3] When the leakage is less prominent, or when it is intermittent, clinical diagnosis becomes much more difficult. Diagnostic tools have therefore been introduced to facilitate diagnosis in these more challenging cases.

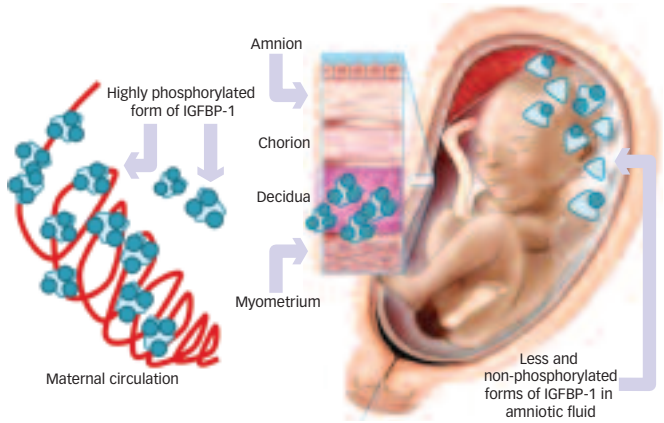
The optimal test should be specific for the amniotic fluid and should not be affected by potentially contaminating substances present in the vagina – including urine, blood, cervical mucus and seminal fluid – that in many diagnostic methods can cause false negative and false positive results. Vaginal medications and other vaginally administered substances may also interfere with the diagnosis.

Dye injection is considered as the gold standard to confirm the status of the membranes, but its invasiveness makes it impossible to use in clinical routine.[4] Ultrasonography can be used to assess the level of amniotic fluid, but it requires quite a significant loss of amniotic fluid and is therefore not adequate to identify cases where the rupture is very small and very little amniotic fluid leaks from the cervix. The rate of false positive results seen with ultrasonography is increased by the fact that oligohydramnios can be caused by other reasons than PROM.[5] The combination of visible pooling of fluid in the posterior fornix, elevated pH level of vaginal fluid and ferning is widely used to diagnose PROM, particularly in the US.[6] If two out of these three parameters are positive, the membranes are considered ruptured. Unfortunately, all three tests are prone to interference by contaminating substances: blood causes false positive results in pH testing and false negative results in ferning; cervical mucus may interfere with ferning, while seminal fluid may give false positive results in pH testing.[7]

These limitations in the accuracy of the different methods may lead to false diagnosis, and the consequences of false diagnosis may be serious: a false positive result may cause unnecessary interventions, even induction of labour; a false negative result may increase the risk of maternal morbidity, premature labour or poor foetal outcome. One of the major complications of PROM is infection, and the risk may increase if the rupture is not accurately identified.[8]

To overcome these problems, various protein markers were tried for PROM diagnosis. The idea was to identify a protein that is present at high concentrations in amniotic fluid, but is far less abundant in other fluids. Such proteins are, for example, diamine oxidase (DAO),[9] alpha-foetoprotein (AFP),[10] prolactin (PRL),[11] foetal fibronectin (fFN)[12] and human chorionic gonadotropin (hCG).[13] However, because these proteins are also found in other fluids, even if in smaller quantities, there is still a risk of false positive results. Some of these proteins also show varying concentrations at different stages of pregnancy; for

Figure 1: Location of the Different Phosphorylation Isoforms of Insulin-like Growth Factor-binding Protein 1 in Pregnancy



Phosphorylation forms of insulin-like growth factor-derived protein-1 (IGFBP-1) are different in amniotic fluid, in maternal circulation and in decidual tissue. Non-phosphorylated IGFBP-1 and IGFBP-1 with low phosphorylation level are found in amniotic fluid, whereas the highly phosphorylated form of IGFBP-1 predominates in decidual cells and in circulation. These different forms of IGFBP-1 can be detected by specific monoclonal antibodies, thus providing the possibility of different diagnostic applications.

example, AFP levels decrease strongly during the last trimester, reducing their accuracy in diagnosing PROM.

Insulin-like Growth Factor-binding Protein-1

To overcome the limitations of the methods described above, insulin-like growth factor-binding protein-1 (IGFBP-1) was introduced as a novel marker for PROM diagnosis in the mid 1990s. IGFBP-1 is an extensively studied protein that is secreted and synthesised by foetal and adult liver cells and by decidualised endometrial cells during pregnancy. IGFBP-1 plays a role in endometrial/decidual function and endometrium–trophoblast interaction.[14] During pregnancy, the concentration of IGFBP-1 increases in the maternal circulation and, starting from the second trimester, it is one of the major proteins in amniotic fluid.[15]

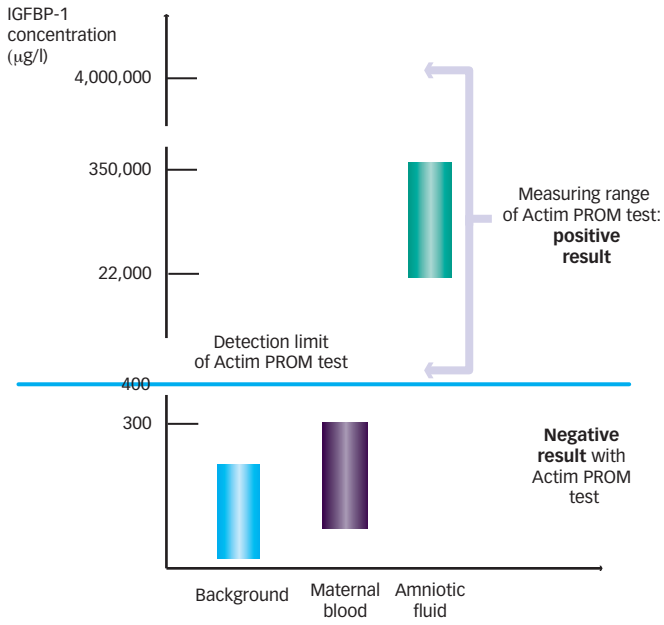
IGFBP-1 has different phosphorylation isoforms that exist in different proportions in various body fluids and tissues.[16–18] In amniotic fluid, the predominant form of IGFBP-1 is the non-phosphorylated form, but other phosphorylation forms are also present – with the exception of the most highly phosphorylated form (see *Figure 1*). That form originates from the decidual tissues, and it is the major form found in maternal circulation (see *Figure 1*).[15,17–19]

The different phosphoisoforms of IGFBP-1 can be distinguished by different monoclonal antibodies.[17] This has enabled the development of two specialised tests: the Actim® Partus test (Medix Biochemica), designed for assessing the risk of pre-term delivery (which has been discussed previously in this journal)[20] and the Actim® PROM test (Medix Biochemica), designed for detecting PROM. This article focuses on the latter.

Properties of the Actim® PROM Test

IGFBP-1 is very abundant in amniotic fluid, where its concentration is 100–1,000 times higher than in maternal serum;[21] insignificant background levels are found in the vagina. Its presence in vaginal secretions can thus be used as a proof of the presence of amniotic fluid, which in turn indicates that the membranes have ruptured.

Figure 2: Concentrations of Insulin-like Growth Factor-binding Protein 1 in Biological Fluids and Measuring Range of Actim® PROM Test



The detection limit of the Actim® PROM test is above the known insulin-like growth factor-derived protein-1 (IGFBP-1) concentrations in biological fluids other than amniotic fluid. This minimises the risk of potentially contaminating substances in the vagina interfering with the test.

Table 1: Peer-reviewed Clinical Studies Evaluating the Performance of Actim® PROM in Detecting Rupture of the Foetal Membranes

Study	n	Testing Weeks	Sensitivity (%)	Specificity (%)
Akercan et al., 2005[3]	87	20–36	100.0	92.0
Darj and Lyrenäs, 1998[44]	75	25–42	96.0	93.0
Erdemoglu and Mungan, 2004[25]	151	26–37	97.0	97.0
Gaucherand et al., 1997[26]	100	19–41	95.0	98.0
Hupfner and Diener, 1997[46]	54	12–42	100.0	93.0
Jain and Morris, 1998[45]	100	24–42	100.0	89.0
Kubota and Takeuchi, 1998[23]	90	15–41	95.0	93.0
Ragosch et al., 1996[27]	75	22–41	100.0	83.0
Rutanen et al., 1996[22]	130	15–37	100.0	95.0

The Actim PROM test is designed to identify the presence of IGFBP-1 in vaginal extracts. It is an immunochromatographic dipstick test and has a detection limit of 25 µg/l in the extracted sample. This level corresponds to approximately 400 µg/l in the original sample (see *Figure 2*). Since the lowest known IGFBP-1 level in amniotic fluid is 10 500 µg/l, the test allows to identify the presence of a very small amount of amniotic fluid, even if the sample is diluted with the presence of other fluids often found in the vagina. This detection limit is above the highest known level found in potentially contaminating biological fluids (see *Figure 2*).[22]

The sample is collected with a sterile polyester swab from the vagina and is extracted in the specimen extraction solution. The sample can be collected directly from the vagina without the use of a speculum or

Table 2: Peer-reviewed Clinical Studies Evaluating the Performance of Actim® PROM against Two Traditional Methods (Vaginal pH and Ferning of Vaginal Fluid) in Detecting Rupture of the Foetal Membranes

Study	n	Testing Weeks	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
			Actim PROM	pH	Actim PROM	pH	Actim PROM	pH	Actim PROM	pH
Erdemoglu and Mungan, 2004[25]	151	26–37	97.0	97.0	97.0	16.0	NA	NA	NA	NA
Gaucherand et al., 1997[26]	100	19–41	95.0	90.7	98.0	77.2	97.6	75.0	96.5	91.7
Kubota and Takeuchi, 1998[23]	90	15–41	95.0	73.3	93.0	72.4	NA	NA	NA	NA
Ragosch et al., 1996[27]	75	22–41	100.0	94.0	83.0	63.0	83.0	69.0	100.0	93.0
			Actim PROM	Ferning	Actim PROM	Ferning	Actim PROM	Ferning	Actim PROM	Ferning
Kubota and Takeuchi, 1998[23]	90	15–41	95.0	42.0	93.0	76.0	NA	NA	NA	NA

NA = not available; NPV = negative predictive value; PPV = positive predictive value.

from the posterior fornix during a sterile speculum examination. The extracted sample is then tested with the dipstick, which is immersed in the sample extract. The sampling and extraction times are short (10–15 seconds each) and the result of the dipstick test is available within five minutes of dipping. This rapidity is comfortable for the patient and provides rapid answers for the clinician.

Since the IGFBP-1 levels remain high throughout the pregnancy, the test can be used during the entire relevant range of gestational ages.

Over the past 15 years, the Actim PROM test has been evaluated in several peer-reviewed clinical studies that have constantly demonstrated its high accuracy in diagnosing PROM in various patient populations (see *Table 1*). Direct comparisons have also been done with traditional methods and have confirmed that Actim PROM is superior to them (see *Table 2*).[23–27]

A particular advantage of Actim PROM is that the diagnosis can be based on the result of a single test that is rapidly performed and provides a clear answer within minutes. In contrast, with the traditional methods, several tests need to be interpreted separately and combined. The fact that the results of these methods are affected by contaminating factors in a conflicting way (for example, blood causes false positives for pH, but false negatives for ferning) further complicates their interpretation.

Several clinical studies have confirmed that vaginal bleeding does not interfere with the accuracy of the Actim PROM test.[22,23,25,28] This is based on the fact that the predominant form of IGFBP-1 existing in blood and maternal tissues, highly phosphorylated IGFBP-1, is not present in amniotic fluid[17] and is not recognised by the key antibody of the test. In addition, the detection limit of the test has been optimised to be above the highest known IGFBP-1 concentration in maternal blood, further limiting the risk of false results (see *Figure 2*).

Seminal fluid and urine do not contain detectable amounts of IGFBP-1, ensuring that these contaminating substances do not affect the test results.[21] In addition, several medical and hygiene products that can potentially contaminate a vaginal sample have been tested and shown not to interfere with the test results.

All these properties make it possible to use the Actim PROM test in all women presenting with signs and/or symptoms of membrane rupture, ensuring proper diagnosis of the entire patient population.

Alternative Tests

Another rapid diagnosis test, the AmniSure® ROM test (AmniSure International LLC) has been available for nearly a decade. It is based on the detection of a protein called placental alpha-microglobulin-1 (PAMG-1), which is described as a decidual protein with elevated concentration in amniotic fluid also found in low levels in other body fluids. In spite of its long presence on the market, the characterisation of this protein marker still remains quite limited and no studies exist confirming the concentrations in different body fluids quoted for the product. In the most often cited papers describing the PAMG-1 levels in amniotic fluid, blood and other body fluids,[29–31] the reported concentrations are very different from those generally reported for the AmniSure ROM test.[32]

Studies on AmniSure ROM have shown a high accuracy of the test in PROM diagnosis. In contrast with the studies on Actim PROM, however, they have systematically included a selected patient population, constantly excluding patients with vaginal bleeding.[33–37] As it is known that 20–25 % of women with suspected membrane rupture may have bleeding, this limitation makes it difficult to estimate the accuracy of the test in true clinical situations. In contrast, evaluations of Actim PROM have systematically included bleeding patients and several studies have directly addressed the question and demonstrated that blood does not affect the test results.

Direct side-by-side evaluations of the AmniSure ROM and Actim PROM tests have provided variable results;[38–41] however, no statistically significant difference has been demonstrated between the two tests among the selected patient groups suitable for AmniSure ROM.

Recently, alternative IGFBP-1 tests have also been introduced, but so far no peer-reviewed evidence of their accuracy is available. It should be stressed that it is not possible to compare the accuracy of different tests or markers without taking into consideration the assay conditions (patient population, sampling, sample handling, antibodies used in the test as well as the detection limit of the test). As a consequence, each commercial test should be evaluated separately using actual clinical samples and properly designed diagnostic methods.[42]

Discussion

Diagnosing PROM is a challenging task in the clinic, particularly in cases where patient samples contain contaminants. The development of accurate protein marker-based tests has greatly improved the diagnosis and subsequent management of PROM cases. The Actim PROM test was the first of these reliable, easy-to-use and non-invasive

tests. With the appearance of other similar tests, there has been some controversy regarding the assessment and comparison of the accuracies of the different methods.[32,42–43]

Estimation of the clinical sensitivity and specificity of tests in PROM diagnosis is complicated by the lack of a safe gold standard test that could be used to confirm the status of the membrane. As a consequence, the diagnosis is based on a combination of clinical observation and the uncertain traditional methods. In different clinics, the methods vary considerably, causing variability in the apparent specificities and sensitivities as well as making it difficult to compare different studies side by side. This is particularly true when comparing different methods with each other.

Vaginal leakage or suspicion of membrane rupture is one of the most common complaints and one of the most typical clinical questions during pregnancy. The Actim PROM test provides an easy and patient-friendly method to reliably screen both pre-term and term patients in clinically unclear situations and identify the true rupture cases. ■

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