Michela Centra*, Giuliana Coata, Elena Picchiassi, Luisa Alfonsi, Samanta Meniconi, Vittorio Bini, Maria Rosaria Di Tommaso, Mauro Cozzolino, Fabio Facchinetti, Francesca Ferrari, Maria Teresa Gervasi, Silvia Rusconi, Tullia Todros, Valentina Frisina, Nicola Rizzo, Maria Bisulli and Gian Carlo Di Renzo

Evaluation of quantitative fFn test in predicting the risk of preterm birth

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Abstract

Objective: To evaluate diagnostic accuracy of quantitative fetal fibronectin (qfFN) test in predicting preterm birth (PTB) risk <34 weeks' gestation or within 14 days from testing. We explored the predictive potential of the test in five-predefined PTB risk categories based on predefined qfFN thresholds (<10, 10–49, 50–199, 200–499 and ≥500 ng/mL).

Methods: Measurement of cervicovaginal qfFN with Rapid fFN 10Q System (Hologic) in 126 women with singleton pregnancy (23–33 weeks' gestation) reporting signs and symptoms indicative of preterm labour (PTL).

Results: For PTB prediction risk <34 weeks' gestation, sensitivity decreased from 100% to 41.7% and specificity increased from 0% to 99.1% with increasing fFN thresholds. Positive predictive value (PPV) increased from 9.5% to 83.3% with increasing qfFN thresholds, while negative predictive value (NPV) was higher than 90% among the fFN-predefined categories. Diagnostic accuracy results showed an area under a receiving operator characteristic (ROC) curve of 84.5% (95% CI, 0.770–0.903). For delivery prediction within 14 days from the testing, sensitivity decreased from 100% to 42.8% and specificity increased from 0% to 100% with increasing fFN thresholds. Diagnostic accuracy determined by the ROC curve was 66.1% (95% CI, 0.330–0.902).

Conclusions: The QfFN thresholds of tests are a useful tool to distinguish pregnant women for PTB prediction risk <34 weeks' gestation.

Keywords: Preterm birth prediction (PTB); quantitative fetal fibronectin (qfFN); symptomatic women.

***Corresponding author: Michela Centra,** Department of Obstetrics and Gynecology, University of Perugia, S. Andrea delle Fratte, 06132 Perugia, Italy, Tel.: +390755853528, Fax: +390755287182, E-mail: michela.centra@libero.it

Giuliana Coata, Elena Picchiassi, Luisa Alfonsi, Samanta Meniconi and Gian Carlo Di Renzo: Department of Obstetrics and Gynecology, University of Perugia, Perugia, Italy

Vittorio Bini: Department of Internal Medicine, University of Perugia, Perugia, Italy

Maria Rosaria Di Tommaso and Mauro Cozzolino: Department of Health Sciences, University of Florence, Florence, Italy

Fabio Facchinetti and Francesca Ferrari: Mother-Infant Department, University of Modena and Reggio Emilia, Modena, Italy

Maria Teresa Gervasi and Silvia Rusconi: Department of Obstetrics and Gynecology, University of Padua, Padua, Italy

Tullia Todros and Valentina Frisina: Department of Surgical Sciences, University of Turin, Turin, Italy

Nicola Rizzo and Maria Bisulli: Department of Obstetrics and Gynecology, University of Bologna, Bologna, Italy

Introduction

Preterm birth (PTB), occurring in approximately 11% of pregnancies, is the primary cause of perinatal death and neonatal morbidity and remains one of the most critical problem in obstetrics [1]. Although all births before 37 weeks of gestation are defined as preterm, the highest rate of morbidity and mortality occurs for deliveries before 34 weeks of gestation. Preterm labour (PTL) signs and symptoms usually used to identify women at risk for PTB are poor predictive indicators for an imminent delivery [2]. So PTB identification is an arduous problem for clinicians and it is crucial to be able to distinguish pregnant women at risk from those not at risk for PTB in order to improve neonatal morbidity and mortality through a successful pharmacological treatment and to reduce healthcare costs.

Several diagnostic biophysical and biochemical methods have been suggested for predicting PTB. Among biochemical markers, fetal fibronectin (fFN), detectable in cervicovaginal fluid, is one of the most studied and utilised tests in the clinical practice. fFN is an adhesive glycoprotein (450 kDa) that acts as a bonding agent for the maternal-fetal layer binding the maternal membranes to the fetal membranes. In pregnancy, cervicovaginal fFN is detectable during the first 22 weeks of gestation, is

undetectable between 22+0 and 34+0 weeks of gestation and again detectable after 34 gestational weeks until term. fFN has been shown to be a strong predictor for PTB in cervicovaginal fluid between 22 and 34 weeks' gestation, [3–5], however, recently, it has been demonstrated that fFN is valuable also from 18 weeks of gestation [6].

The most used fFN bedside test (Rapid fFN TLI_{IQ}; Hologic, Marlborough, MA, USA) is a qualitative test that provides a positive or negative result based on a single fFN threshold of 50 ng/mL, although limited data regarding the selection and the accuracy of this cut-off point are available. It follows that the test in symptomatic women has a good negative predictive value (NPV) >90% [7–12], but has a low positive predictive value (PPV) from <20% to 40% [7, 13, 8, 11] for predicting delivery before 34 weeks' gestation or within 14 days from testing with the consequence that a high number of pregnant women with a positive qualitative fFN test will not deliver preterm.

Some studies showed that PTB risk increases with increasing cervicovaginal fFN concentration [9, 14, 15] and that the quantitative measurement of the fFN level could improve the accuracy in identifying women at risk of PTB. It has also been observed that introducing pre-specified threshold concentrations of fFN could further increase the potential prediction of the fFN test [14, 16–18].

The purpose of the present study was to evaluate the accuracy of a new bedside quantitative fFN test (qfFN testing; Rapid fFN 10Q System, Hologic), used between 23+0 and 34+0 weeks of gestation, in predicting women at risk for PTB before 34 weeks' gestation or within 14 days from testing. We explored the predictive potential of the test in five predefined PTB risk categories, based on predefined fFN threshold concentrations; PTB risk classes were classified from low (fFN<10 ng/mL) to high PTB risk (fFN≥500 ng/mL) (Table 1).

Patients

We performed a prospective multicentre study to evaluate whether the quantitative measurement of cervicovaginal fFN, using a new bedside quantitative Rapid fFN 10Q System (Hologic), increases the diagnostic accuracy for PTB in pregnant women with signs and symptoms suggestive of PTL.

Table 1: PTB risk categories based on fFN concentrations.

Risk categories	fFN concentrations
1 – Low	<10 ng/mL
2 – Lower middle	10–49 ng/mL
3 – Middle	50–199 ng/mL
4 – Upper middle	200–499 ng/mL
5 – High	≥500 ng/mL

The multicentre study was conducted in six Italian hospitals (Perugia, Florence, Turin, Bologna, Modena and Padua). The study-coordinating centre of Perugia validated the accuracy of data entry using supporting documentation from participating centres.

We prospectively enrolled 146 women with singleton pregnancy between 23+0 and the 33+0 weeks of gestation with self-reported signs and symptoms indicative of PTL. Gestational age was calculated on the last menstrual period and then confirmed by ultrasound.

The recruited patients reported signs and symptoms of PTL, as regular uterine contractions with or without pain, low abdominal cramping, low back pain and pelvic pressure. Besides, they presented clinically intact amniotic membranes as determined by speculum examination.

The Local Ethics Committee approved the study and informed written consent was obtained from each pregnant woman before the enrolment.

According to the protocols of each hospital, all recruited patients underwent a full clinical examination, including cervical length, cervical dilatation, amniotic membrane status and patient history investigation.

Pharmacological treatments such as atosiban, corticosteroids and magnesium sulfate were administered to all pregnant women enrolled in the study.

Materials and methods

Cervicovaginal sample for qfFN analysis was taken before cervical-vaginal examination. According to the fFN test manufacturer's instructions and the hospital protocols, we excluded patients with cervical dilation >3 cm, rupture of amniotic membranes, cervical cerclage, known or suspected placenta praevia, vaginal bleeding, twin or multiple pregnancy, sexual intercourse within 24 h and pregnant women with previous vaginal examination. Cervicovaginal fluids were sampled by using a Rapid fFN 10Q Specimen Collection Kit (Hologic, Marlborough, MA, USA) composed of a sterile polyester swab and an extraction buffer vial. The swab was gently inserted into the vaginal cavity at the level of vaginal fornix for 10 s and then inserted into the collection vial containing the extraction buffer. We eliminated all the samples with blood-stained swabs because of interference with fFN measurement.

After removing the swab from the vial, 200 μL of sample was immediately put in a Rapid fFN 10Q cassette (Hologic) and analysed, using the Rapid fFN 10Q analyser (Hologic), and results of the concentration of fFN were obtained in 10 min by TLilQ Analyzer software, version 2.0.0. The analyser detects a range of fFN concentrations from 0 to 500 ng/mL. Concentration of fFN higher than 500 ng/mL is shown as >500 ng/mL. The

internal controls were assessed using the Control Kit Rapid fFN (Hologic) which consists of a negative control for fFN concentration <50 ng/mL and a positive control for fFN concentration >50 ng/mL.

Results of fFN quantification, expressed in ng/mL, were grouped into five pre-specified incremental categories of PTB risks (Table 1, <10, 10–49, 50–199, 200–499 and ≥500 ng/mL), as described by Abbott et al. [18].

Results of the qfFN test were blinded to the clinician's management decisions.

For each patient, results of fFN concentrations and clinical data including demographic, obstetric and gynaecological characteristics were entered in a database.

We followed all the pregnancies until delivery and the test-to-spontaneous delivery interval was documented. Time to delivery was defined as the interval between the time and date when results of the qfFN test were performed and the time and date of spontaneous term or preterm delivery.

Statistical analysis

The diagnostic accuracy of fFN levels in predicting PTB was determined using receiver operating characteristic (ROC) curves, with cut-offs corresponding to the best combination of high sensitivity and high specificity. A preliminary power analysis indicated that a sample of 10 from the positive group and 100 from the negative group achieve 82% power to detect a difference of 0.27 between the area under the ROC curve (AUC) under the null hypothesis of 0.50 and an AUC under the alternative hypothesis of 0.77 using a two-sided z-test at a significance level of 0.05. Areas of ROC curves were compared with the Delong method [19].

The Shapiro-Wilk test was used to assess the normal distribution of variables and the Mann-Whitney test and the χ^2 test were used for comparisons of continuous and categorical variables, respectively. For an easier understanding, data are presented as mean±SD.

To calculate the odds ratios (ORs) linked to fFN levels, logistic regression (LR) models were fit for the prediction of PTB at delivery; the goodness of fit of LR models was checked using the Hosmer and Lemeshow test.

All statistical analyses were performed using IBM-SPSS® version 22.0 (IBM Corp., Armonk, NY, USA, 2011). A two-sided P-value <0.05 was considered significant.

Results

A total of 126 women with singleton pregnancy who met the inclusion criteria were included in the study. We excluded 20 cases because we did not obtain pregnancy outcome.

Twelve women (9.5%) delivered before 34 weeks of gestation. Mean (±SD) gestational age at the time of qfFN testing for women who delivered before 34 weeks' gestation was 27.42±4.31 and for those who delivered after 34 weeks of gestation was 28.97±2.91 (P=0.2). Mean (±SD) gestational age at delivery was 29.83±2.88 and 38.08±1.96 weeks (P≤0.001) before and after 34 weeks of gestation, respectively.

Of the women 80.16% had spontaneous onset of labour and 19.84% had pre-labour caesarean delivery.

Cervical length, at the time of qfFN testing was 1.28±0.6 cm in women who delivered before 34 weeks of gestation and 2.47±0.99 cm (P≤0.001) in those who delivered after 34 weeks. The diagnostic accuracy for delivery prediction of the cervical length determined by a ROC curve showed that the area under the ROC curve was 0.837 (95% CI, 0.760–0.898). The cervical length with a cut-off of 15 mm has a sensitivity of 66.67%, a specificity of 84.07%, a NPV of 96.94% and a PPV of 25%.

Concentrations of fFN were obtained using the Rapid fFN 10Q System. In the two groups of women who delivered before and after 34 weeks of gestation, fFN concentrations were 321.67±215.24 ng/mL and 66.82±107.29 ng/mL, respectively (P≤0.001). Based on fFN concentrations, in Table 2 we reported numbers and percentages of women with fFN results falling in each predefined fFN categories for PTB risks. We found the largest number of women

Table 2: Distribution of spontaneous birth within fFN predefined PTB risk categories, before and after 34 weeks' gestation.

	Patients n	1 – Low (<10 ng/mL)	2 – Lower middle (10–49 ng/mL)	3 – Middle (50–199 ng/mL)	4 – Upper middle (200–499 ng/mL)	5 – High (≥500 ng/mL)
		n (%)	n (%)	n (%)	n (%)	n (%)
Pregnant women with delivery <34 weeks' gestation	12	1 (8.3%)	1 (8.3%)	2 (16.7%)	3 (25.0%)	5 (41.7%)
Pregnant women with delivery >34 weeks' gestation	114	47 (41.2%)	31 (27.2%)	24 (21.1%)	11 (9.6%)	1 (0.9%)

Data are expressed as n (%).

who delivered before 34 weeks of gestation (83.4%) was within categories 3–middle, 4–upper middle and 5–high of the fFN-predefined categories (fFN≥50 ng/mL) with the highest percentage for the 5–high class of risk (41.7%). Furthermore, the largest number of women who delivered after 34 weeks of gestation (68.4%) was within categories 1–low, and 2–lower middle of the fFN-predefined categories (fFN≤50 ng/mL) (P<0.001).

The diagnostic accuracy for delivery prediction of the qfFN testing was determined by a ROC curve (Figure 1). Results showed that the area under the ROC curve was 0.845 (95% CI, 0.770–0.903), suggesting a good diagnostic accuracy of the Rapid fFN 10Q System.

The best cut-off among different fFN concentrations resulted in fFN concentration ranging from 200 to 499 ng/mL corresponding to risk categories 4–upper middle. Based on this cut-off point the qfFN test showed a good sensitivity of 66.67%, high specificity and NPV of 89.47% and 96.2%, respectively, and a discrete PPV of 40%.

In Table 3, we reported the results of sensitivity, specificity, NPV and PPV of qfFN testing for each of the five fFN-predefined categories. Specifically, we observed that sensitivity decreases, from lower to higher categories with a value ranging from 100% (class 1) to 41.7% (class 5). The test showed a good specificity that increases with increasing fFN concentrations, from low-fFN concentrations with values of 0% (class 1) to high-fFN concentrations with values of 89.5% and 99.1% (categories 4 and 5). NPV was higher than 90% among fFN-predefined categories, while PPV increases as function of fFN concentrations within predefined categories, from 9.5% for 1–low class to 83.3% for the 5–high class.

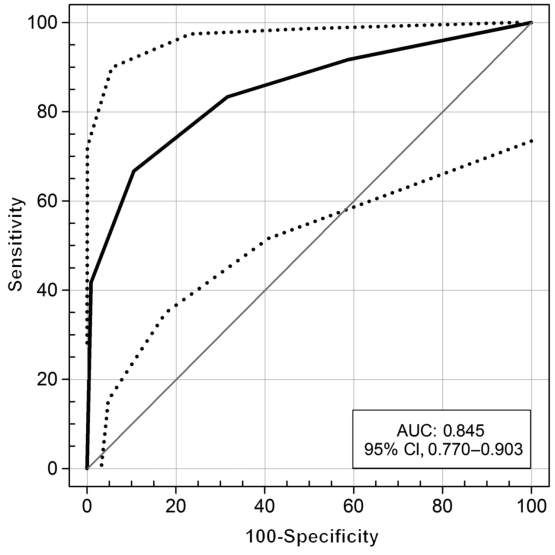

Figure 1: Receiver operating characteristic curve analysis of diagnostic accuracy of qfFN testing for prediction of spontaneous preterm delivery at <34 weeks' gestation.

The diagnostic accuracy of the qfFN test and of the cervical length for the prediction of PTB did not reveal a significant difference as demonstrated by the comparison of the areas under the ROC curves (0.008, 95% CI, 0–0.100, P=0.839).

Odds ratio derived from logistic regression was 1.009 (95% CI, 1.005–1.013), indicating that for each increased ng/mL of fFN concentration, the risk of PTB increase of 1.009 times.

We found that 11 out of 126 women delivered within 14 days from the testing and among these seven delivered before and four delivered after 34 weeks of gestation.

Based on fFN concentrations, in Table 4 we reported numbers and percentages of women who delivered within 14 days from qfFN testing for each predefined fFN categories for PTB risks. We found the largest number of women who delivered within 14 days (81.9%) from fFN testing was within categories 3–middle (n. 3), 4–upper middle (n. 3) and 5–high (n. 3) of fFN-predefined categories (fFN≥50 ng/mL).

We also determined the diagnostic accuracy of the test for the delivery prediction within 14 days from the testing by a ROC curve analysis (Figure 2). The area under the ROC curve was 0.661 (95% CI, 0.330–0.902) showing a low diagnostic accuracy in the prediction of preterm delivery within 14 days.

In Table 5, we reported the results of sensitivity and specificity of qfFN test for the delivery prediction within 14 days from testing for each fFN-predefined categories. In detail, we observed that sensitivity decreases, from lower to higher categories with a value ranging from 100% (class 2–lower middle) to 42.86% (class 5–high) and specificity increases, from low fFN concentration with values of 0% (class 2–lower middle) to high fFN concentration with values of 50% and 100% (categories 4–upper middle and 5–high).

As qfFN test results were masked to clinicians, pharmacological treatments were administered to all the pregnant women enrolled in the study. Pharmacological treatments administered to pregnant women include tocolytics, corticosteroids and tocolytics combined with corticosteroids, as reported in Table 6.

Discussion

The bedside qualitative fFN testing is a useful tool to identify pregnant women at risk for PTB. The presence of fFN in the cervicovaginal secretions in pregnancy has been

Table 3: Prediction of PTB at <34 weeks' gestation.

Predictive variable	1 – Low (<10 ng/mL)	2 – Lower middle (10–49 ng/mL)	3 – Middle (50–199 ng/mL)	4 – Upper middle (200–499 ng/mL)	5 – High (≥500 ng/mL)
Sensitivity, %	100	91.7	83.3	66.7	41.7
95% CI	73.4–100	61.5–97.4	51.6–97.4	34.9–89.9	15.3–72.2
Specificity, %	0	41.2	68.4	89.5	99.1
95% CI	0–3.2	32.1–50.8	59.1–76.8	82.3–94.4	95.2–99.9
Negative predictive value, %	/	97.9	97.5	96.2	94.2
95% CI	/	93.9–102	94.1–100.9	92.6–99.9	90–98.4
Positive predictive value, %	9.5	14.1	21.7	40	83.3
95% CI	4.4–14.6	6.4–21.8	9.8–33.7	18.5–61.5	53.5–113.2

Data are expressed as %.

Table 4: Distribution of spontaneous birth within 14 days from fFN testing for each predefined PTB risk category.

	Total (n)	1 – Low (<10 ng/mL)	2 – Lower middle (10–49 ng/mL)	3 – Middle (50–199 ng/mL)	4 – Upper middle (200–499 ng/mL)	5 – High (≥500 ng/mL)
		n (%)	n (%)	n (%)	n (%)	n (%)
Pregnant women who delivered within 14 days from fFN testing	11	0	2 (18.2%)	3 (27.3%)	3 (27.3%)	3 (27.3%)

Data are expressed as n (%).

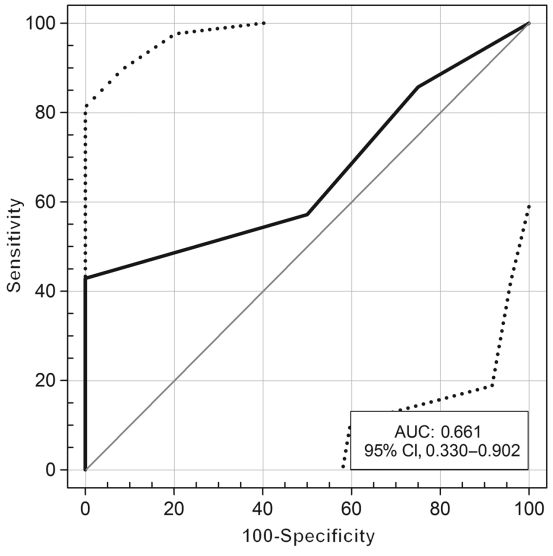

Figure 2: Receiver operating characteristic curve analysis of diagnostic accuracy of qfFN testing for prediction of spontaneous preterm delivery within 14 days of test.

definitively associated with an increased risk of PTB [7, 14, 18]. Numerous studies, however, established that the bedside qualitative fFN testing has a limited diagnostic accuracy in the prediction of spontaneous delivery in symptomatic pregnant women because it is based only on one threshold (50 ng/mL) and for this reason is prone to false-positive and negative results around that cut-off point [15]. For this reason, the testing has a good NPV, but limited PPV <20% [7, 8, 11, 13].

Some studies, which measured cervicovaginal fFN concentration using an enzyme-linked immunosorbent assay (ELISA) analysis, showed that PTB risk is proportional to cervicovaginal fFN concentration in symptomatic and asymptomatic pregnant women [14, 16, 17]. In a recent study, Abbott at al. [18] measured the level of fFN in cervicovaginal fluids of symptomatic woman at risk for PTB, evaluating different fFN thresholds (from 10 to 500 ng/mL). They observed that increasing levels of fFN in cervicovaginal fluids increase the risk for PTB and concluded that quantitative information added its value in predicting PTB.

In the present multicentre study, we evaluated the new bedside qfFN testing (Rapid fFN 10Q System, Hologic) in the prediction of PTB before 34 weeks of gestation or within 14 days of testing in symptomatic pregnant women enrolled between 23+0 and 33+0 weeks of gestation. We measured the entire range of cervicovaginal fFN concentration from 0 to 500 ng/mL and the results were then stratified in five predefined PTB risk categories, in order to assess the diagnostic accuracy of the test for each class. We examined the range from 23+0 to 34+0 weeks of gestation because in this period fFN in cervicovaginal fluid is usually absent and when the fluid is present it is a powerful predictor of PTB [3–5, 20].

Table 5: Prediction of PTB within 14 days from fFN testing.

Predictive variable	1 – Low (<10 ng/mL)	2 – Lower middle (10–49 ng/mL)	3 – Middle (50–199 ng/mL)	4 – Upper middle (200–499 ng/mL)	5 – High (≥500 ng/mL)
Sensitivity, %	/	100	85.7	57.14	42.86
95% CI	/	58.9–100	42.2–97.6	18.8–89.6	10.4–81.2
Specificity, %	/	0	25	50	100
95% CI	/	0.0–59.8	4.1–79.7	8.3–91.7	40.2–100

Data are expressed as %.

Table 6: Pharmacological treatments administered to all pregnant women.

	Patients, n	Pregnant women given tocolytics, n (%)	Pregnant women given corticosteroids, n (%)	Pregnant women given tocolytics + corticosteroids, n (%)	Pregnant women not given drugs, n (%)
Pregnant women with delivery >34 weeks' gestation	114	10 (8.8%)	19 (16.7%)	31 (27.2%)	54 (47.4%)
Pregnant women with delivery <34 weeks' gestation	12	1 (8.3%)	3 (25%)	5 (41.7%)	3 (25%)

Data are expressed as n (%).

In the study, six Italian hospitals participated in recruiting 146 pregnant women between 23+0 and 33+0 weeks' gestation with signs and symptoms for preterm delivery. Each pregnant women underwent fFN testing by the Rapid fFN 10Q System in order to measure the fFN concentration within the cervicovaginal fluids. Based on the fFN concentration ranges, results were grouped in five-predefined PTB risk categories as described in the Material and methods section. Each predefined threshold for qfFN was used to establish the sensitivity, specificity, PPV and NPV for spontaneous delivery before 34 weeks of gestation or within 14 days from the testing.

Among women who delivered at <34 weeks' gestation (9.5% of the total enrolled pregnant women), 83.4% presented a high fFN concentration corresponding to the three higher-predefined categories for PTB risk, while 16.6% of them had low fFN concentration corresponding to the lower predefined PTB risk categories.

The diagnostic accuracy of the qfFN testing for delivery prediction, calculated by a ROC curve analysis, was of 84.5%, indicating a high capacity to identify women at risk for PTB before 34 weeks' gestation. Moreover, we calculated the best threshold of the test in order to increase the PPV, and to enable the identification of true high-risk pregnant for PTB before 34 weeks' gestation that was above 200 ng/mL. The specificity increased with each threshold reaching high values for the risk categories 4 and 5 (89.5% and 99.1%, respectively). The sensitivity of the categories 4 and 5, unlike of the specificity, showed a reduction (66.7% and 41.7%, respectively), probably due to the pharmacological treatments administered to pregnant women. In fact, in this study, results of the test were blinded to the managing obstetrician and intervention (hospitalisation, tocolysis, corticosteroids) was performed based on the standard hospital practice.

The qfFN test improves the PPV for the prediction of PTB before 34 weeks, increasing with each fFN threshold (9.5, 14.1, 21.7, 40.0, 83.3%, respectively), while maintaining a high NPV, confirming the results of Abbott et al. with respect to a single fFN threshold of 50 ng/mL (21.7%, our data not shown). The comparison of diagnostic accuracy of the qfFN test and of the cervical length for the delivery prediction showed a high capacity to identify women at risk for PTB for both.

Women (81.9%) who give birth within 14 days from testing presented a high concentration of fFN corresponding to the three higher-predefined categories for PTB risk, while the 18.2% felt in the lower-middle class category; no cases were observed in the lowest class category. Diagnostic accuracy of the qfFN testing, calculated by a ROC curve analysis, was 66.1%, indicating a low capacity to identify women at risk for PTB within 14 days of testing. Sensitivity and specificity have the same trend observed for PTD delivery <34 weeks of gestation. Sensitivity showed a reduction from lower to higher categories, instead specificity increased with increasing fFN concentrations.

The results of our study are consistent with other studies [18] on quantification of cervicovaginal fFN in symptomatic pregnant women at risk for PTB. We

observed that the relationship between fFN concentration in cervicovaginal fluid and PTB risk is linear, with increased concentrations inferring a greater risk of delivery.

We observed that minimal increase in fFN (1–49 ng/mL) is associated with a low increase in risk of PTB; moderated increase in fFN (50–199 ng/mL) is associated with a moderate increase in PTB risk; finally a significant increase in fFN (200–499 ng/mL and ≥500 ng/mL) is associated with a significant increase in PTB risk. We also observed that a high cut-off, above >200 ng/mL, significantly increases the PPV (with minimal effect on the NPV) for delivery before 34 weeks of gestation. On the other hand, we obtained a poor diagnostic accuracy of the fFN within 14 days of testing probably due to the therapy administration which may have affected the outcome of pregnancy. In this study, results of the fFN test were blinded to the clinician's management decisions and intervention (hospitalisation, tocolysis, corticosteroids) was performed based on the standard hospital practice. Randomised clinical trials have found that tocolytic therapy results in a prolongation of pregnancy of 48 h to 7 days providing time for beneficial measures such as transfer to a level III perinatal centre and administration of corticosteroids for the enhancement of lung maturation [21].

In conclusion, the use of the bedside qfFN testing before 34 weeks' gestation in pregnant women with signs and symptoms of PTB allows: (1) the clinicians to adapt their decisions based on the different fFN testing thresholds; (2) the identification of true low-risk pregnancy who will be reassured that the risk of very PTB is very low because the test has a high NPV; (3) the identification of true high-risk pregnancy for PTB who needed antenatal treatment because the test has a good PPV.

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