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LETTER TO THE EDITOR

Comment on: Guidelines for the management of spontaneous preterm labor: identification of spontaneous preterm labor, diagnosis of preterm premature rupture of membranes and preventive tools for preterm birth

Dear Editor,

With great interest I read the recently published guidelines for the management of spontaneous preterm labor [1]. I was delighted to see that unlike the previous guidelines published in 2006 [2], these new ones also take into consideration the diagnostic marker insulin-like growth factor binding protein-1 (IGFBP-1) that I have worked with since the early 80s. However, I would like to bring the readers' attention to some errors and points that may be misleading regarding evaluation of IGFBP-1 as a marker of ruptured fetal membranes (ROM) and in comparing it with placental α microglobulin-1 (PAMG-1).

Firstly, human IGFBP-1 is a well characterized protein since more than 20 years [3,4]. Its synthesis by the liver and decidua, and levels in amniotic fluid and other body fluids have been thoroughly examined in all stages of pregnancy [5,6] and the data have been published in peer-reviewed journals. Meanwhile, the data available on PAMG-1 is more limited and partly confusing. In the most often cited papers regarding the PAMG-1 levels in amniotic fluid, blood and other body fluids [7–9], the values are quite different from those reported in the guidelines. This makes comparison between IGFBP-1 and PAMG-1 difficult.

IGFBP-1 has been used as a marker of ROM since the mid 90s (Actim PROM test). Since then, several studies have consistently shown that this test identifies membrane rupture with high accuracy. Unfortunately, many of these studies were omitted in the analysis presented in Table I of the guidelines comparing the performance of the different tests [10–12]. As a consequence, the sensitivity and specificity of the IGFBP-1 test remain underestimated. For example, the lowest sensitivity (74%) is from a study by Lockwood 1994 using a quantitative radioimmunoassay with frozen samples in the laboratory with a different detection limit and assay conditions from the current IGFBP-1 based bed-side PROM test [13].

Secondly, the guidelines state that the detection limit of PAMG-1 with Amnisure ROM test (5 ng/ml) is lower than the detection limit of IGFBP-1 with Actim PROM test (25 ng/ml). This comparison is irrelevant, since the quoted levels of PAMG-1 protein in amniotic fluid (2000–25,000 ng/ml) are clearly lower than the known levels of IGFBP-1 (10,500–350,000 ng/ml [14]), which naturally calls for a need of a lower detection limit. In the guidelines the lowest level of IGFBP-1 is quoted to be 27 ng/ml in early pregnancy. Such low levels have not been reported at pregnancy weeks clinically relevant for diagnosis of ROM [15].

Thirdly, the sensitivity and specificity of any test has to be interpreted in the clinical context. The methods used for estimation of the accuracy and reliability of the PAMG-1 test compared to the IGFBP-1 test are questionable for several reasons. For example, samples of pure blood-free amniotic fluid obtained during intraoperative amniocentesis at cesarean section were diluted with 0.9% saline and serial dilutions were tested using both tests [16,17]. The study design does not correspond to the bed-side situation where amniotic fluid is contaminated by vaginal discharge or other possible fluids like urine, semen or blood that may affect the test result causing false positives, if the test is too sensitive. A high rate of positive PAMG-1 test results has been found among patients with intact membranes and labor at term [18] and in patients with a short cervix [19]. This data has not been considered when analyzing the specificity of PAMG-1 test. Also, the publication on the intra-amniotic dye test and its comparison with the PAMG-1 test is a congress abstract only, with no information on the numbers of patients or the study design [20].

Finally, IGFBP-1 test results have repeatedly been shown to be unaffected by the presence of blood [10,21,22]. Indeed, the monoclonal antibody used in the Actim PROM test does not recognize the highly phosphorylated IGFBP-1 which is the predominant isoform in maternal and fetal blood and decidua [4]. Since blood may be present in approximately 25% of cases with suspected PROM, this information is critical in order to estimate the accuracy and clinical usefulness of the marker. Suspected rupture of membranes in the presence of bleeding is the most challenging situation in the clinic, since the therapeutic measures differ depending on whether the membranes in such a case are intact or not. Yet, no information is available on the accuracy of the PAMG-1 test in patients with suspected membrane rupture and bleeding since patients with bleeding have systematically been excluded in PAMG-1 clinical studies, suggesting that PAMG-1 test cannot be used in such challenging cases.

The statement that presence of blood up to 50% does not interfere with the PAMG-1 test result is only based on a conference poster, reporting serial dilutions of peripheral blood in 0.9% saline in vitro [23]. Again, the study design is not equivalent to the clinical situation where amniotic fluid in cervicovaginal swab sample is mixed with vaginal secretion and other possible contaminants. Also, this high rate of positive Amnisure results in the presence of blood raises a question on the validity of the reported range in the maternal blood (0.5–2 ng/ml) that should not react in a test with a detection limit of 5 ng/ml.

Considering all the points above, the currently available data does not unequivocally support the superiority of the PAMG-1 test as compared with the IGFBP-1 test.

***Declaration of interest*:** The author is the inventor of the patent for the IGFBP-1 based PROM test.

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Editor's reply:

On behalf of the EAPM Study Group on Preterm Birth, we thank Dr. Rutanen for her comments regarding the recently published Guidelines on the management of spontaneous preterm labor. Her work with Medix Biochemica on the development of the IGFBP-1 protein marker test for use in diagnosing ROM is certainly appreciated.

With that said, we are somewhat concerned with Dr. Rutanen's statement that the recommendations of the Guidelines are "misleading" to the reader. We will try to address Dr. Rutanen's concerns below.

It is true that not all of the studies favored by the inventor/manufacturer of the IGFBP-1 test were referenced in the Guidelines. The literature review process utilized in the Guidelines is widely accepted by the scientific community. Such process has been also utilized in several recent independent review articles on the PROM problematic, which are referenced in the Guidelines. The referenced review articles independently opted not to include some studies favored by Dr. Rutanen and her colleagues in the review process. This is not unusual, as multiple factors (i.e. inclusion/exclusion criteria, reference method used and the sample size of each study) typically play a role in the selection process of top review publications. The Guidelines do not aim to provide a meta-analysis of the literature, but rather an "expert view of a European problematic". A critical, objective and expert analysis of the literature was performed before drawing any conclusion made in the Guidelines.

Dr. Rutanen questions the antigen metrics of PAMG-1 and IGFBP-1 tests mentioned in the Guidelines. Our response to her concern is that the Guidelines are merely stating facts available in the literature about the different tests and the antigens they claim to detect; the placental α-microglobulin-1 (PAMG-1) information provided is highlighted by Gaucherand et al. (2011), who also published on IGFBP-1 test in the past.[1] The stated concentration ranges and sensitivity thresholds of the IGFBP-1, PAMG-1 and other protein markers and their tests are not provided with any implicit or explicit reference to performance metrics of the said tests and, therefore, we do not see a problem with the approach we have taken. We agree that such a link, if made, could be debatable.

The topic of blood interference on the PAMG-1 and IGFBP-1 tests is one that may warrant further clarification. While it is true that some IGFBP-1 studies did not exclude patients with active vaginal bleeding, these studies did not specifically focus on the problem of interference by vaginal bleeding. As stated

by the Manufacturer of the IGFBP-1 test, "In the case of heavy vaginal bleeding the amount of IGFBP-1 in blood may be so high that the test gives a positive result."[2] Rutanen et al. (1996) also state that a, "positive IGFBP-1 test should be interpreted with caution in cases with heavy vaginal bleeding."[3] Mere absence of active vaginal bleeding as an exclusion criterion in a clinical study does not seem sufficient to claim that the IGFBP-1 test is "not affected by the presence of blood," as Dr. Rutanen prompts. To date, the closest attempt toward isolating the varying effects of different blood admixtures to a PROM patient sample was provided by Wilfong with a convincing serial dilution protocol.[4] Theoretically, false positives are possible with both tests as maternal blood concentrations of the respective antigens are higher than both tests' detection limits.11,[9]For this reason we do not recommend the use of either test in the presence of heavy vaginal bleeding.

It is indisputable that there is a difference between *in vitro* and *in vivo* testing, but we respectfully disagree with Dr. Rutanen that the recently published Gaucherand (2011) study comparing the Actim® PROM Test and AmniSure® ROM test is irrelevant and does not have clinical implications.[2] The study sought four primary end points: sensitivity, detection limit, response time, and reproducibility between the two tests. In all of these categories, the test based on PAMG-1 (AmniSure® ROM Test) was superior to that based on IGFBP-1 (Actim® PROM Test). The finding that the test based on PAMG-1 is more sensitive in its detection of amniotic fluid than that based on IGFBP-1 is particularly relevant for clinical practice as biochemical tests are specifically used in the most challenging situations when amniotic fluid leakage is small and both clinical and ultrasound examinations are not informative. The finding that the results of the test based on PAMG-1 are more reproducible is particularly important in clinical situations where a single test is performed in a suspected PROM patient that results in a false-negative diagnosis. This false diagnosis could lead to severe adverse neonatal outcomes because appropriate management was prohibited.[2] Furthermore, the *in vitro* differences highlighted by Gaucherand in this study seemed to be echoed *in vivo* in the study by Kwek et al. (2010) showing that the test based on PAMG-1 had a higher sensitivity and specificity for the diagnosis of ROM than that based on IGFBP-1 in their tested population.

With particular respect to the comparison study between the PAMG-1 test and the intra-amniotic dye test, Dr. Rutanen is correct that the abstract does not include the sample size or study design. That said, at the time of the World Congress of Perinatal Medicine in 2009 held in Berlin, Germany, the poster presented on this study did reveal the sample size and study design. Therein it was indicated that the study contained 63 patients and in all patients, the results of the PAMG-1 test agreed completely with those of the indigo carmine intra-amniotic injection test. To date, this is the highest known sample size for any study (published or abstracted) comparing the intra-amniotic dye test to another diagnostic for ROM. Above-mentioned findings are seconded by the most recent edition of the American online Guidelines for the diagnosis of PROM called "Up-to-Date". Up-to-Date authors pronounced that in the United States, the use of indigo carmine intra-amniotic injection is now rarely indicated as a result of the availability of the test based on PAMG-1 (AminSure® ROM Test).[5] Similar to the Up-To-Date authors, we felt that this information was critical to include in our Guidelines, given the invasive and high-risk nature of the intra-amniotic dye test and the possibility that this information could help reduce or eliminate its use.

Regarding Dr. Rutanen's conclusions based on the findings of Romero et al. (2009),[11] we are quite surprised that she would be concerned that a positive biomarker test result, when clinical assessment tells membranes are intact, represents a false positive result. Rutanen et al. (1996) were in fact among the first to highlight that biomarker tests are more sensitive than standard clinical assessment: "In patients with suspected but clinically unconfirmed ROM, the positive test result is associated with increased risk of preterm delivery, suggesting that microruptures of fetal membranes can also be detected by the PROM TEST."[6] In no way it is suggested in Dr. Rutanen's 1996 publication that positive IGFBP-1 results were false and, therefore, reduce specificity, as she suggests so readily for the PAMG-1 test in the present Letter to the Editor.

Furthermore, citing the Lee/Romero et al. (2009)[7] study as a reason to doubt the specificity of the PAMG-1 test is irrelevant to the discussion for the following two reasons: (1) Lee/Romero study focuses on the term laboring patient only, while the Guidelines focus on the non-laboring patient, (2) As the clinical outcome of a term patient in labor is quite clear (i.e. delivery within a very short period of time), the value of the this article in the scientific community has come to be seen as a further indication of superior sensitivity of the PAMG-1 test over standard conventional methods.

The value of the positive PAMG-1 test in the patient with clinically intact membranes was best addressed by Lee et al. (2007)8 when it was demonstrated that in 20 out of 23 cases where there was a positive PAMG-1 test and negative clinical assessment, the patient followed the clinical course of one who was ruptured. Consequently, the standard clinical assessment in this study had a sensitivity of only 87.4% with an NPV of 54.5%. Interestingly, it was also found that all patients with clinically intact membranes, signs and symptoms of preterm labor and a positive PAMG-1 test delivered within 7 days of initial testing.[8] All studies to-date on the significance of a positive PAMG-1 test in the presence of clinically intact membranes have demonstrated either a significantly shorter time to delivery[8], significantly shorter time to SROM[9], or significant association with adverse neonatal outcomes.[10]

The aim of the Guidelines is simple and straightforward: to provide a non-bias, independent opinion of various issues in the management of spontaneous preterm birth, as reflected in the literature. We thank Dr. Rutanen and her group for their comments on our work.

Sincerely,
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