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Combination of selected biochemical markers and cervical length in the prediction of impending preterm delivery in symptomatic patients

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Summary

The pathophysiology of preterm delivery (PTD) is complex and multifactorial.It occurs in 8–12% of all deliveries, and the rate of PTD has increased during the past years in spite of intensive efforts towards early detection and prompt treatment. Fifty-eight pregnant women were eligible to join the study if they attended the University Clinic for Gynecology and Obstetrics, Skopje and were admitted to Department of High Risk pregnancy Unit with symptoms of preterm labor (PTL) (symptoms of uterine activity judged by the assessing physician to be indicative of PTL) at 24.0 to 36.6 weeks gestation.Test specimens for fetal fibronectin (fFN), phosphorylated insulin like growth factor binding protein 1 (phIGFBP-1), IL-6, and IL-2R and measuring the cervical length via transvaginal ultrasound were performed for each patient. The best statistical model for predicting PTL in the present study was to use a combination of the phIGFBP-1 test, a positive fFN test, cervical length less than 21.5mm, levels of IL-6 higher than 1,305 pg/ml in the cervico-vaginal fluid (CVF), and serum levels of C-reactive protein (CRP) higher than 6.1mg/L which was excellent at identifying the patients that were to deliver within 14 days of admittance.

Key words: Preterm delivery; Fetal fibronectin; Cytokines; Predictors; ph-IGFBP-1.

Introduction

Prevention of preterm delivery (PTD) is a major obstetrical challenge. This is not only due to assisted conception, as increased PTD rates have been demonstrated also among spontaneous pregnancies. The pathophysiology is complex and multifactorial. The rate of preterm births has been estimated at around 14.9 million, which accounts for 11.1% of all live births worldwide[1]. Individual countries incidence rates are highly dependent on the degree of development and range from 5% in most developed European countries to 18% in several African countries[1]. It occurs in 8–12% of all deliveries, and the rate of preterm delivery has increased during the past years in spite of intensive efforts towards early detection and prompt treatment [2]. Currently, cervico-vaginal fetal fibronectin (fFN) test and trans- vaginal ultrasound measurement of cervix length are recommended by the American Congress of Obstetricians and Gynecologists for the prediction of PTD [2]. However, there is an urgent need for new markers that may be easier and more sensitive than current methods because the majority of women who had undergone a transvaginal ultrasound examination and pelvic examination can experience discomfort [3].

Fetal and neonatal morbidity and mortality rates are strongly associated with gestational age at birth. Specifically, infants born before 32 weeks of gestation are at risk of sequela [4-6]. More than 70% of women presenting with symptoms of preterm labor (PTL) do not progress to active labor and delivery [7, 8]. It is essential to identify pregnant women with threatened PTL who will deliver preterm, and differentiate them from the women who will continue their pregnancies to full term. The past three decades have seen a plethora of attempts at developing methods to correctly predict preterm delivery, such as obstetric history, symptoms, epidemiological risk factors, maternal indicators such as age and anthropometric parameters, pregnancy characteristics such as bleeding, different physical examination parameters and biological markers etc., but most of these methods are neither sensitive nor specific enough [9, 10]. Any method potent enough to accurately distinguish women who are likely to deliver preterm infants from those who have the symptoms and clinical presentation, but are unlikely to delivery prematurely, would certainly go a long way towards preventing unnecessary and potentially risky medical interventions and reduce costs and burden to the medical system.

Attempts to predict PTD based on maternal and biochemical data, and interventions to reduce PTD rates, have been largely unsuccessful [11–13]. The necessity of finding

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reliable prediction models is urgent, and a multiple-markers test indicative of the multifactorial etiology of PTD is likely to be more successful [14, 15]. There is a wealth of literature suggesting that cervical length measured by ultrasound and fFN have the potential to improve the prediction of PTD [16-18]. In order to institute specific therapy more appropriately, it is important to have adjuvant tests to help predict who is most likely to deliver preterm.The detection of phosphorylated insulin like growth factor binding protein 1 (phIGFBP-1) in the cervical secretions of women presenting with PTL has been shown to be associated with an increased risk of PTD [19-28]. Recently, cervico-vaginal concentrations of phIGFBP-1 have been shown to correlate with the risk of PTD [19-28]. Disruption to the chorio-decidual interface results in elevated levels in cervical secretions. Potentially contaminating body fluids with fFN—such as semen and urine—contain only trace quantities of phIGFBP-1 [19].

Another group of biochemical markers involved in the prediction of PTD is inflammatory molecules suck as cytokines. Cytokines may be involved in the etiology of preterm birth through their influence on prostaglandin synthesis and secretion [29]. A number of studies have reported increased concentrations of certain cytokines, most notably interleukin 6 (IL-6) in the serum and amniotic fluid of patients with PTL [30-34]. Several studies have investigated IL-6 detection in the cervico-vaginal fluid (CVF) and demonstrated that the presence of IL-6 in the CVF is associated with PTD [35-37].

Increased serum concentrations of the C-reactive protein (CRP) in the first trimester do not increase the overall risk of PTD [38], but in symptomatic patients' serum CRP levels have a low sensitivity (38%) and high specificity (94%) in the prediction of PTD before 34 weeks of gestation [39].

Increased serum levels of the soluble interleukin 2 receptor (IL-2R) have been associated with development of preeclampsia and eclampsia, as well as PTD and intrauterine growth restriction [40].

Most of these markers can be used in conjunction with the evaluation of the cervical length via transvaginal ultrasonography. Studies conducted in the general population of pregnant women have shown that the decrease in cervical length is a predictor of PTD, although it has a low predictive value (6-47.6%) [41-45].

However, threatened PTD and PTD requiring treatment at high-level medical facilities are increasing in Macedonia. In these circumstances, to extract unknown factors related to preterm delivery, the authors carried out a study using patients diagnosed with threatened preterm delivery admitted to the Department of high risk pregnancy at University Clinic of Obstetrics and Gynecology Skopje, which is a tertiary medical organization and they combined different independent predictive factors of premature delivery.

The aim of this study was to evaluate the usefulness of different biochemical markers, namely: fFN, phIGFBP-1, IL-6, IL2-R, and CRP, in the prediction of preterm delivery within 14 days of admission in symptomatic patients, as well as the predictive and diagnostic value of the combination of markers along with the cervical length evaluated via transvaginal ultrasonography. The hypothesis of the study was that the combination of markers would yield better results in predicting PTD within 14 days of admission than each marker.

Material and Methods

Fifty-eight pregnant women were eligible to join the study if they attended the University Clinic for Gynecology and Obstetrics, Skopje and were admitted to Department of High Risk pregnancy Unit with symptoms of preterm labor (symptoms of uterine activity judged by the assessing physician to be indicative of PTL) at 24.0 to 36.6 weeks gestation. They were recruited in period of six months from September 2013 to March 2014. They were with symptoms or complaints suggestive of PTL including uterine contractions, intermittent lower abdominal pain, and pelvic pressure. Recruited patients had intact amniotic membranes determined by speculum examination and minimal cervical dilatation ($\leq$ 3 cm).Women were excluded if they had ruptured membranes, antepartum hemorrhage, active labor, a cervical cerclage in place, and suspected chorioamnionitis (defined by fever, abdominal pain, leukocytosis).

Consenting women were treated according to usual hospital protocol. The authors took a detailed history, performed tocography, and a speculum exam to obtain test specimens for fFN, phIGFBP-1, IL-6, and IL-2R and drew blood to determine the adequate serum concentrations of the respective markers and measured the cervical length via transvaginal ultrasound.

The obtained data was digitized, and all statistical tests were performed using SPSS version 13.0. The authors used descriptive statistical analysis to display the following parameters: mean, standard deviation, coefficient of variation, and interval of variation. The categorical variables were tested using Chi square and Fischer exact tests, and the quantitative variables were analyzed with the independent sample test and Mann-Whitney's U test. To determine the correlation between the variables, the authors used Spearman Rank Ordered Correlation test and Pearson's coefficient of linear correlation. The also used binary logistic regression to determine the predictive role of the analyzed parameters in the prediction of preterm labor. Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were computed for each biochemical markers and cervical length.

Results

The main demographic characteristics of the study population are shown in Table 1. Mean maternal age was 30.12 years. Mean gestational age was 31.55 weeks at recruitment. Mean height was 164.34 cm. Mean weight was 74.05 kg. Mean BMI was 27.54. Of the 58 patients enrolled in the study, nine had history of previous PTD. The authors also evaluated the number of previous spontaneous abortions, parity, and smoking of the patients with threatened preterm labor.

Thirty-six patients (62.07%) delivered within 14 days from admission. Table 2 presents the distribution of the patients that delivered or remained pregnant within 14 days of admission in regards to the results of the fFN. From the 36

Table 1. — *Demographic characteristics of study population (n=58).*

Variable	Mean ±SD (range)
Maternal age (years)	30.12 ± 4.82 (20 - 40)
Gestation age at examination (w)	31.55 ± 3.95 (22 - 36)
BMI	27.54 ± 4.93 (18.7 - 43.8)
	n(%)
Parity	
Nulliparous	13 (22.41)
Multiparous	45 (77.59)
Previous preterm delivery	10 (17.24)
Smoker	11 (18.96)

Table 2.— *Detection of fetal fibronectin in the CVF of the studied population.*

Variable	Outcome within 14 days of admission		*p* value
	Undelivered (n=22)	*Delivered (n=36)*	
Fetal fibronectin (%)			
Positive	7 (31.82%)	27 (75.0%)	0.0011
Negative	15 (68.18%)	9 (25.0%)	

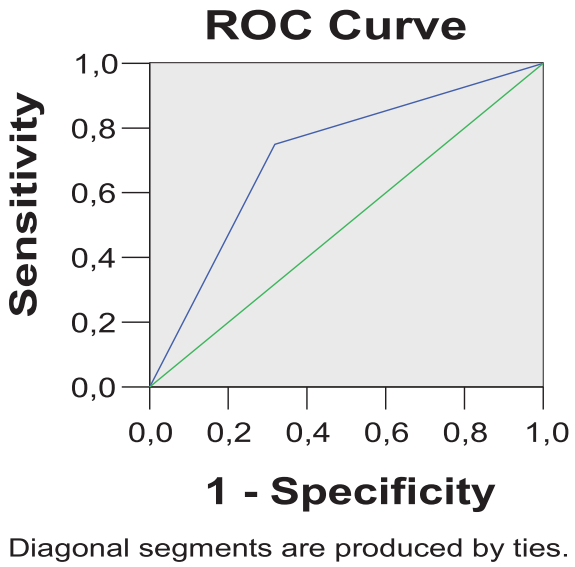

Figure 1.— ROC curve for the performance of the fFN test in the prediction of preterm delivery.

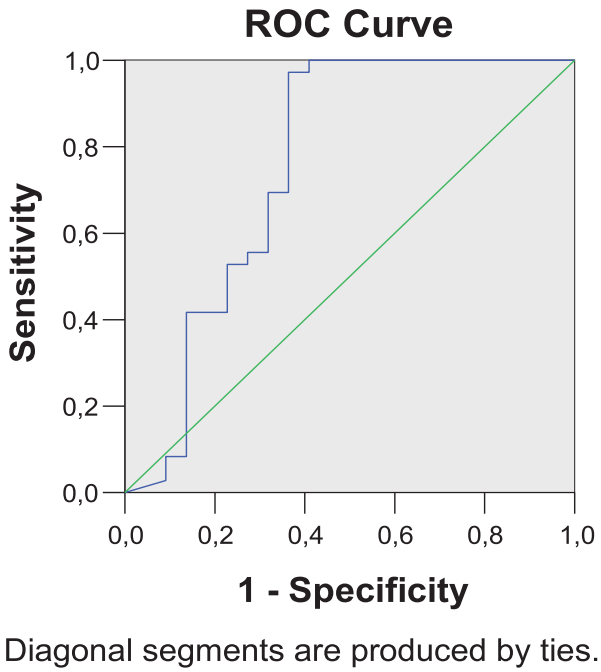

Figure 2.— ROC curve for the performance of IL-6 in the CVF as a predictor of preterm delivery.

patients that were delivered within 14 days of admission, 27 patients (75%) had a positive fFN test, while 15 patients (68.18%) of the 22 patients that remained pregnant after 14 days from admission had a negative fFN test. The Chi-square statistical test confirmed that this observed difference was statistically significant ($p = 0.0011$).

The fFN test is a significant predictor of preterm delivery. Patients with a positive fFN test have an OR of 6.429 (95% CI 1.991 - 20.758) to deliver prematurely. The diagnostic performance of the fFN test in the present study was as follows: sensitivity = 75%; specificity = 68.2%; PPV = 79%; NPV = 62.5%; positive likelihood ratio (LR+) = 2.46; negative likelihood ratio (LR-) = 0.37; AUC = 0.716; 95% CI = 0.575-0.856. Figure 1 shows the ROC curve for the diagnostic performance of the fFN test.

In the group of patients that delivered within 14 days of admission, the authors found significantly higher concentrations of IL-6 in the CVF ($p = 0.0011$). The average measured concentration of IL-6 was 3,139.8 ± 2,646.2 pg/ml in the group of patients that delivered within 14 days, while the average concentration in the group that remained pregnant longer than 14 days was 1,755.7 ± 3,165.7 pg/ml.

Using the data, the authors calculated a ROC curve in order to determine the cut-off value for the concentration of IL-6 in the CVF that accurately predicts preterm delivery (Figure 2). The results determined that the best cut-off value for the concentration of IL-6 in the CVF that correctly predicted preterm delivery in this study was 1,305 pg/ml, which gave the test a sensitivity of 69.4%, specificity of 68.2%, a LR+ of 2.18 and a LR- of 0.45. The calculated rate of PTD was 78.13% in patients with a concentration of IL-6 in the CVF higher than 1,305 pg/ml, and 42.31% in the patients with concentrations lower than the cut-off.

The difference in the concentration of IL-6 in the CVF, classified above or below the determined cut-off of 1,305 pg/ml, between the two groups of patients was statistically significant ($p = 0.005$)

Table 3. — *Detection of phIGFBP-1 in the CVF of the studied population.*

Variable	Outcome within 14 days of admission		*p* value
	Undelivered (n=22)	*Delivered (n=36)*	
PhIGFBP-1 n (%)			
Positive	14 (63.64%)	12 (33.33%)	0.02
Negative	8 (36.36%)	24 (66.67%)	

Table 4. — *Serum levels of the soluble IL-2R in the studied population.*

Variable	Outcome within 14 days of admission		*p* value
	Undelivered (n=22)	*Delivered (n=36)*	
Serum concentration of IL-2R (U/ml)	382.8±138.6 (range 169-748)	471±197.6 (range 199-1327)	0.044

The patients that gave birth within 14 days of admission were also statistically more likely to have a positive phIGFBP-1 test ($p = 0.02$). The distribution of patients with regards to the phIGFBP-1 test is shown in Table 3.

All but one pregnant women that remained pregnant after 14 days of admission had a serum level of IL-2R below 500 U/ml and the difference in concentrations between the two groups was statistically significant ($p = 0.044$, Table 4.).

The authors calculated an optimal cut-off value for the IL-2R levels of 388.5 pg/ml, which yielded a sensitivity of 69.4%, specificity of 68.2%, LR+ 2.18, LR- 0.45, and an AUC of 0.688 (Figure 3).

The group of patients that were delivered within 14 days of admission had significantly higher serum levels of CRP, when compared to the patients that remained pregnant after 14 days ($p = 0.001$). The average CRP concentration in the PTD group was 11.9 ± 16.85 mg/l versus 5.67 ± 5.5 mg/l in the group of patients that remained pregnant after two weeks.

The optimal cut-off value for the CRP serum concentrations that correctly predicted PTD in this group of patients was 6.1 mg/l, which gave the test a sensitivity of 72.2%, specificity of 72.7%, LR+ 2.64, LR- 0.38, and an AUC of 0.756 (Figure 4).

The patients that were delivered within 14 days of admission in the present study group had an average cervical length of 18.78 ± 5.8 mm, which was significantly lower than the average cervical length (23.87 ± 6.36 mm) of patients that remained pregnant after 14 days ($p = 0.0028$). The shortest cervical lengths that the authors measured in the two groups were five and 12 mm, respectively. The univariate logistic regression revealed that the best cut-off value for the cervical length in the present study was 21.5 mm, which yielded a sensitivity of 30.6% and a specificity of 36.4%. The ROC curve for the diagnostic performance of the transvaginally measured cervical length is shown in Figure 5.

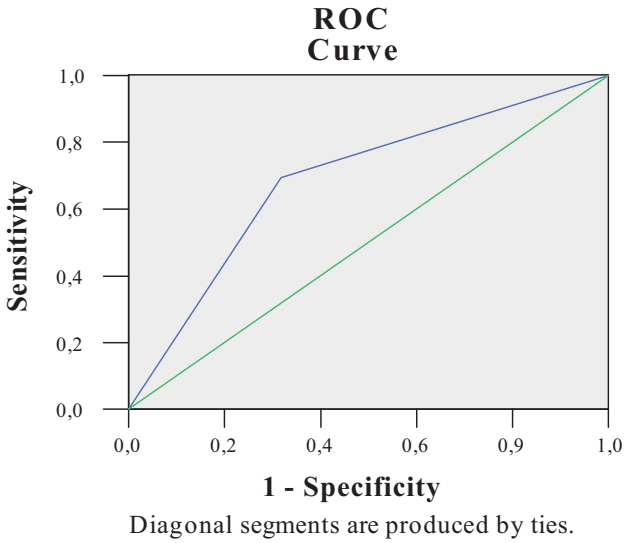

Figure 3.— ROC curve for the performance of IL-2R serum concentration as a predictor of preterm delivery.

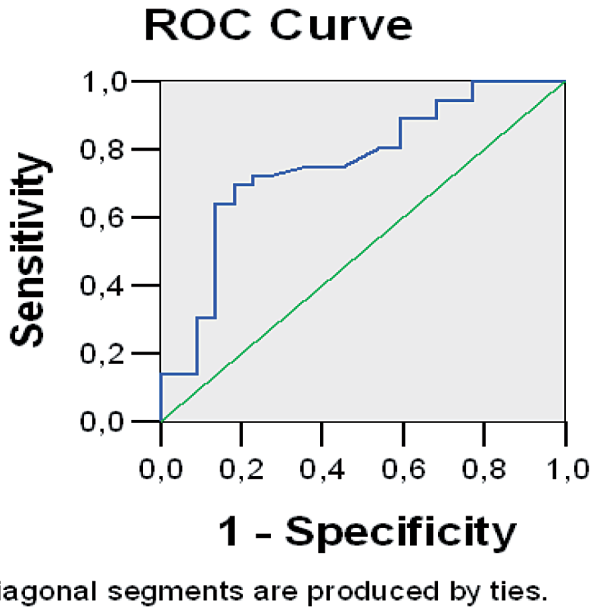

Figure 4.— ROC curve for the performance of CRP serum concentration as a predictor of preterm delivery.

Table 5 summarizes the diagnostic performance of each individual test.

Combination of markers

The authors used multivariate logistic regression to devise models based on the combination of different tests. Table 6 summarizes the diagnostic performance of each tested combination. The combination of cervical length (measured transvaginally) of less than 21.5, positive fFN and phIGFBP-1 tests, as well as serum concentrations of

Table 5. — *Individual test diagnostic performance in the prediction of PTD within 14 days of admission.*

Test	PPV	NPV	LR+	LR-	AUC	OR (PTD)
CL	75%	54%	2.54	0.42	0.711	3.5
CRP	69.4%	59%	2.54	0.42	0.756	6.06
fFN	79%	62.5%	2.36	0.37	0.716	6.43
phIGFBP-1	75%	54%	1.83	0.52	0.652	3.5
IL-6	78.1%	57.69%	2.18	0.45	0.759	3.87
IL-2R	78.12%	57.7%	2.18	0.45	0.688	4.87

Table 6. — *Diagnostic performance of different combinations of markers in the prediction of PTD within 14 days of admission.*

Combination of markers	Sensitivity (%)	Specificity (%)	Area under the curve (AUC)	*p* (preterm birth)
fFN + phIGFBP-1	100	40.9	0.799	0.94
CL + fFN	91.7	40.9	0.784	0.526
CL + phIGFBP-1 + fFN	91.7	54.5	0.830	0.69
CL + IL-6	86.1	40.9	0.751	0.57
ffN + IL-6	97.2	63.6	0.759	0.867
fFN + phIGFBP-1 + IL-6	97.2	68.2	0.823	0.774
CL+ fFN + phIGFBP-1 + IL-6	83.3	59.1	0.850	0.9396
CL+ CRP + fFN + phIGFBP-1+ IL-6	86.1	72.7	0.867	0.9853
CL+ CRP + fFN + phIGFBP-1 + IL-6 + IL-2R	88.9	77.3	0.912	0.995

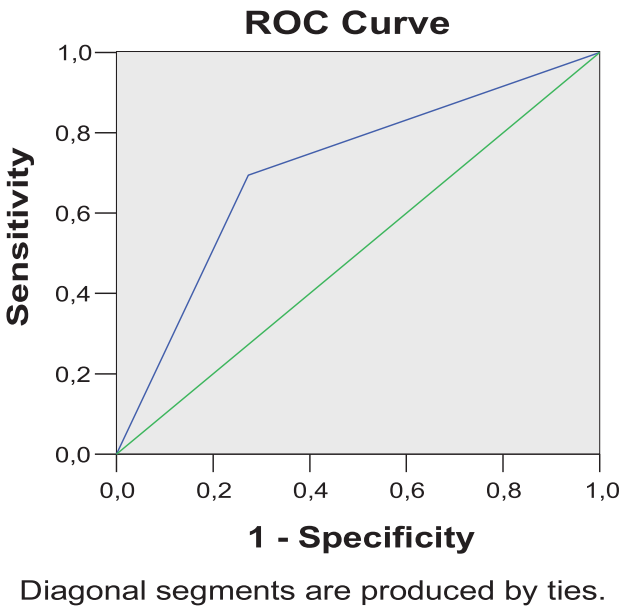

Figure 5.— ROC curve for the performance of cervical length as a predictor of preterm delivery.

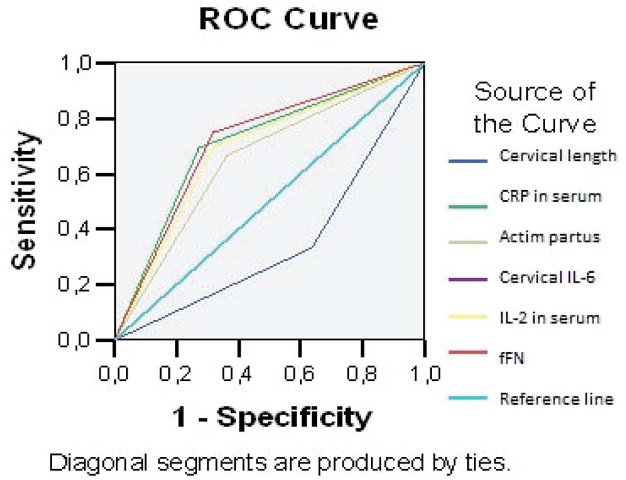

Figure 6.— ROC curves for the diagnostic performance of different combinations of predictive markers for preterm delivery.

CRP and IL-2R and CVF concentration of IL-6 above their respective cut-off values yielded the best calculated probability form PTD within 14 days of admission ($p = 0.995$).

Figure 6 demonstrates the ROC curves of the individual combinations. The combination of the six markers has an AUC of 0.912 (95% CI 0.837 - 0.987) which indicates that this particular combination of predictive markers makes a precise classification of cases of PTL into two groups of patients: one in which the patients are very likely to deliver within 14 days of admission and another in which the patients are very likely to remain pregnant after 14 days.

Discussion

Despite advances in obstetric care, PTD remains a major cause of neonatal morbidity and mortality. With women presenting an acute risk of PTD, tocolysis, steroids, and in utero transfer to a center with neonatal intensive care are recommended [46]. This involves unnecessary treatment and complex management in a relevant number of symptomatic women who eventually will not deliver preterm. Therefore, there is a need for assessment tools to reliably identify cases who are at highest risk of early delivery, and those who are not and can avoid treatment.

This fact clearly illustrates the necessity of devising a model that correctly predicts imminent PTD. Given the multifactorial etiology of PTD as a syndrome, it is safe to assume that predictive models that utilize multiple specific markers have a better chance of succeeding.

There have been a limited number of studies that investigated the relationship between different biochemical markers in the maternal serum and CVF and the occurrence of PTD. One such study [17] published a predictive model based on the serum and CVF inflammatory markers in the early second trimester (12-25 weeks of gestation) in asymp-

tomatic patients with a previous PTD. Their model correctly predicted 69% of the recurrent PTDs. Holst *et al.* [8] conducted a study on 89 patients and analyzed 27 protein markers associated with PTD in the amniotic fluid and CVF and devised a prediction model based on amniotic macrophage inflammatory protein-1 beta, cervical interferon-gamma, and monocyte chemotactic protein-1. The authors demonstrated that their model correctly identified the symptomatic patients that would deliver prematurely within seven days of testing.

Transvaginal ultrasonographic cervical length measurement is a commonly used method of evaluating patients with symptoms of PTL. The generally accepted cut-off value of 25 mm or less is considered to be a relevant predictor of an impending PTD in patients with symptoms of PTL [47-49]. The patients that delivered within 14 days of admission in the present study group had an average cervical length of 18.78 ± 5.8 mm and the optimal cut-off value for the cervical length in this study was 21.5 mm, which yielded a sensitivity of 30.6% and a specificity of 36.4%.

Previous studies also demonstrated that patients with PTL and clinical chorioamnionitis have elevated concentrations of IL-6 in the amniotic fluid and umbilical cord blood serum [22-27]. A recent large study conducted by Woodworth *et al.* [28] focused on the diagnostic accuracy of IL-6 detected in the CVF as a predictor to PTD. The authors analysed 660 CVF samples for IL-6 and concluded that the IL-6 test with a cut-off of 250 pg/ml had a sensitivity of 35%, specificity 87%, PPV 19%, NPV 98%, LR+ of 4.83, and LR- of 0.41. These results over-perform the test in the present analysis. The present regression analysis gave a significantly higher cut-off value for the concentration of IL-6, as opposed to the now almost universally-accepted value of 250 pg/ml, first determined by Lockwood *et al.* [9]. This may be due to the fact that the authors calculated the likelihood of delivery within 14 days as opposed to seven days, the small sample size, and the fact that the study recruited a high-risk group of patients that already had symptoms of PTL, a high proportion of which (over 60%) delivered within 14 days.

A multitude of published studies undoubtedly demonstrated the clinical relevance of fFN testing for the assessment of patients at risk of PTD. One of the more relevant such studies was a double-blinded study that evaluated the use of fFN in patients with threatened PTL [29]. The study enrolled 763 patients and used a cut-off of 50 ng/ml. The calculated NPV for delivery within 14 days of admission was 99.2%. For patients that tested positive for fFN, the authors calculated the risk of delivery at 38.8%, although the PPV was only 13.4%. Most authors agree that the greatest value for fFN testing in symptomatic preterm patients is its high NPV with the potential to reduce unnecessary intervention.

Conclusion

The best statistical model for predicting PTL in the present study was to use a combination of the phIGFBP-1 test, a positive fFN test, cervical length less than 21.5 mm, levels of IL-6 higher than 1,305 pg/ml in the CVF, and serum levels of CRP higher than 6.1 mg/l which was excellent at identifying the patients that were to deliver within 14 days of admittance.

The combination of these tests performed better than each individual test in the present population and decreased the false positive rate, which in turn reduced the chances for inappropriate patient treatment, bringing down the costs. Still, the present study was hindered by a small sample size and burdened by recruiting only high-risk symptomatic patients which influenced the results.

The study is only the beginning of this type of research in the present population. Further research is required in terms of the evaluation of cost-benefits of using such tests to prevent subsequent unnecessary interventions in the low-risk group, as well as achieve the benefits from such intervention in the high-risk groups of patients.

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