OBSTETRICS

Evaluation of a quantitative fetal fibronectin test for spontaneous preterm birth in symptomatic women

Danielle S. Abbott, MBBS; Samara K. Radford, B Med Sci; Paul T. Seed, MSc; Rachel M. Tribe, PhD; Andrew H. Shennan, MD

OBJECTIVE: The purpose of this study was to determine whether quantification of cervicovaginal fluid fetal fibronectin (fFN) improves diagnostic accuracy of spontaneous preterm birth (sPTB) in symptomatic women.

STUDY DESIGN: A prospective blinded predefined secondary analysis of a larger study of cervicovaginal fluid fFN concentration (nanograms per milliliter) in women symptomatic of preterm labor (n =300 women; 22-35 weeks' gestation) with a Hologic 10Q system (Hologic, Marlborough, MA). Clinicians were blinded to the result until after the delivery, but the qualitative Hologic TLI_{IQ} fFN result was made available.

RESULTS: The positive predictive value for sPTB (<34 weeks' gestation) increased from 19%, 32%, 61%, and 75% with increasing thresholds (10, 50, 200, and 500 ng/mL, respectively). Compared with <10 ng/mL fFN, the relative risk of delivery was 5.6 (95% confidence interval [CI], 1.05–29.57), 7.9 (95% CI, 1.38–45.0), 22.8 (95% CI, 3.84–135.5), and 51.3 (95% CI, 12.49–211.2; $P < .01$).

CONCLUSION: Quantitative fFN provides thresholds (10 and 200 ng/mL) in addition to the qualitative method (50 ng/mL) to discriminate the risk of sPTB in symptomatic women.

Key words: fetal fibronectin, preterm birth

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Spontaneous preterm birth (sPTB) remains a challenging problem in obstetrics and is associated with significant neonatal morbidity and death.[1] Contractions and preterm labor symptoms are poor indicators in symptomatic women as to who will actually deliver preterm.[2] Accurate discrimination of those women who are at greatest risk would facilitate treatment, including targeted administration of steroids or in utero transfer, thereby potentially reducing neonatal death.[3,4] There is a paucity of accurate tests to identify symptomatic pregnant women who are at highest risk of delivery, but the most commonly used test is a qualitative bedside test (Rapid fFN TLI_{IQ}; Hologic, Marlborough, MA) that analyses cervicovaginal fetal fibronectin (fFN). FFN is a glycoprotein found in amniotic fluid, placental tissue, and the extracellular component of the decidua basalis adjacent to the placental intervillous space. It is released after mechanical- or inflammatory-mediated damage to the membranes or placenta before birth.[5] Cervicovaginal fluid fFN has a high negative predictive value for delivery in symptomatic women within 2 weeks of testing[6,7] and has been established as a useful test in women who have symptoms that are suggestive of preterm labor. Positive prediction, however, is modest (<20%), and because most women have a good outcome, many are over treated.[6]

The current qualitative test provides positive or negative result based on a threshold of 50 ng/mL.[8] Qualitative tests that are based on a single threshold are prone to increasing false-positive/-negative results around the threshold, and clinicians do not equally weigh false-positive and false-negative tests. Studies that have used enzyme-linked immunosorbent assay (ELISA)–based quantification of fFN suggest that concentrations of fFN within cervicovaginal secretions correlate with the risk of sPTB and that a knowledge of the fFN concentration may improve prediction.[9,10,11] The aim of this study was to evaluate a novel bedside quantitative system and to ascertain the predictive potential of prespecified threshold concentrations of fFN for the prediction of sPTB.

Materials and Methods

This was a prospective observational blinded study that involved a consecutive series of 300 symptomatic women with singleton pregnancies who underwent fFN sampling between 22^{+0} and 35^{+6} weeks' gestation for symptoms that are suggestive of threatened preterm labor. Such women had all presented themselves to an emergency assessment unit with symptoms of threatened preterm labor that the attending clinician believed warranted an fFN test. According to hospital protocol and the licensing recommendations on fFN testing, women with previous vaginal examination, sexual intercourse (within 24 hours), cervical dila-

From the Division of Women's Health, King's College London, Women's Health Academic Centre, Kings Health Partners, St. Thomas' Hospital, London, England, UK.

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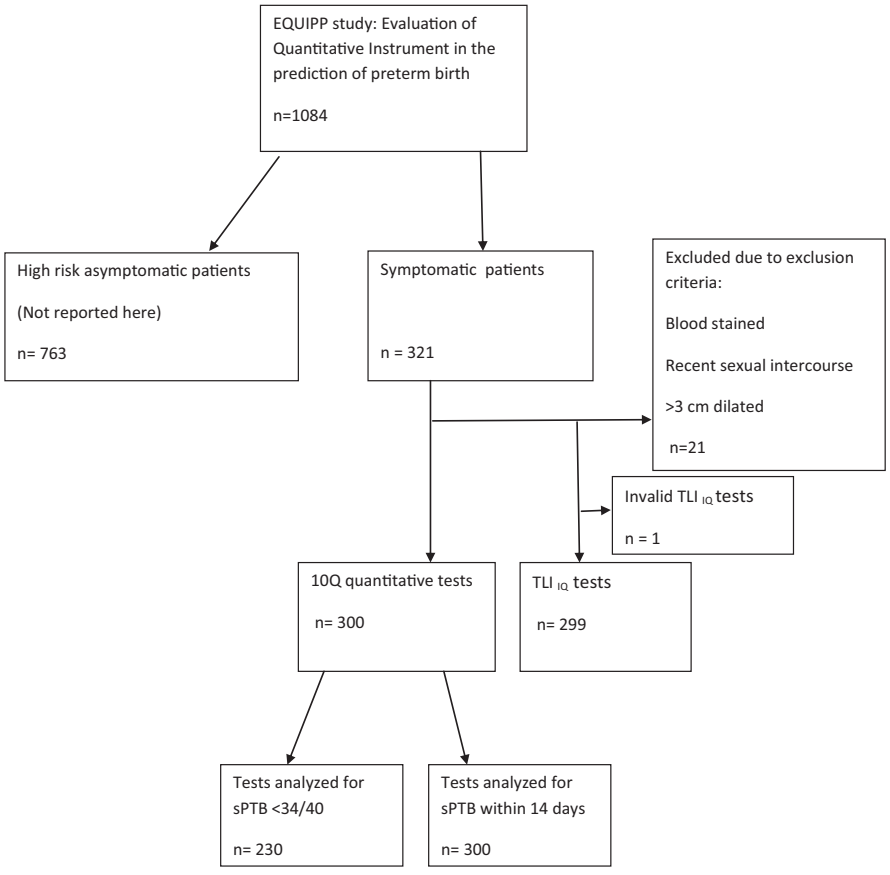
FIGURE 1
Flow diagram of participants demonstrating those excluded



Standards for the reporting of diagnostic accuracy studies (*STARD*) flow diagram illustrates the number of participants who were involved in study and those who were excluded according to defined exclusion criteria.

sPTB, spontaneous preterm birth.

TLIIQ; Hologic, Marlborough, MA.

Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

tion >3 cm, frank bleeding, or rupture of membranes (on speculum examination) were excluded from the study.

The study was conducted from October 2010 through April 2012 at 5 hospitals in the United Kingdom. This investigation was a predefined substudy of evaluation of a quantitative instrument for prediction of preterm birth, which is a study of quantitative fFN for the prediction of sPTB and which was undertaken over the same period of recruitment. The study was approved by the South East London Research Ethics Committee, and all local research ethics committees that were associated with participating centers. Informed written consent was obtained from all participants. Gestational age was confirmed by early obstetric ultrasound examination.

Women with blood-stained swabs were excluded from analysis because of interference with fFN measurement. One aliquot (200 μL) of the sample was analyzed with the conventional qualitative Rapid fFN TLI_{IQ} analyzer (Hologic), and another sample was analyzed with the quantitative Rapid fFN 10Q analyzer (Hologic) according to manufacturer's instructions.

The reliability of the Rapid 10Q analyzer that was measured at 2 concentrations of fFN (53 and 156 ng/mL) demonstrates precision as 5.9% and 7.5% and accuracy as 98.1% and 93.1%, respectively. Experiments that were performed during product development confirmed a good correlation between ELISA and 10Q tests (slope = 0.97; r^2 = 0.82; personal communication, Hologic with Jerome Lapointe, September 2010).

All clinicians were trained in the use of the fFN analyzers, and the 2 tests were run concurrently. Categoric TLI_{IQ} data (positive/negative) were provided to clinicians, but 10Q results remained double-blinded to the patient and clinician (a random result code was generated by the analyzer) until after delivery. Thresholds of 10, 50, 200, and 500 ng/mL were predefined before analysis, based on the literature.[10] Statistical analysis was performed with STATA software (version 11.2; StataCorp LP, College Station, TX). Descriptive characteristics were calculated for baseline demographics. Thresholds for fFN were used to establish sensitivity, specificity, positive predictive value, and negative predictive value for spontaneous delivery within 14 days (primary endpoint) and a predefined outcome of delivery at <34 completed weeks' gestation; receiver operator characteristic curves were generated. Results of fFN quantification were then grouped into the 5 prespecified incremental categories (0-9, 10-49, 50-199, 200-499, and ≥500 ng/mL), and the corresponding sPTB rates were calculated. The relative risk (relative to fFN of 0-9 ng/mL category) and exact 95% confidence intervals were calculated. A χ^2 test was used to determine statistical significance between fFN categories.

The category (0-9 ng/mL) was selected because the 10Q analyzer displayed a lower limit of detection than ELISA and was therefore equivalent to previous reports of "zero" with ELISA in terms of prevalence. Greater than 500 ng/mL was defined as the highest category because this was the upper limit to be reported by the 10Q analyzer and was anticipated to be prevalent in a symptomatic population.

Secondary outcomes that were analyzed included steroid administration and particularly evaluated steroid administration within 2 weeks of delivery. When the predictive value of each fFN concentration category for outcome <34 weeks' gestation was assessed, all tests between 33^{+0} and 33^{+6} weeks' gestation were excluded from the analysis to re-

TABLE 1
Demographic and obstetric characteristics of symptomatic women (n = 300)

Characteristic	Value
Age, y[a]	29 ± 6
Body mass index, kg/m^2	26 ± 6
Ethnicity, n (%)	
White	153 (53)
Black	94 (31)
Other	53 (16)
Previous preterm birth, n (%)	55 (18)
Previous premature prelabor rupture of membranes, n (%)	12 (4)
Previous second-trimester miscarriage, n (%)	15 (5)
Previous cervical surgery, n (%)	15 (5)
Smoking history, n (%)	
Current	37 (12)
Exsmoker	38 (13)
Never	223 (76)
History of domestic violence, n (%)	29 (14)
History of recreational drugs, n (%)	5 (2)

[a] Data are given as mean ± SD.

Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

TABLE 2
Prediction of spontaneous preterm birth within 14 days

	Fetal fibronectin threshold			
Predictive variable	10 ng/mL	50 ng/mL	200 ng/mL	500 ng/mL
Sensitivity, %	82.4	76.5	58.8	35.3
Specificity, %	59.3	81.1	93.9	97.5
Negative predictive value, %	98.2	98.3	97.4	96.1
Positive predictive value, %	10.9	19.7	37.0	46.2
Likelihood ratio				
Plus	2.02	4.04	9.69	14.12
Minus	0.30	0.29	0.44	0.66
Receiver operator characteristic curve area	0.71	0.79	0.76	0.66
Relative risk (relative to fetal fibronectin 0-9 ng/mL)	0.9	4.3	16.1[a]	26[a]

[a] χ^2 test, $P < .01$.

Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

duce false-positive test results because of proximity of test to endpoint. Data from women with iatrogenic delivery before the gestation of interest were also excluded. This analysis is of a subgroup of symptomatic patients from the larger study of quantitative fFN, which was powered on asymptomatic patients (Figure 1). No formal power calculation was performed therefore for this subgroup analysis. Because this is the first study to evaluate the quantitative fFN system, the study was carried out in accordance to guidelines for the evaluation of the performance of a new diagnostic test, and standards for the reporting of diagnostic accuracy studies criteria were adhered to throughout (Figure 1).[12]

RESULTS

Demographic and obstetrics characteristics for the study participants are described in Table 1. A total of 300 women with singleton pregnancies were eligible for analysis (21 women were removed according to the listed exclusion criteria; Figure 1). The mean gestational age of testing was 29^{+4} weeks' gestation (SD, 3.82 weeks), and 5.7% of the study population delivered spontaneously within 14 days of testing. There were no adverse events related to the test. Overall, there was a sPTB rate of 8.7% at <34 weeks' gestation and 12% at <37 weeks' gestation.

The median gestational age at delivery was 38^{+5} weeks' gestation (SD, 3.22). Of the 300 participants, 209 of the participants (70%) had a spontaneous onset of labor; 91 women (30%) underwent induction or had a prelabor cesarean delivery. Concentrations of fFN were obtained for all women with the use of the 10Q system. A TLI_{IQ} test was unsuccessful for 1 case; of the 299 TLI_{IQ} tests that were performed, 67 patients (22%) had a positive cervicovaginal fluid fFN result.

Stepwise logistic regression confirmed which of the preselected cutoffs had the greatest predictive power ($R^2 = 0.13$; $P = .02$) for delivery at <34 weeks' gestation. These were <10 ng/mL (very-low risk) and ≥200 ng/mL (high-risk). The ≥50 ng/mL threshold was retained because it is the standard threshold that is used for the TLI_{IQ} test. The threshold of ≥500 ng/mL was included because it was the upper limit of the device. Tables 2 and 3 summarize the data for the prediction of delivery at <14 days and sPTB at <34 weeks' gestation for each of the 10Q fFN thresholds. The number of women with results falling within in each of the prespecified fFN categories is shown in Table 4. In most participants (57%), the fFN concentration was <10ng/mL.

The relative risk of a sPTB within 14 days of a test increased as fFN concentration increased, compared with the fFN 0-9 ng/mL group: fFN 10-49 ng/mL, 0.9 (95% confidence interval [CI], 0.09–8.57; $P = .93$); 50-199 ng/mL, 4.3 (95% CI, 0.91–20.6; $P = .05$); fFN 200-499 ng/mL, 16.1 (95% CI, 4.0–64.8; $P < .01$); and fFN ≥500 ng/mL, 26 (95% CI, 7.3–92.2; $P < .01$).

The rate of sPTB at <34 weeks' gestation was associated strongly with the concentration of fFN and increased from 1.5% in the lowest category (0-9 ng/mL) to 75% in the highest category (≥500 ng/mL; Table 4). The relative risk of a sPTB at <34 weeks' gestation was significantly increased in each of the higher fFN concentration categories group when compared with the fFN 0-9 ng/mL group: fFN 10-49 ng/mL, 5.6 (95% CI,1.05–29.57; $P < .01$); 50-199 ng/mL, 7.9 (95% CI, 1.38–45.0; $P < .01$); fFN 200-499 ng/mL, 22.8 (95% CI, 3.84–135.5; $P < .01$); and fFN ≥500 ng/mL, 51.3 (95% CI, 12.49–211.2; $P < .01$).

TABLE 3
Prediction of spontaneous preterm birth at <34 weeks' gestation

	Fetal fibronectin threshold			
Predictive variable	**10 ng/mL**	**50 ng/mL**	**200 ng/mL**	**500 ng/mL**
Sensitivity, %	90.0	70.0	55.0	45.0
Specificity, %	64.0	85.7	96.7	98.6
Negative predictive value, %	98.5	96.8	95.8	95.0
Positive predictive value, %	19.4	31.8	61.1	75.0
Likelihood ratio				
Plus	2.52	4.90	16.5	31.5
Minus	0.16	0.35	0.47	0.56
Receiver operator characteristic curve area	0.77	0.78	0.76	0.72
Relative risk (relative to fetal fibronectin 0-9 ng/mL)	5.6[a]	7.9[b]	22.8[b]	51.3[b]

χ^2 test.
[a] $P < .05$; [b] $P < .001$.
Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

Steroid administration within 2 weeks and <34 weeks of delivery correlated with increasing cervicovaginal fluid fFN concentration, although there were still a number of women who were given steroids antenatally with a negative fFN test result (Table 5). For prediction of delivery within 14 days and at <34 weeks' gestation, the 10Q analyzer performed more accurately then the current qualitative TLI_{IQ}, as illustrated by the receiver operation characteristic curves in Figures 2 and 3, respectively.

Comment

This is the first study, to our knowledge, to report the use of a novel bedside Rapid fFN 10Q system to quantify fFN concentrations within cervicovaginal fluid and to determine the subsequent risk of sPTB. The results clearly demonstrate that quantitative information has added value over the currently used qualitative fFN bedside test.

These data are supportive of previous reports from our group and others who reported improved prediction of preterm birth using values for the fFN concentration in symptomatic and asymptomatic high-risk[10] and low-risk women.[11] However, these were performed with the use of ELISA, which is a time-consuming and laboratory-based method, to measure cervicovaginal fluid fFN concentrations. In contrast, the Rapid fFN 10Q analyzer produces quantitative fFN results quickly (within 10 minutes), requires minimal personnel training, and can be implemented within the clinical environment.

TABLE 4
Spontaneous preterm birth rate within fetal fibronectin categories

Fetal fibronectin category, ng/mL	n (%)	Spontaneous preterm birth at <34 weeks' gestation, %	Spontaneous preterm birth at <14 days gestation, %
0-9	170 (57)	1.5	1.8
10-49	62 (21)	8.2	1.6
50-199	41 (14)	11.5	7.7
200-499	14 (5)	33	29
≥500	13 (4)	75	46

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The largest previous study of symptomatic women included 725 singleton pregnancies and used a threshold of 50 ng/mL for a positive test result that was measured by ELISA testing for fFN and demonstrated an sPTB rate of 3.9 % within 2 weeks, which was marginally lower than that found (5.7%) in this present prospective study.[6] Peacemen et al[6] reported a positive predictive value of 16% for the prediction of delivery within 2 weeks,[6] whereas we report a similar positive predictive value at a lower threshold of quantitative fFN (10 ng/mL) and a slightly higher positive predictive value at a threshold of 50 ng/mL.

In the present study, changing the threshold from the conventional 50 ng/mL to 200 ng/mL led to a 2-fold increase in the positive predictive value for the prediction of delivery at <34 weeks' gestation with minimal effect on the negative predictive value. The use of a higher threshold could allow clinicians to alter decision-making based on risk:benefit ratio for different interventions. We observed, for example, that inappropriate steroid administration correlated with the fFN concentration when clinicians were blinded to absolute values of cervicovaginal fluid fFN, which suggests that knowledge of fFN concentrations may allow steroid use to be more appropriate and targeted at women with high values of fFN and the greatest risk of a spontaneous preterm delivery. Conversely, more stringent prediction of low-risk individuals (eg, <10 ng/mL) may be used to support early discharge. Decisions regarding in utero transfer could be tailored similarly, and protocols could be developed to recommend management at specific fFN-indicated risk levels. The addition of cervical length (which currently is not measured routinely in the United Kingdom) may add further value and should be verified in future studies.

This study provides a clear demonstration that the concentration of fFN, as detected by the bedside analyzer, may have considerable impact in the relation to clinical outcome and prognosis and

TABLE 5
Steroid use without delivery within 2 and <34 weeks

	Fetal fibronectin category, n (%)				
Steroids given[a]	0-9 ng/mL (n = 37)	10-49 ng/mL (n = 19)	50-199 ng/mL (n = 22)	200-499 ng/mL (n = 10)	500 ng/mL (n = 11)
Delivery ≥2 weeks	36 (97)	19 (100)	19 (86)	7 (70)	5 (45)
Delivery ≥34 weeks	35 (95)	16 (84)	18 (82)	6 (60)	2 (18)

[a] Nonparametric trend test, $P < .0001$.

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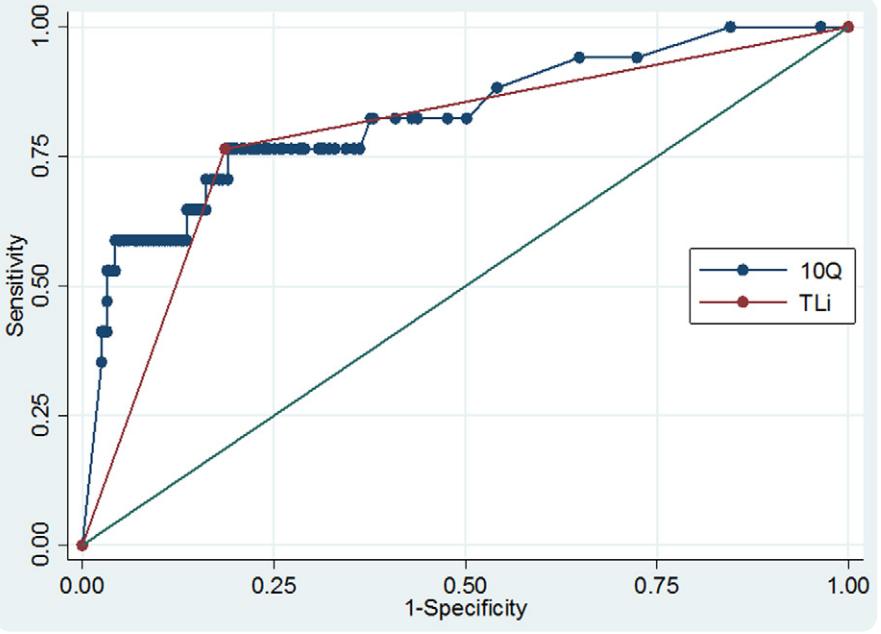
should enable clinicians to treat the patient with a personalized approach. The qualitative system (Rapid fFN TLI_{IQ} analyzer) was clinically useful for the identification of individuals with a "negative" test result; however, our results demonstrate that, even these individuals, may be at different levels of risk. Positive TLI_{IQ} test results, which may have been criticized for low prediction, may now be used more informatively with a risk of a spontaneous preterm delivery that ranges from 20-75%, according to the threshold used. Decision-making could be improved by the consideration of a positive test result beyond 200 ng/mL. Clinicians are familiar with treating patients using a continuous variable, even with single thresholds defining a disease (eg, blood pressure); therefore, a risk stratification that can alter management is likely to be welcomed and adopted in the clinical community.

The major strength of these observations is that the data were derived from a prospectively collected blinded dataset. However, the relatively small number of women with markedly elevated fFN levels (>200 ng/mL) is a potential limitation, and a larger study is indicated to provide adequate numbers of women in the higher concentration category. The sPTB rates and associated relative risks in the present study are, however, of a similar range to those observed previously with the use of quantitative fFN by ELISA in high-risk asymptomatic women.[10]

Receiver operator characteristic analysis has demonstrated consistently that the conventional 50-ng/mL threshold that was used in the current qualitative fFN test appears to be appropriate[7,11] and again is confirmed in the present study. However, the data reported here, which were obtained with the Rapid fFN 10Q combined with other approaches for the measurement of quantitative fFN, also suggest that additional discriminatory information is available when absolute concentrations of fFN are provided. Based on the results of this study, we would recommend a review of the fFN threshold that is used to predict sPTB in symptomatic women and the implementation of quantitative fFN measurement to improve positive prediction (with little sacrifice to the negative predictive value) and to aid clinical treatment. A formal evaluation of clinical outcomes and management according to different thresholds based on the quantitative analyzer is warranted. This could enable clinicians to tailor decisions and treat patients on an individual basis, which will lead to more appropriate administration of steroids, admission, or in utero transfer. ■

FIGURE 2
ROC curve demonstrates the performance of qualitative TLI_{IQ} fetal fibronectin test within 14 days

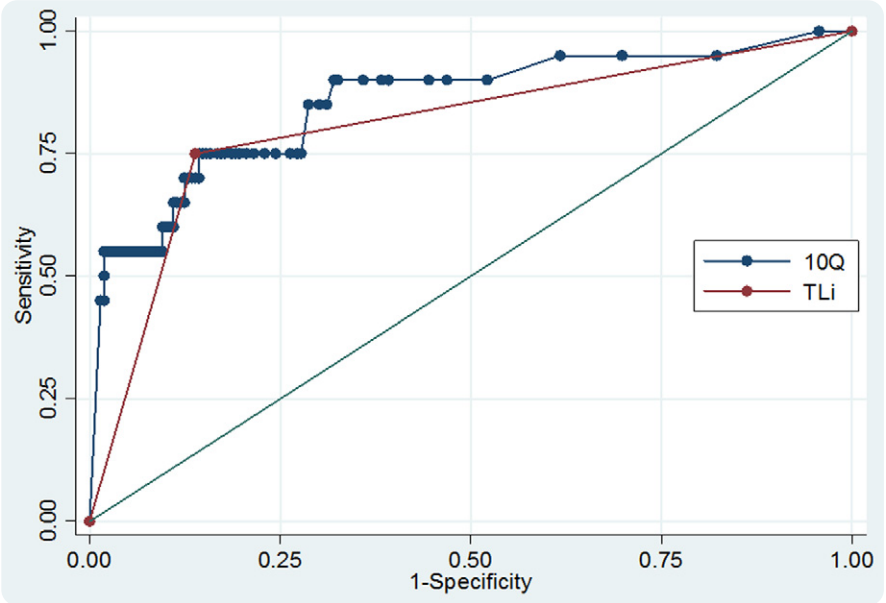


The *red circles* on the *red line* indicate 1 clear threshold of 50 ng/mL vs the quantitative fetal fibronectin 10Q test. The *blue circles* on the *blue line* indicate a range of thresholds for prediction of spontaneous preterm delivery within 14 days of test.

ROC, receiver operating characteristic.

Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

FIGURE 3
ROC curve demonstrates the performance of qualitative TLI_{IQ} fetal fibronectin test within <34 weeks



The *red circles* on the *red line* indicate 1 clear threshold of 50 ng/mL vs the quantitative fetal fibronectin 10Q test. The *blue circles* on the *blue line* indicate a range of thresholds for prediction of spontaneous preterm delivery at <34 weeks' gestation.

Abbott. Quantification of fFN in prediction of sPTB. Am J Obstet Gynecol 2013.

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