



## Serum fibronectin and C-reactive protein in prediction of preterm delivery

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### To the Editor:

Premature birth (PB) represents the main cause of perinatal mortality and morbidity worldwide (1,2). The proinflammatory cytokines became of interest for obstetricians in the context of theories referring to inflammation or intrauterine infections (IAI), which can lead to premature labor. An increased cytokines level induces elevations in the C-reactive protein (CRP) level, a liver protein synthesized soon after tissue damage or in case of infections (3). Medical research studies on PB included maternal CRP on the list of biomarkers used for sustaining the diagnosis of chorioamnionitis, especially in pregnant women presenting preterm rupture of chorioamniotic membranes (PROM). In other studies, CRP levels exceeding 7mg/l are associated with an increased risk of PB in pregnant women with intact membranes (2,4).

Fibronectin (FN) is a ubiquitous glycoprotein present in the extracellular matrix, exhibiting an important role in embryogenesis: being produced by trophoblasts it acts as a real “glue” and ensures the adhesion of the placenta to the decidua. Fetal fibronectin (fFN) in the cervicovaginal fluid has been intensely studied, having a high accuracy in prediction of spontaneous PB during the following 7-10 days (5).

The purpose of this study was to determine if the maternal serum FN and CRP levels in women with imminence of preterm labor are useful tools for prediction and diagnosis of PB.

The study was performed between January 2012 and December 2013. We included in this case-control study 92 patients presenting

monofetal 22-34 weeks pregnancy, admitted to the Obstetrics-Gynecology Clinic of the Emergency County Hospital in Tîrgu-Mureş: a group of 52 pregnant women with imminent preterm symptoms and a control group of 40 asymptomatic pregnant women who came for a prenatal visit.

From the multitude of biomarkers which predict PB, in our study we chose fibronectin because it was the most efficient biomarker. Based on the proteinaceous nature of fibronectin and theories that consider even subclinical IAI triggers of PB, we associated the study of FN with the study of CRP. We developed a model for the determination of these biomarkers in maternal serum, for pregnant women between 22-34 weeks. It included maternal serum sampling collected along with routine laboratory analysis, which is more easily performed, compared to the invasive investigations: amniocentesis or fetal sampling.

Blood samples were collected from the study group when they were admitted to the hospital and in case of the control group, when they came for the prenatal visit. The dosage of these biomarkers was performed once for all the patients. The average gestational age was  $28.55 \pm 4.12$  weeks (study group) and  $30.33 \pm 3.58$  weeks (control group), while the maternal age was:  $28.67 \pm 6.74$  years for the study group and  $30.63 \pm 4.88$  years in the control group.

We obtained demographic, obstetric and medical history of the pregnant women based on the data of a questionnaire attached to the informed consent document.\*

The novelty of our study consisted in determining serum fibronectin using the IBL-International GMBH kit; the recommended reference range for adults being 24-124 µg/ml.

## Letter to the Editor

Statistical analysis was performed using GraphPad InStat3. Data were considered as nominal or quantitative variables. A chi-square test was used in order to compare the frequencies of nominal variables. Quantitative variables were compared using t, Mann-Whitney, ANOVA or Kruskal-Wallis tests, when appropriate. Based on the cut-off points, performance parameters of diagnostic validity were estimated and ROC curves were determined. The level of statistical significance was set at  $p < 0.05$ .

Of the 92 22-34 weeks pregnant women who met the study inclusion criteria, 52 patients were hospitalized with a diagnosis of imminent preterm birth (56.52%) and 40 patients had a normal progression of pregnancy (43.47%). Based on overall analysis of the pregnant women and studying the medical documentation, we found that 18 patients presented  $PB \leq 7$  days of being admitted to the hospital. A number of 34 patients gave birth over this interval and they

were included in the subgroup with  $PB \geq 7$  days.

Statistical analysis showed that the mean level of serum fibronectin in the control group was  $302.5 \pm 86.32 \mu\text{g/ml}$  with a significant difference compared to the control group  $p < 0.05$ . Serum fibronectin levels were significantly higher in the subgroup of patients with  $PB \leq 7$  days of hospitalization (Table I).

Mann Whitney test showed significantly higher CRP levels ( $p < 0.01$ ) in the group of pregnant women with imminence of PB versus control group, with the median value  $2.49 \text{ mg/l}$  (1.01-13.1). Application of Kruskal-Wallis test for both groups showed significantly increased serum CRP levels in pregnant women with  $PB \leq 7$  days (Table I).

The Chi<sup>2</sup> test showed that 58.2% of the pregnant women in the study group were women with multiple births, while in the subgroup with  $PB \leq 7$  days the majority of women were nulliparous (80.6%). Our study has shown that PROM

**Table I. Performance of noninvasive tests for diagnose preterm birth risk**

Variables	Study Group		PB $\leq 7$ days	
	FN tests	CRP tests	FN tests	CRP tests
Biomarkers values (mean $\pm$ SD)/ (median value)	332.2 $\pm$ 65.63 $\mu\text{g/ml}$	5.18 mg/l (0.74-169.4)	360.6 $\pm$ 45.30 $\mu\text{g/ml}$	10.98 mg/l (2.43-169.4)
AUC (area under ROC curve)	0.59	0.68	0.70	0.85
p value	0.10	0.0059	0.0044	0.0001
Cut-off value	318.12 $\mu\text{g/ml}$	2.56 mg/l	365.32 $\mu\text{g/ml}$	5.2 mg/l
Sensitivity (IC 95%)	73.8% 59-84.4	76% 61.8-86.9	61% 35.7-82.7	77.78% 52.4-93.6
Specificity (IC 95%)	45% 29.3-61.5	56.52% 34.5-76.8	75% 58.8-87.3	78.26% 56.3-92.5
Positive Predictive Value (PPV) (IC 95%)	63.3% 49.8-75.5	79.2% 65.0-89.5	52.2% 29.8-74.3	73.7% 48.8-90.9
Negative Predictive Value (NPV) (IC 95%)	56.3% 37.7-73.6	52% 31.3-72.2	81.1% 64.8-92.0	81.8% 59.1-95.0
LR+ (IC 95%)	1.33 0.9-1.9	1.75 1.2-2.6	2.44 1.6-3.7	3.58 2.6-5.0
LR – (IC 95%)	0.6 0.4-1.1	0.42 0.2-0.8	0.52 0.2-1.1	0.28 0.09-0.9

was present in 11.10 % of cases with symptoms and PB $\leq$ 7 days. Complications in the PB $\leq$ 7 days group were: PROM 19%, genitourinary infections 17%, arterial hypertension (HTN) 11%, diabetes mellitus (DM) 5%, placenta praevia 5% and others 25%.

In our study we calculated performance parameters for the studied biomarkers by referring to the presence of labor in the study group versus the control group. Statistical analysis of the study group has shown that the sensitivity, specificity and PPV of CRP are higher than that of FN (Table I); these values indicate that both proteins are useful tools for predicting PB. NPV for both biomarkers were similar: 81.1% FN / 81.8% CRP. To assess the ability of these biomarker tests to predict PB, ROC curves for the study group and the subgroup  $\leq$ 7 days were plotted and compared (Table I). The highest recorded FN level was 399.52  $\mu$ g/ml: 27.78% Sn, 97.50% Sp, LR(+)11.11, LR(-)0.74, while the highest CRP level was 11.63mg/l, 50% Sn, 95.65% Sp, LR(+)11.50, LR(-)0.52.

Ideal biomarkers for PB should meet several conditions: to be biologically plausible, to have high sensitivity and specificity, to be associated with a progressive clinical parameter (preterm labor), to be determined by a reliable and reproducible method (6). Correct diagnosis of premature labor is important, early differentiation between true and false labor determining the management of PB. A meta-analysis which tested over 30 novel biomarkers led to the prediction accuracy of PB in asymptomatic pregnant women. Their conclusion was that none of the studied biomarkers met the criteria in order to be considered clinically useful in predicting PB due to the heterogeneity of the included studies. fFN in cervico-vaginal fluid has been most strongly associated with spontaneous PB and has the best accuracy in predicting PB within the following 7-10 days (7).

Most authors considered fFN as the gold standard for predicting preterm delivery; fFN measured using the ELISA technique is considered positive above 50ng/ml. A previous study described the following fFN test's range of performance: 97-98% Sn, 70-97% Sp, PPV 74-93% and NPV 98-100 % (5). Unfortunately, a poor PPV makes fFN look more like a bronze standard (9). Several prior studies indicated the absence of a non-invasive gold standard for PROM diagnosis, leading to the need of identifying biomarkers that could be associated to this diagnosis (9).

Several authors indicate that CRP is pathological in pregnancy if it's level is between 7 and 20 mg/l. In the absence of specific symptoms chorioamnionitis is difficult to be identified, but the majority of patients are diagnosed after delivery (2,4). Spontaneous PROM is present in 8-10% of term births and 30-35% of PB are triggered by PROM. The cause-effect interrelation between PROM and IAI in PB remains constant in the opinion of most authors (10).

Our study indicated high sensitivity (76%) and PPV (79.2 %), with moderate specificity (56.52%) for CRP, indicating significant diagnostic value for this inflammatory marker in PB. However, our study had some limitations including a small sample size and a large range of gestational age.

Pregnant women with PB $\leq$ 7 days presented elevated CRP levels (10.98.mg/l) and PROM in 11.10% of cases. Although most pregnant women with PB had intact membranes, the elevated CRP in this group indicated the possibility of subclinical infections. Pregnancy complications for this subgroup confirmed data in the literature and the hypothesis that IAI are potential triggers of PB.

Other authors have studied several biomarkers but they indicated fFN as having the highest predictive value, followed by CRP tests with average levels of 7.99 $\pm$ 3.56 mg/l in term births versus 18.13 $\pm$ 9.13 mg/l in PB (3).

In our study population the assessment of FN performance parameters showed high sensitivity (73.8%) and moderate specificity (45%), but sufficient for assessing the risk of PB. In the subgroup with  $PB \leq 7$  days, elevated FN levels were associated with an increased risk of PB. Thus if the level of serum FN is 365.32  $\mu\text{g/ml}$ , the LR(+) is 2.44, the FN level of 399.52  $\mu\text{g/ml}$  indicating that pregnant women have an 11.11 times higher risk of giving birth prematurely than the control group.

Serum FN and CRP determinations help us in the selection of real premature labor and enable the implementation of appropriate diagnostic and therapeutic behavior.

This study provided new information, suggesting that elevated maternal serum FN and CRP are associated with a high risk of PB. The results obtained in our study are encouraging, serum FN derived from pregnant women with symptoms of PB being a useful marker, with PPV (52.2%) and high NPV (81.1 %), comparable with fFN in the prediction of  $PB \leq 7$  days.

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The study was approved by the Ethics Committee of the University of Medicine and Pharmacy in Tîrgu-Mureș; all patients eligible for the

proposed trial completed the informed consent document, according to the Helsinki Declaration of the World Medical Association in 2013 on research performed on human subjects.

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### Conflict of interest

The authors declare that there are no conflicts of interest.

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