GinPolMedProject ©
1 (51) 2019: 014-018 • REVIEW ARTICLE

Comparison of tests using placental alpha-microglobulin 1 (PAMG-1) and type 1 insulin-like growth factor binding protein (IGFBP-1) in the diagnosis of premature rupture of membranes

Dorota Knapik, Anita Olejek

Department of Gynecology, Obstetrics and Gynecologic Oncology in Bytom, Medical University of Silesia in Katowice, Poland

AUTHORS' CONTRIBUTION: **(A)** Study Design · **(B)** Data Collection · **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

SUMMARY

A diagnosis of premature rupture of membranes (PROM) is mainly based on the interview and physical examination. In a doubtful situation, various tests of cervico-vaginal secretions and ultrasound are used. At present, the most widely advertised and commercially produced tests of cervico-vaginal secretions are AmniSure® (based on the analysis of placental alpha-microglobulin-1, i.e. PAMG-1) and Actim Prom® (based on the analysis of type 1 insulin-like growth factor binding protein, i.e. IGFBP- 1). PAMG-1 is a protein present only in the amniotic fluid, whereas IGFBP-1 is a protein whose concentrations in the amniotic fluid largely exceed those in the plasma. Both markers have the potential to become a standard in the diagnosis of PROM.

Concentrations of PAMG-1 range from 2,000 to 25,000 ng/mL in the amniotic fluid and from 0.05 to 0.2 ng/mL in normal vaginal secretions. The manufacturer proposes a concentration of 5 ng/mL as the cut-off point, which seems to effectively prevent any false-negative results. The technology is not affected by the presence of blood and can be used at any gestational age. IGFBP-1 is a protein produced by the decidua and the fetal liver. Its concentration in the amniotic fluid is around 27 ng/mL shortly after conception and reaches a level above 100,000 ng/mL in advanced pregnancy; for comparison, the maternal plasma levels are fairly constant and range from 58 to 600 ng/mL.

Having analyzed the data from the literature, it may be concluded that the mean sensitivity and specificity values for the test with IGFBP-1 (obtained by 7 different research groups) were 93.5% and 92.5%, respectively, while for the test with PAMG-1: 96.2% and 97.2%, respectively. Both tests have therefore sufficient parameters to fulfill the tasks set for them. The PAMG-1 test is superior due to the lower influence of blood in the sample.

Key words: pregnancy; amniotic fluid; rupture of membranes; diagnosis

Address for correspondence: Dorota Knapik
Department of Gynecology, Obstetrics and Gynecologic Oncology in Bytom, Medical University of Silesia in Katowice, Poland, Stefana Batorego 15, 41-902 Bytom
Tel. +48 602-654048, fax: +48 322732731
e-mail: sek.kan@sccs.pl, knapikdorota@gmail.co

Word count: 2343 **Tables:** 0 **Figures:** 0 **References:** 26

Received: 18.12.2018
Accepted: 22.02.2019
Published: 28.03.2019

INTRODUCTION

A diagnosis of premature rupture of membranes (PROM) is mainly based on the interview and physical examination [1]. In a doubtful situation, various tests of cervico-vaginal secretions [2] and ultrasound [3] are used. At present, the most widely advertised and commercially produced tests of cervico-vaginal secretions are AmniSure® (an American test based on the analysis of PAMG-1) and Actim Prom® (a Finnish test based on the analysis of IGFBP-1). AmniSure® is popular especially in the United States, whilst Actim Prom® is more common in Europe (particularly in Germany and Scandinavia) [4]. Without doubt, the test based on IGFBP-1 assay is older and therefore much more explored. But which exhibits a better predictive value? It is difficult to say as the manufacturers of both tests provide conflicting data regarding their usability. The study analyzes all data on this subject found in the medical literature.

PLACENTAL ALPHA-MICROGLOBULIN-1 (PAMG-1)

Placental alpha-microglobulin-1 (PAMG-1) is a protein found in the amniotic fluid. The isolation of monoclonal antibodies has made it possible to use a test to detect PAMG-1 in the amniotic fluid in concentrations ranging from 2,000 to 25,000 ng/mL. This makes it possible to detect very low concentrations of the amniotic fluid in vaginal secretions (ranging from 0.2 to 2.5 mg/L of the amniotic fluid in 1 mL of vaginal secretions). The concentration of PAMG-1 in normal vaginal secretions ranges from 0.05 to 0.2 ng/mL (maximally up to 3 ng/mL when blood is present in secretions or when the patient has vaginal bacterial infection). The manufacturer proposes a concentration of 5 ng/mL as the cut-off point, which seems to effectively prevent any false-negative results [5].

The application of PAMG-1 as a marker was described for the first time in 2005 in the United States. The studies included 203 pregnant patients with gestational age between 15 and 42 weeks with preterm PROM (PPROM) confirmed with standard methods (including US) and the PAMG-1-based test. Inconsistency between the test results and the standard assessment was noted in only 7 cases. The sensitivity of the test was evaluated at 98.9%, and specificity amounted to 100% [6]. Another study was published in 2007 by Lee et al. [7]. PAMG-1 was used to diagnose all consecutive patients with symptoms of PROM who were admitted to hospital in one year. The study included 184 pregnant patients with suspected PPROM. During the first examination, PPROM was identified in 76% of the patients using standard methods (fluid leakage, pH test and microscopic analysis of vaginal secretions) and in 88% of the patients using the PAMG-1-based test. Further observation confirmed PPROM in almost all the patients in whom traditional methods failed to detect amniotic fluid leakage. Based on this work, the authors hypothesized that the application of the PAMG-1 test is significantly superior to standard tests in the diagnosis of PPROM.

The test based on PAMG-1 is currently commercially available as AmniSure®. It produces a positive result with a PAMG-1 level >5 ng/mL. It has been available in this form since 2003. The manufacturer underlines that the application of the test requires no specula. It is also emphasized that the level of PAMG-1 detectability has been established well above the "background," i.e. the physiological level in cervico-vaginal secretions. The manufacturers also point that this technology is not affected by the presence of blood and can be used at any gestational age. Moreover, the test may be stored at room temperature. In the past several years, more and more studies have emerged evaluating the reliability of this test in comparison to traditional methods [7, 8] or to its major competitor, the IGFBP-1-based test [9–12]. It is very popular in the United States where it has been approved by the Food and Drug Administration (FDA); it is slightly less popular in Europe. The results of recent studies indicate that the PAMG-1 test may be more accurate than the IGFBP-1 test, which is used in numerous European countries [5,10,12], but not all authors share this view [9,11]. The PAMG-1 test is also mentioned in the latest RCOG Guidelines from 2010, citing the first study on this issue by Cousins et al. [6].

TYPE 1 INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN (IGFBP-1)

Type 1 insulin-like growth factor binding protein (IGFBP-1) is a protein produced by the decidua and fetal liver. It is found in the amniotic fluid in very high concentrations (approximately 100–1,000 times higher than its plasma levels). The production of this protein increases approximately 4,000–5,000-fold between weeks 11 and 16 of gestation. IGFBP-1 detection in vaginal secretions unequivocally indicates amniotic fluid leakage. The IGFBP-1 concentration in the amniotic fluid is around 27 ng/mL shortly after conception and reaches a level above 100,000 ng/mL in advanced pregnancy; for comparison, maternal plasma levels are fairly constant and range from 58 to 600 ng/mL. The measurements are not disturbed by the presence of blood in vaginal secretions [5].

The IGFBP-1 protein has been applied in PPROM diagnosis in the form of a commercially available Actim Prom® test, which is produced in Finland. In accordance with the guidelines of the German Society of Obstetrics and Gynecology, this test is currently recommended in the diagnosis of PPROM in Germany. The IGFBP-1 test is not a novelty. Its use was reported in the United States in 1994 in an article from the New York *Mount Sinai School of Medicine* [13]. The authors investigated healthy pregnant women and patients with a certain diagnosis of PROM, and observed significant differences in IGFBP-1 levels (553 vs 3 ng/mL, p=0.0002). The next studies were performed in Sweden in 1998. A multicenter trial included 6 obstetric units [14]. In total, 174 women were examined, of whom 46 had confirmed PPROM, while 99 were suspected of having PROM. In women with a certain diagnosis of PROM, the sensitivity of the test reached 95.7% and specificity – 93.1% [14]. In the same year, studies assessing the value of the IGFBP-1 test were conducted by Kubota and Takeuchi in Japan [15]; the sensitivity and specificity exceeded 90%. It occurred that the results of the IGFBP-1 test are not disturbed by various contaminants, cervical dilation or even uterine contractions [15]. The authors of both these studies independently conclude that the test is a useful marker in PROM diagnosis [14,15].

The usefulness of the IGFBP-1 test was also confirmed later by Erdemoglu et al. [16] and Akercan et al. [17] in Turkey. The former authors conducted a study in a large group of 151 pregnant women: PROM was confirmed in 36, ruled out in 35 and suspected in 80 of the patients between week 20 and 42 of gestation. It was attempted to confirm PROM in a nitrazine test and with the AFI index (measured by the four-quadrant method with AFI of <80 cm considered as oligohydroamnios). The sensitivity and specificity values for the IGFBP-1 test turned out to be significantly higher than those for the nitrazine test and AFI index, and its positive outcome was associated with a 12-fold greater risk of pregnancy termination within the following 7 days [16].

The most interesting investigation was presented by Akercan et al. who examined 87 pregnant women between week 20 and 36 of gestation and grouped them into patients with clinically evident PROM (n=25), clinically suspected PROM (n=42) and women with intact fetal membranes (n=20) [17]. The IGFBP-1 test was positive in all women with clinically evident PPROM (sensitivity 100%) and negative in 19 out of 20 women with intact fetal membranes (sensitivity 95%). However, the most interesting results were obtained in the third group, i.e. 36 women with clinically suspected PROM, where the test was positive in 13 women (36%) and negative in 23 women (63%). In those with the positive test result, pregnancy ended in week 31, and in those with the negative result – in week 39. This resulted in evident highly significant differences in birth weight and postnatal condition. Data of particular interest concern 11 of the 13 (85%) women with the positive test result who delivered within two weeks after the test was performed, while the women with the negative result continued their pregnancy for over two following weeks in all the cases (p=0.001). The test exhibited the sensitivity of 100% and specificity of 92% for the latency of less than two weeks [17].

The IGFBP-1 test is positive with an IGFBP-1 level exceeding 25 ng/mL. The test has been known and available since early 1990s. At present, it is used in approximately 70 countries, particularly in Europe and Asia, and, for over 5 years, also in the USA where it has been approved by the FDA. This test is also mentioned in a Polish textbook edited by Grzegorz Bręborowicz [18].

TEST COMPARISON

Both markers have the potential to become a standard in the diagnosis of PPROM. PAMG-1 is a protein present only in the amniotic fluid, whereas IGFBP-1 is a protein whose concentrations in the amniotic fluid largely exceed those in the plasma. The IGFBP-1 concentration in the amniotic fluid is greater than 100,000 ng/mL in the second and third trimesters of gestation, with maternal blood levels never exceeding 1,000 ng/mL [5]. The available literature concerning both these tests is abundant. The authors of the present report managed to find 16 publications: five on the reliability of the IGFBP-1 test [13–17], 6 on the reliability of the PAMG-1 test [6–8, 19–21] and five comparative analyses of the two tests [5,9,10–12]. This material is not easy to interpret. In studies regarding only IGFBP-1 test, the sensitivity and specificity values were: 95.7% and 93.1% [14], 95.2 and 90.5% [15], 97% and 97% [16] as well as 100% and 92%, respectively [17]. However, the results of the oldest study by Lockwood et al. [13] are not fit for comparison with those obtained in other studies as the commercially available Actim Prom test was not used then, IGFBP-1 levels were determined in a laboratory, and the cut-off point applied in the study was lower than that adopted by the Actim Prom manufacturer. These data may be supplemented with information from three (of five) studies that compare the IGFBP-1 and PAMG-1 tests, which yielded sensitivity and specificity values for IGFBP-1 of: 87.5% and 94.4% [12], 89.8% and 97.5% [9] as well as 89.3% and 82.7%, respectively [11]. However, the results of two of the comparative studies could not be analyzed either, because they had not been conducted in conditions typical for PPROM: the amniotic fluid was collected during a cesarean section, and samples were then diluted [5,10]. When analyzing all the available results, it can be stated that the mean sensitivity of the IGFBP-1 test calculated by seven different research groups was 93.5% (range from 82.7% to 100%), while its mean specificity amounted to 92.5% (from 82.7% to 97.5%).

For comparison, in studies regarding only the PAMG-1 test, the sensitivity and specificity values were: 98.7% and 87.5% [7], 97.3% and 98.6% [8], 98.9% and 100% [6] as well as 94.4% and 98.6%, respectively [20]. In one of the publications regarding only the PAMG-1 test, the sensitivity and specificity were not

analyzed [21]. As before, the results of two of the comparative studies could not be analyzed as they had been carried out in *in vitro* conditions [5,10]. As previously, these data may be supplemented with information from three studies that compared the IGFBP-1 and PAMG-1 tests, which yielded the following sensitivity and specificity values for PAMG-1: 92.7% and 100% [12], 94.3% and 97.5% [9] as well as 97.3% and 98.7%, respectively [11]. When considering all the available results, it can be stated that the mean sensitivity of the PAMG-1 test calculated by seven different research groups was 96.2% (range from 92.7% to 98.9%), while its mean specificity amounted to 97.2% (from 87.5% to 100%).

In comparative analyses conducted by various authors, better parameters were usually obtained for the PAMG-1 test. This is the conclusion of the authors of all the studies in this group [5–12]. It must be pointed out, however, that the methods of two of these studies were completely detached from the conditions in which PROM is typically diagnosed [5,10], and careful interpretation of the remaining papers indicates that the authors do not disclose their unequivocal opinions on this matter [9,11,12]. In one of these studies, the conclusions include a statement about the superiority of the PAMG-1 test parameters, but in the final conclusion the authors evidently indicate that this does not mean that the test is superior [12]. The authors of the remaining two studies demonstrate that the observed difference is of no clinical relevance and that both tests are equally reliable [9,11].

The results of the studies comparing the PAMG 1 and IGFBP tests published before April 2013 were included in the meta-analysis of Palacio et al. [22]. In total, 762 pregnant patients with confirmed PROM and 1,385 pregnant patients with suspected PROM were analyzed. The authors concluded that there were no significant differences in the accuracy of the two tests in patients with confirmed PROM, but that AmniSure performed better in the case of suspected PROM [22].

Which of these two tests, IGFBP-1 or PAMG-1, should be chosen then? A valuable opinion is expressed in the discussion section of an article by Albayrak et al. [9] from 2011. The authors draw attention to the cost of the test. In their conditions (Turkey, 2011), AmniSure was approximately three times more expensive than Actim Prom (this difference is not that large in the Polish conditions). In their final conclusions, the authors clearly indicate that both tests have sufficient parameters to fulfill the tasks set for them. However, the AmniSure test is also superior due to the lower influence of blood in the sample [23].

All the above considerations do not include any analysis of the usability of the newest tests, recently introduced to the diagnostic work-up of premature rupture of fetal membranes, based on the application of two biomarkers: IGFBP and alpha-fetoprotein (ROM plus, Amnioquick Duo+). However, the introduction of these tests has brought no breakthrough; their accuracy has been proven to be very high, but comparable to that of the PAMG-1 test [24–26].

REFERENCES

1. Royal College of Obstetricians and Gynaecologists (RCOG). Guideline No. 44, November 2006. Preterm Prelabour Rupture of Membranes. http://www.neonatalformulary.com/pdfs/uk_guidelines/AMPICILLIN-RCOG_guideline_on_PPROM.pdf [wydruk u autora].
2. **Knapik D, Olejek A.** Analiza wydzieliny szyjkowo-pochwowej w diagnostyce przedwczesnego pęknięcia błon płodowych. *Ginekol Pol* 2011;82:50-55.
3. **Carroll SG, Papiaoannou S, Nicolaides KH.** Assessment of fetal activity and amniotic fluid volume in the prediction of intrauterine infection in preterm prelabor amniorrhexis. *Am J Obstet Gynecol* 1995;172:1427–1435.
4. **van der Ham DP, van Teeffelen AS, Mol BW**. Prelabour rupture of membranes: overview of diagnostic methods. *Curr Opin Obstet Gynecol* 2012;24:408-412.
5. **Chen FC, Dudenhausen JW.** Comparison of two rapid strip tests based on IGFBP-1 and PAMG-1 for the detection of amniotic fluid. *Am J Perinatol* 2008;25:243-246.
6. **Cousins LM, Smok DP, Lovett SM et al.** AmniSure placental alpha microglobulin-1 rapid immunoassay versus standard diagnostic methods for detection of rupture of membranes. *Am J Perinatol* 2005;22:317-320.
7. **Lee SE, Park JS, Norwitz ER et al.** Measurement of placental alpha-microglobulin-1 in cervicovaginal discharge to diagnose rupture of membranes. *Obstet Gynecol* 2007;109:634-640.
8. **Abdelazim IA, Makhlouf HH.** Placental alpha microglobulin-1 (AmniSure(®) test) for detection of premature rupture of fetal membranes. *Arch Gynecol Obstet* 2012; 285:985-989.
9. **Albayrak M, Ozdemir I, Koc O et al.** Comparison of the diagnostic efficacy of the two rapid bedside immunoassays and combined clinical conventional diagnosis in prelabour rupture of membranes. Eur J Obstet Gynecol Reprod Biol 2011;158:179-182.
10. **Pollet-Villard M, Cartier R, Gaucherand P et al.** Detection of placental alpha microglobulin-1 versus insulin-like growth factor-binding protein-1 in amniotic fluid at term: a comparative study. *Am J Perinatol* 2011;28:489-494.
11. **Abdelazim IA, Makhlouf HH.** Placental alpha microglobulin-1 (AmniSure test) versus insulin-like growth factor binding protein-1 (Actim PROM test) for detection of premature rupture of fetal membranes. *J Obstet Gynaecol Res* 2013;39:1129-1136.
12. **Tagore S, Kwek K.** Comparative analysis of insulin-like growth factor binding protein-1 (IGFBP-1), placental alpha-microglobulin-1 (PAMG-1) and nitrazine test to diagnose premature rupture of membranes in pregnancy. *J Perinat Med* 2010;38:609-612.

13. **Lockwood CJ, Wein R, Chien D et al.** Fetal membrane rupture is associated with the presence of insulin-like growth factor-binding protein-1 in vaginal secretions. *Am J Obstet Gynecol* 1994;171:146-150.
14. **Darj E, Lyrenäs S.** Insulin-like growth factor binding protein-1, a quick way to detect amniotic fluid. *Acta Obstet Gynecol Scand* 1998;77:295-297.
15. **Kubota T, Takeuchi H.** Evaluation of Insulin-Like Growth Factor Binding Protein-1 as a diagnostic Tool for Rupture of the Membranes. *J Obstet Gynecol Res* 1998;24:411-417.
16. **Erdemoglu E, Mungan T.** Significance of detecting insuling-like growth factor binding protein-1 in cervicovaginal secretions: Comparison with nitrazine test and amniotic fluid volume assessment. *Acta Obstet Gynecol Scand* 2004;83:622-626.
17. **Akercan F, Cirpan T, Kazandi M et al.** The value of the insulin-like growth factor binding protein-1 in the cervical-vaginal secretion detected by immunochromatographic dipstick test in the prediction of delivery in women with clinically unconfirmed preterm premature rupture of membranes. *Eur J Obstet Gynecol Reprod Biol* 2005;121: 159-163.
18. **Bręborowicz G.** Położnictwo i ginekologia. Warszawa: PZWL; 2005, tom I, str.125-129, 178-182, 439-444.
19. **Lee SM, Lee J, Seong HS et al.** The clinical significance of a positive Amnisure test in women with term labor with intact membranes. *J Matern Fetal Neonatal Med* 2009; 22:305-310.
20. **Birkenmaier A, Ries JJ, Kuhle J et al.** Placental á-microglobulin-1 to detect uncertain rupture of membranes in a European cohort of pregnancies. *Arch Gynecol Obstet* 2012;285:21-25.
21. **Neil PR, Wallace EM.** Is Amnisure® useful in the management of women with prelabour rupture of the membranes? *Aust N Z J Obstet Gynaecol* 2010;50:534-538.
22. **Palacio M, Kühnert M, Berger R et al.** Meta-analysis of studies on biochemical marker tests for the diagnosis of premature rupture of membranes: comparison of performance indexes. *BMC Pregnancy Childbirth* 2014;14:183.
23. **Ramsauer B, Duwe W, Schlehe B et al.** Effect of blood on ROM diagnosis accuracy of PAMG-1 and IGFBP-1 detecting rapid tests. *J Perinat Med* 2015;43:417-22.
24. **Eleje GU, Ezugwu EC, Ezebialu IU et al.** Performance indices of AmnioQuick Duo+ versus placental á-microglobulin-1 tests for women with prolonged premature rupture of membranes. *Int J Gynaecol Obstet* 2018 Nov 2. doi: 10.1002/ijgo.12703.
25. **Eleje GU, Ezugwu EC, Eke AC et al.** Accuracy and response time of dual biomarker model of insulin-like growth factor binding protein-1/ alpha fetoprotein (Amnioquick duo+) in comparison to placental alpha-microglobulin-1 test in diagnosis of premature rupture of membranes. *J Obstet Gynaecol Res* 2017;43:825-833.
26. **Igbinosa I, Moore FA, Johnson C, Block JE.** Comparison of rapid immunoassays for rupture of fetal membranes. *BMC Pregnancy Childbirth* 2017;17:128.