

ADVANCING PRETERM BIRTH PREDICTION: A PROSPECTIVE MULTICENTRIC STUDY INVESTIGATING THE PREDICTIVE PERFORMANCE OF PARTOSURE, FETAL FIBRONECTIN, AND CERVICAL LENGTH

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ADVANCING PRETERM BIRTH PREDICTION: A PROSPECTIVE MULTICENTRIC STUDY INVESTIGATING THE PREDICTIVE PERFORMANCE OF PARTOSURE, FETAL FIBRONECTIN, AND CERVICAL LENGTH (Abstract): The prediction of preterm birth (PTB) is still a controversial topic, and many efforts have been made to identify the best predictive markers. The **aim** of this study was to prospectively assess the predictive performance of placental alpha macroglobulin-1 (PAMG-1), fetal fibronectin (fFN), and cervical length, both individually and combined, for the prediction of spontaneous preterm birth at 7 and 14 days in a cohort of pregnant patients with threatened preterm labor. **Material and methods:** Between October 2022 and April 2023, we performed a prospective observational study that evaluated 80 pregnant patients with singleton pregnancies, between 24 and 32 completed weeks of gestation. We qualitatively determined PAMG-1 (PartoSure test) and fFN from the vaginal fluid. Clinical data along with cervical length measurements were also retrieved. The predictive performance of the evaluated markers was determined using logistic regression and ROC analysis. **Results:** PartoSure obtained the best results for the prediction of PTB in the next 7 or 14 days. On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for the evaluated outcomes. A combined algorithm that comprised PartoSure, fFN, and cervical length measurements achieved the best predictive performance for the prediction of PTB at 14 days. **Conclusions:** The combined algorithm is superior in terms of predictive performance for the prediction of PTB in the next 7 or 14 days. The PartoSure test could be used individually for the prediction of PTB, but clinicians need to correlate its results with other clinical findings. **Keywords:** PRETERM BIRTH PREDICTION; PARTOSURE; PAMG-1; FETAL FIBRONECTIN; CERVICAL LENGTH.

Preterm birth (PTB), defined as delivery before 37 completed weeks of gestation, is one of the most important public health issues, associated with increased morbidity and mortality rates worldwide (1). According to one of the most recent studies published in the literature, the number of preterm births worldwide declined by 5.26% from 16.06 million in 1990 to 15.22 million in 2019, and the number of fatalities decreased by 47.71% from 1.27 million in 1990 to 0.66 million in 2019 (2).

Even though some significant improvements have been made for the prediction and prevention of this disorder, there is still no accurate test that will correctly identify the majority of patients who will deliver prematurely (3). Maternal risk factors such as personal history of preterm birth, cervical excisional procedures, smoking status, use of assisted reproductive techniques, vaginal and urinary tract infections, thrombotic disorders, or ischemic placental disease have been proposed as predictors of PTB (4-8). Moreover, sonographic markers such as short cervical length, uterocervical angle, uterine artery pulsatility index, as well as cervical and elastography parameters, have proven to be associated with increased risk of preterm birth and adverse pregnancy outcomes (9-12).

Placental alpha macroglobulin-1 (PAMG-1), which is comprised in the PartoSure test, is synthesized by decidual cells that line the uterus and is subsequently released into the amniotic fluid (13). Typically, the concentration of PAMG-1 in vaginal discharge is minimal. However, research has demonstrated that the detection of PAMG-1, either qualitatively or quantitatively, in vaginal discharge is indicative of impending delivery (14, 15).

Fetal fibronectin (fFN) is an important constituent of extracellular matrix, and it is located at the interface between the decidua and chorionic tissues (16). The release of fFN into cervicovaginal secretions occurs as a result of the disruption of the interface caused by inflammation, abruption, or uterine contractions (17). This phenomenon can serve as a foundation for predicting preterm birth.

It is estimated that approximately 65-75% of PTBs occur spontaneously, whereas the remaining cases are iatrogenic in nature (18). The occurrence of spontaneous PTB can be attributed to various pathophysiological mechanisms (19). Consequently, accurately predicting PTB has been a persistent obstacle, given that relying solely on clinical symptoms does not provide sufficient predictive precision (3, 20). In a recent meta-analysis by Melchor *et al.*, PAMG-1, fFN, and cervical length showed a variable predictive performance between studies, due to the influence of demographic and clinical factors (21).

Nikolova *et al.*, comparatively evaluated the predictive performance of PAMG-1, fFN, and cervical length for the prediction of preterm birth in a time-frame between 7-14 days in a cohort of patients 203 patients at risk (22). The authors concluded that PAMG-1 (Sensitivity- Se: 80%; Specificity- Sp: 95%) was the best predictor of PTB within 7 days compared to fFN (Se: 50%; Sp: 72%) and cervical length (Se: 57%; Sp: 73%).

The current literature data is reported heterogeneously, and the predictive performance of combined algorithms was not studied in prospective studies. Therefore, the aim of this multicentric study was to prospectively assess the predictive performance of PAMG-1, fFN, and cervical

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length, both individually and combined, for the prediction of spontaneous preterm birth at 7 and 14 days in a cohort of pregnant patients with threatened preterm labor

MATERIAL AND METHODS

We performed a prospective observational study that evaluated pregnant patients with singleton pregnancies, between 24 and 32 completed weeks of gestation who self-reported pelvic pressure, uterine contractions with or without pain, intermittent lower abdominal pain, or any other sign, symptom, or complaint that might be indicative of imminence of preterm birth. Between October 2022 and April 2023, 80 pregnant patients who were evaluated at “Sf. Ioan cel Nou” Emergency Hospital, Suceava, and at “Cuza-Voda” Clinical Hospital of Obstetrics and Gynecology, Iasi, Romania, were enrolled.

Ethical approval for this study was obtained from the Institutional Ethics Committees of “Grigore T. Popa” University of Medicine and Pharmacy Iasi (No. 101/08.07.2021), and “Sf. Ioan cel Nou” Emergency Hospital, Suceava (No. 21/26.10.2022). Informed consent was obtained from all participants included in the study. All methods were conducted in accordance with relevant guidelines and regulations.

The speculum examination of the enrolled individuals revealed that they had cervical dilatation (less than 3 cm) and clinically intact amniotic membranes. Patients with overt fetal membrane rupture, advanced cervical dilatation (more than 3 cm), presence of blood on speculum examination, suspected placenta previa, cervical cerclage in place, patients less than 18 years old, or who were unable to offer their informed consent were excluded from the

study.

Complete obstetrical and medical examinations were performed at admission. Before cervical examination, we collected vaginal fluid samples for PartoSure (Germantown, MD 20874, USA), and fetal fibronectin tests (Antagen Pharmaceuticals Inc, Boston, MA 02118, USA). The samples were processed according to the manufacturer’s instructions, and both PAMG-1 and fFN were determined qualitatively (test and control line positives). We proceeded with digital and cervical examinations.

The patients underwent standard anatomy scans as well as transvaginal ultrasound performed by experienced obstetricians, with at least level 2 qualification in ultrasound examination, using an E10 scanner with a 4.8 MHz transabdominal probe, and a 5-15 MHz intravaginal probe (GE Medical Systems, Milwaukee, WI, USA).

The following data were recorded: gestational age, clinical symptoms, cervical length, presence of PAMG-1 and fFN, and obstetrical outcomes. The primary outcome was preterm birth in the next 7 days, while the secondary outcome was preterm birth in the next 14 days after admission.

In the first stage of the statistical analysis, descriptive statistics were used for the characterization of our cohort of patients. In the second stage of the analysis, we evaluated and compared the predictive performance of PAMG-1, fFN, and cervical length for the prediction of PTB in a 7-days and 14 time-frames using logistic regression and ROC analysis. Cut-off values, adjusted to gestational age at diagnosis, were identified according to the Youden index. The combined algorithm comprised all three markers and its predictive performance was also calculated. The statistical analyses were performed using *STATA SE*

(version 17, 2022, StataCorp LLC, College Station, TX, USA). A p value less than 0.05 was considered statistically significant.

RESULTS

This study enrolled 80 patients with imminence of preterm birth. Their demographic characteristics are presented in table I. The median age for this cohort of patients was 29.5 years (interquartile range, IQR: 24-35), while the median BMI was 26.43 kg/m² (IQR: 22.86- 28.48). The majority of these patients came from a rural background (52.5%), and their clinical characteristics revealed high rates of vaginal infections (22.5%) as well as a significant personal history of preterm birth (17.5%) and other adverse pregnancy outcomes such as preeclampsia and intrauterine growth restriction (13.75%).

The predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 7-days' time-frame was calculated, and it is presented in table II and figure 1. PartoSure obtained the best results when evaluated individually for the prediction of PTB in the next 7 days, with a sensitivity of 71.79%, specificity of 48.78% and an AUC value of 0.602. The combined model achieved a sensitivity of 74.89%, a specificity of 90.24%, and an

AUC value of 0.769.

TABLE I.

Demographic and clinical characteristics of patients included in the study

Parameter	Median and IQR or n (%)
Age, years	29.5 (24- 35)
BMI, kg/m ²	26.43 (22.86- 28.48)
Medium	Rural= 42 (52.5%) Urban= 38 (47.5%)
Preterm birth history	14 (17.5%)
Confirmed urinary tract infection	7 (8.75%)
Confirmed vaginal infection	18 (22.5%)
Gestational hypertension	3 (3.75%)
Personal history of adverse pregnancy outcomes	11 (13.75%)
Gestational diabetes	2 (2.5%)
Autoimmune disorders	1 (1.25%)

IQR- interquartile range; BMI-body mass index.

The predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 14-days' time-frame was calculated and it is presented in table III and figure 2. PartoSure obtained the best results when evaluated individually for the prediction of PTB in the next 14 days, with a sensitivity of 53.66%, specificity of 87.18% and an AUC value of 0.704. The combined model achieved a sensitivity of 70.73%, a specificity of 94.87%, and an AUC value of 0.828.

TABLE II.

The predictive performance of individual and combined tests for the prediction of PTB at 7 days.

Test	Sensitivity	Specificity	LR+	LR-	AUC and 95% CI
PartoSure	71.79	48.78	1.40	0.57	0.602 (0.48- 0.70)
fFN	46.15	73.17	1.72	0.73	0.596 (0.48- 0.70)
Cervical length (cut-	38.46	17.07	0.463	3.604	0.204 (0.11- 0.30)
Combined	74.89	90.24	7.35	0.31	0.769 (0.66- 0.86)

LR+- positive likelihood ratio; LR- - negative likelihood ratio;

AUC- area under the receiver operating curve; CI- confidence interval; fFN- fetal fibronectin.

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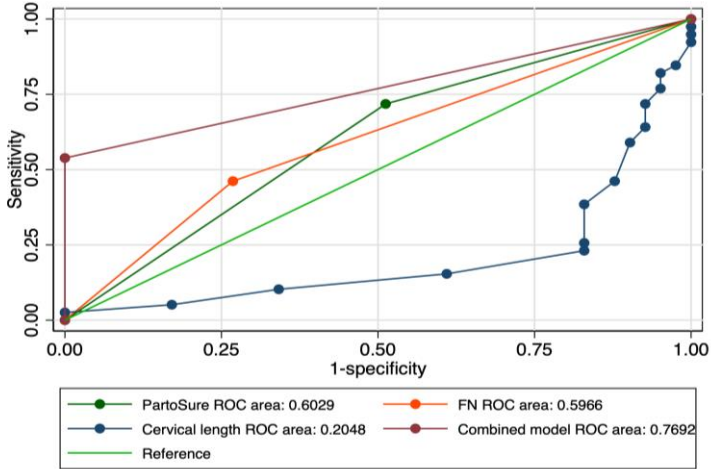


Fig. 1. Multiple ROC comparisons of individual and combined tests for the prediction of PTB in the next 7 days. (ROC- receiver operating characteristic curve; FN- fetal fibronectin)

TABLE III.

The predictive performance of individual and combined tests for the prediction of PTB at 14 days

Test	Sensitivity	Specificity	LR+	LR-	AUC and 95% CI
PartoSure	53.66	87.18	4.18	0.53	0.704 (0.61- 0.79)
fFN	43.90	79.49	2.14	0.70	0.616 (0.51- 0.71)
Cervical length	36.59	69.23	1.18	0.91	0.500 (0.37- 0.62)
Combined	70.73	94.87	13.79	0.30	0.828 (0.72- 0.90)

LR+- positive likelihood ratio; LR- - negative likelihood ratio;

AUC- area under the receiver operating curve; CI- confidence interval; fFN- fetal fibronectin.

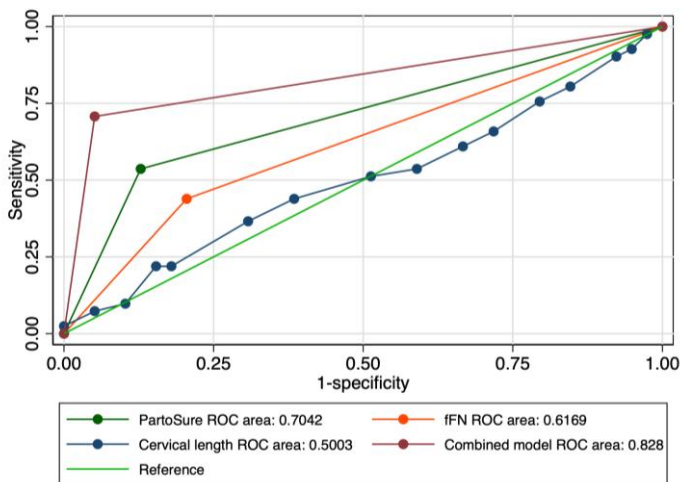


Fig. 2. Multiple ROC comparison of individual and combined tests for the prediction of PTB in the next 14 days. (ROC- receiver operating characteristic curve; fFN- fetal fibronectin)

DISCUSSION

The present study evaluated the predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 7-days and 14-days' time-frames. Our results indicated that PartoSure achieved the best results in terms of sensitivity and specificity for the prediction of PTB at both 7 days and 14 days intervals from its detection. Its sensitivity was higher for the evaluated outcome at 7 days, while its specificity increased when used to predict PTB at 14 days. On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for PTB at 7-and 14-days intervals after its measurement.

Our results are similar to those reported in previous literature. Nikolova *et al.*, reported the following sensitivities for PartoSure, fFN, and cervical length for predicting imminent spontaneous PTB within 7 days: 80%, 50%, and 57%, along with their corresponding specificities: 95%, 72%, and 73% (22). Moreover, the authors indicated lower predictive performance of PartoSure (Se- 63%, Sp- 96%), fFN (Se-42%, Sp-72%), and cervical length (Se-54%, Sp-74%) for the prediction of imminent spontaneous PTB within 14 days.

A recent review by Rouholamin *et al.*, indicated that PartoSure test had the highest specificity, positive predictive value (PPV), and positive likelihood ratio (LR+) for the prediction of PTB in all categories of pregnant patients, but, at the same time, outlined the need to correlate its results with the clinical findings and other diagnostic procedures (15).

We also calculated the predictive performance of a combined algorithm that comprised PartoSure, fFN, and cervical length for the prediction of imminent spontaneous PTB within 7 and 14 days. Our

results indicated relative similar sensitivities and specificities, although the overall accuracy was higher for the prediction of PTB at 14-days (AUC of combined algorithm: 0.828; 95%CI: 0.72- 0.90). As far as we know, this is the first study in the literature that evaluated the predictive performance of combined algorithms, so comparative data is not available.

PartoSure (PAMG-1) and fFN were both qualitatively determined in our study. Ravi *et al.*, evaluated the predictive performance of various cut-offs of fFN and compared the results with qualitative PAMG-1 for the prediction of PTB in the next 7 to 14 days (23). Their results indicated that PAMG-1 test was the best predictor for the evaluated out-comes, and reported a sensitivity of 67%, and a specificity of 96% for the prediction of PTB in the next 7 days. Moreover, the authors reported that a conventional cut-off for fFN of 50 ng/ml provided enough accuracy for the prediction of the evaluated outcomes (Se- 67%, and Sp-77%).

This study has several limitations: small cohort of patients and qualitative determination of PAMG-1 and fFN. On the other hand, this is the first study that evaluated the predictive performance of a combined algorithm in a prospective setting. Further studies, on larger cohorts of patients, could determine the cost-effectiveness of combined strategies for the prediction of PTB.

CONCLUSIONS

The results of this study indicated that PartoSure achieved the best results in terms of sensitivity and specificity for the prediction of PTB at both 7-days and 14-days intervals from its detection.

On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for PTB at 7-

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and 14-days intervals after its measurement.

A combined algorithm that comprised PartoSure, fFN and cervical length measurements achieved the best predictive performance for the prediction of PTB at 14 days.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no con-

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D. Nemescu, A. Caraeleanu and Luiza-Maria Cobzeanu had equal contribution to the first and correspondent authors and should be regarded as main authors of the manuscript.

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