Original Research

Quantitative Fetal Fibronectin to Predict Preterm Birth in Asymptomatic Women at High Risk

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OBJECTIVE: To evaluate the diagnostic accuracy of cervicovaginal fluid quantitative fetal fibronectin, measured by a bedside analyzer, to predict spontaneous preterm birth before 34 weeks of gestation.

METHODS: We conducted a prospective masked observational cohort study of cervicovaginal fluid quantitative fetal fibronectin concentration in asymptomatic women at high risk of spontaneous preterm birth (n=1,448; 22–27 6/7 weeks of gestation) measured using a rapid bedside analyzer. The routine qualitative result (positive–negative) was made available to clinicians at the time of testing, but the quantitative result remained blinded until after delivery.

RESULTS: Spontaneous preterm birth (less than 34 weeks of gestation) increased from 2.7%, 11.0%, 14.9%, 33.9%, and 47.6% with increasing concentration of fetal fibronectin (less than 10, 10–49, 50–199, 200–499, and 500 ng/mL or greater, respectively). A threshold of 200 ng/mL had a positive predictive value of 37.7 (95% confidence interval [CI] 26.9–49.4) with specificity 96% (95% CI 95.3–97.3). Women with a fetal fibronectin concentration of less than 10 ng/mL had a very low risk of spontaneous preterm birth at less than 34 weeks of gestation (2.7%), no higher than the background spontaneous preterm birth rate of the general hospital population (3.3%). The quantitative fetal fibronectin test predicted birth at less than 34 weeks of gestation with an area under the curve (AUC) of 0.78 (95% CI 0.73–0.84) compared with the qualitative test AUC 0.68 (95% CI 0.63–0.73). Quantitative fetal fibronectin discriminated risk of spontaneous preterm birth at less than 34 weeks of gestation among women with a short cervix (less than 25 mm); 9.5% delivered prematurely less than 10 ng/mL compared with 55.1% greater than 200 ng/mL ($P<.001$).

DISCUSSION: Alternative risk thresholds (less than 10 ng/mL and greater than 200 ng/mL) improve accuracy when using quantitative fetal fibronectin measurements to define risk of spontaneous preterm birth. This is particularly relevant for asymptomatic women with a short cervix.

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Accurate identification of women at high risk of spontaneous preterm birth, the leading cause of neonatal morbidity and mortality worldwide,[1] remains

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a challenge. Prediction of women at highest risk would enable targeted surveillance and guide prophylactic intervention (eg, progesterone[2,3] or cervical cerclage[4]) or timely antenatal therapy (eg, corticosteroids[5]). Because only a minority of "high-risk" women subsequently deliver early,[6] an accurate test would provide reassurance for the majority.

Cervicovaginal fluid fetal fibronectin measurements are a good negative predictor of spontaneous preterm birth in both symptomatic[7] and asymptomatic high-risk women[8,9] after 22 weeks of gestation. Traditionally it has been a qualitative test that generates a positive–negative result based on a threshold of 50 ng/mL.[10] The positive predictive value (PPV), however, is suboptimal (less than 20%), limiting utility.[11] Furthermore, by using a single threshold for a continuous variable, important risk discrimination may be missed.

Several retrospective studies using enzyme-linked immunosorbent assay measurement of fetal fibronectin have indicated that risk of preterm birth is proportional to cervicovaginal fluid fetal fibronectin concentration.[11,12] Our recent results from women symptomatic of preterm labor have demonstrated enhanced positive prediction utilizing different thresholds.[13]

We undertook a multicentre study (Evaluation of a Quantitative Instrument for the Prediction of Preterm Birth) to prospectively evaluate the predictive accuracy of quantitative fetal fibronectin for preterm birth using a novel bedside analyzer (Rapid fFN 10Q analyser) in asymptomatic high-risk women. We hypothesized that quantification would advance prediction by providing additional risk discrimination.

MATERIALS AND METHODS

This prospective masked observational study was undertaken from October 2010 to September 2013 at five teaching hospitals in the United Kingdom. Women with singleton pregnancies and risk factors for spontaneous preterm birth, who underwent qualitative cervicovaginal fluid fetal fibronectin sampling between 22 0/7 and 27 6/7 weeks of gestation and who were asymptomatic for threatened spontaneous preterm birth were invited to participate. The inclusion criteria were one or more of previous spontaneous preterm birth or preterm rupture of membranes at less than 37 weeks of gestation, previous spontaneous second-trimester miscarriage, previous cervical surgery (large loop excision of the transformation zone, loop electrosurgical excision procedure, laser or cone excision), or an incidental finding of a cervical length of 25 mm or less in the index pregnancy. Samples from women who reported prior sexual intercourse (within 24 hours), or confirmed or suspected rupture of membranes, or who had frank bleeding visible on the swab were excluded from analysis as a result of known interference with fetal fibronectin measurement.[14]

The study was approved by the South East London Research Ethics Committee (REC no. 10/H0806/68, London, United Kingdom). Written informed consent was obtained from all participants. Gestational age was confirmed by early obstetric ultrasonography (11–14 weeks of gestation). Participant baseline demographics, obstetric history, and risk factors were entered onto an online secure study-specific database (www.medscinet.net/ptbstudies).

A series of power calculations were carried out designed to demonstrate that quantitative fetal fibronectin provided additional risk information compared with the standard threshold of 50 ng/mL. Based on previous work from our group,[9] quantitative fetal fibronectin was grouped into four prespecified incremental categories (less than 10, 10–49, 50–199, greater than 200 ng/mL), anticipated in ratios 80:10:7:3. A total sample size of 1,350 at standard levels of significance ($\alpha = 0.05$) was required to achieve 80% power to detect a difference in the rate of spontaneous preterm birth before 34 weeks of gestation of 5% and 12% in 0–9 and 10–49 ng/mL fFN categories and preterm birth rates of 23.5% and 50% in the fFN categories 50–199 and greater than 200 ng/mL.

During speculum examination, a polyester swab was inserted into the posterior fornix of the vagina (10 seconds) to collect a sample of cervicovaginal fluid. The swab was placed into the test buffer solution and analyzed immediately. One aliquot (200 microliters) of the sample was analyzed using the conventional qualitative Rapid fFN TLI_{IQ} analyzer; another 200-microliter aliquot was analyzed using the new quantitative Rapid fFN 10Q analyzer according to manufacturer's instructions. All clinicians received appropriate training to use the analyzers. The two tests were run concurrently. A qualitative result (TLI_{IQ} positive–negative) was provided to clinicians, but quantitative results (10Q) remained masked to the clinician and participant (a result code was generated by the analyzer) until all outcome data had been collected. Based on our previous research, test thresholds of 10, 50, and 200 ng/mL were predefined before study data analysis.[12,13] The reliability of the Rapid 10Q analyzer has previously been reported.[13] Cervical length measurement (mm) was taken after the swab using transvaginal ultrasonography performed by trained staff and the mean of three values used in the analysis.

Repeated fetal fibronectin (qualitative and quantitative) measurements were taken on each visit to the antenatal clinic if consent was obtained. For the purpose

of this primary analysis, the first test obtained during the gestational testing window (22 0/7–27 6/7 weeks of gestation) was used. If the sample was ineligible (eg, recent bleeding or sexual intercourse), it was excluded from analysis and the next available sample within the assessment window that fulfilled all criteria was used. If no appropriate sample was available, the patient was excluded.

Pregnancy outcome details were obtained from handheld notes, reviewed by trained research midwives, and entered onto the study database. Data entry was checked for inaccuracies contemporaneously by senior research midwives. Women were considered to have the outcome of interest (spontaneous preterm birth) if they had spontaneous onset of labor, or experienced preterm premature rupture of membranes with subsequent premature delivery. Women with iatrogenic delivery before the specific gestational endpoint under consideration (30, 34, or 37 weeks of gestation) were excluded from the analysis (n=7 less than 30 weeks of gestation, n=15 less than 34 weeks of gestation, n=41 less than 37 weeks of gestation). Ordered logistic regression confirmed no relationship between iatrogenic delivery and qfFN category (P=.457), thus excluding the possibility of iatrogenic delivery as a competing risk.

Statistical analysis was performed using Stata 11.2. Descriptive characteristics were calculated for baseline demographics. Results of fibronectin quantification were grouped into the four prespecified incremental categories and the corresponding spontaneous preterm birth rates calculated. As a result of the high prevalence of levels greater than 500 ng/mL, an additional category of 500 ng/mL or greater was introduced preanalysis. Each predefined threshold for quantitative fetal fibronectin was used to establish sensitivity, specificity, PPV, negative predictive value (NPV), likelihood ratio for spontaneous delivery before 34 weeks of gestation (primary endpoint), and predefined outcomes of delivery before 30 completed weeks of gestation and within 4 and 8 weeks of testing. Receiver operating characteristic (ROC) curves were created for each endpoint, The relative risk (RR) (relative to fetal fibronectin of less than 10 ng/mL) and exact 95% confidence intervals were calculated using binomial regression with a log link (using MQL Fisher scoring). The utility of cervical length measurement after stratification by quantitative fetal fibronectin category was explored as well as the utility of quantitative fetal fibronectin after stratification by the presence and absence of a short cervix (less than 25 mm). This study is reported in accordance with Standards for the Reporting of Diagnostic Accuracy studies.

RESULTS

A consecutive series of 1,572 women meeting the eligibility criteria for recruitment were enrolled (Fig. 1) and analysis performed once the predefined sample size was achieved. After applying exclusion criteria, 50 women who consented for the study were excluded. Analysis was performed on 95% (1,448/1,522) of women for whom outcome data were available. Demographic and obstetric characteristics for the study participants are displayed in Table 1. Valid concentrations of fetal fibronectin were obtained for all women with the use of the 10Q test and there were no adverse events reported in relation to test use. Paired qualitative TLI_{IQ} results were available for all but five women where the test was invalid or the test was not performed or documented. Clinical management was based on TLI_{IQ} result alone.

The mean gestational age at testing was 23 2/7 weeks of gestation (standard deviation 8 days) and

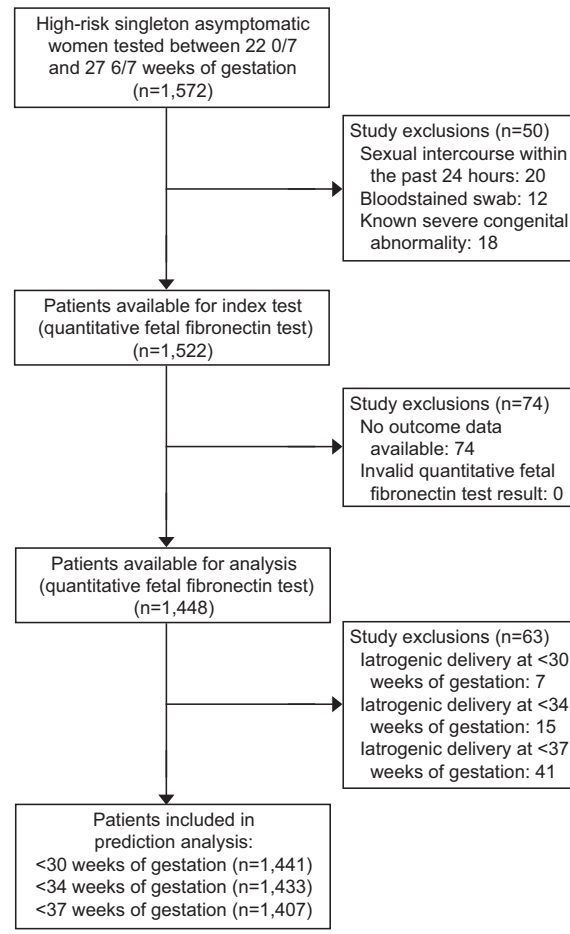

Fig. 1. Flow diagram of participants through the study.
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Table 1. Demographic and Obstetric Characteristics of High-Risk Asymptomatic Women Tested for Cervicovaginal Fluid Fetal Fibronectin Concentration (n=1,448)

Characteristic	Value
Age (y)	32±5.1
BMI (kg/m^2)	25.4±5.2
Ethnicity	
White	873 (60)
Black African/Caribbean	343 (24)
Asian	146 (10)
Other	86 (6)
Previous spontaneous preterm birth	537 (37)
Previous prelabor PROM	282 (19)
Previous 2nd-trimester miscarriage	292 (20)
Previous cervical surgery	622 (43)
Incidental short cervix (less than 25 mm)	228 (16)
Uterine anomaly	50 (3.5)
Smoking history	
Current	93 (6)
Ex-smoker	261 (18)
Never	1,094 (76)
History of domestic violence	34 (2)
History of recreational drugs	21 (1)
History of GBS (self-reported)	121 (8)

BMI, body mass index; PROM, premature rupture of membranes; GBS, group B streptococcus.
Data are mean±standard deviation or n (%).

median gestational age at testing was 22 6/7 weeks (interquartile range 22 3/7–22 5/7 weeks of gestation). The median gestation at delivery was 38 3/7 weeks of gestation (interquartile range 37 6/7–40 3/7 weeks of gestation). The spontaneous preterm birth rate was 3.1% at less than 30 weeks of gestation, 7.0% at less than 34 weeks of gestation, and 13.8% at less than 37 weeks of gestation; 30 women (2.1%) delivered within 4 weeks of testing and 62 (4.3%) delivered within 8 weeks of testing.

The proportion of women with spontaneous preterm birth according to quantitative fetal fibronectin category is shown in Table 2. In this cohort, 1,000 (nearly 70%) of fetal fibronectin concentrations were within the lowest category (less than 10 ng/mL). As fetal fibronectin concentration increased, so too did the spontaneous preterm birth rate for all gestational endpoints, increasing from 27 per 1,000 (2.7%) at less than 34 weeks of gestation in the lowest risk category (less than 10 ng/mL) to 10 of 21 (47.6%) at less than 34 weeks of gestation in the highest risk category (greater than 500 ng/mL, $P<.01$) (Table 2). This is no higher than the background spontaneous preterm birth rate at less than 34 weeks of gestation of 3.3% of all women booked for their pregnancies in St. Thomas' Hospital London (main recruiting center) in 2011.

The diagnostic accuracy of quantitative fetal fibronectin for predicting spontaneous preterm birth at less than 34 weeks of gestation and less than 30 weeks of gestation with the use of prespecified thresholds of 10 ng/mL, 50 ng/mL, 200 ng/mL, and 500 ng/mL is shown in Tables 3 and 4. A threshold of 10 ng/mL had a higher sensitivity for spontaneous preterm birth before 30 and 34 weeks of gestation than the traditional 50-ng/mL threshold for women tested between 22 0/7 and 27 6/7 weeks of gestation with a correspondingly high NPV (Table 3). Use of a higher threshold of (200 ng/mL) considerably improved the specificity of the test together with a marked improvement in PPV compared with the traditional 50-ng/mL threshold while retaining a high NPV (Table 3).

Table 2. Spontaneous Preterm Birth Rates in Asymptomatic High-Risk Women According to Quantitative Fetal Fibronectin Categories

qfFN Category (ng/mL)	n (%)	Spontaneous Preterm Birth*		
		Less Than 30 Wk of Gestation	Less Than 34 Wk of Gestation	Less Than 37 Wk of Gestation
Less than 10	1,000 (69.1)	10 (1.0)	27 (2.7)	81 (8.1)
10–49	249 (17.2)	8 (3.2)	27 (11.0)	50 (20.1)
50–199	121 (8.4)	6 (5.0)	18 (14.9)	32 (26.4)
200–499	57 (3.9)	13 (22.8)	19 (33.9)	26 (45.6)
500 or greater	21 (1.5)	8 (38.1)	10 (47.6)	11 (52.4)
Total†	1,448 (100)	45 (3.1)	101 (7.0)	200 (13.8)

qfFN, quantitative fetal fibronectin.
Data are n (%).
* All comparisons for each gestational endpoint are statistically significant ($P<.01$) except 10–49 ng/mL compared with 50–199 ng/mL and 200–499 compared with 500 or greater ($P>.1$ for all gestational endpoints).
† Women with iatrogenic deliveries before the gestation of analysis were excluded (n=7 less than 30 weeks of gestation, n=15 less than 34 weeks of gestation, n=41 less than 37 weeks of gestation).

Table 3. Prediction of Spontaneous Preterm Birth Before 34 Weeks of Gestation According to Quantitative Fetal Fibronectin Threshold (n=1,433)

	Fetal Fibronectin Threshold (ng/mL)			
Predictive Variable	10 or Greater	50 or Greater	200 or Greater	500 or Greater
Sensitivity	73.3 (63.5–81.6)	46.5 (36.5–56.7)	28.7 (20.1–38.6)	9.9 (4.9–17.5)
Specificity	72.2 (69.7–74.6)	88.7 (86.8–90.3)	96.4 (95.3–97.3)	99.2 (98.5–99.6)
PPV*	16.7 (13.3–20.5)	23.7 (18.0–30.3)	37.7 (26.9–49.4)	47.6 (25.7–70.2)
NPV*	97.3 (96.1–98.2)	95.6 (94.3–96.7)	94.7 (93.4–95.8)	93.6 (92.1–94.8)
Positive likelihood ratio*	2.64 (2.28–3.05)	4.10 (3.17–5.31)	7.97 (5.27–12.1)	12.0 (5.20–27.6)
Negative likelihood ratio*	0.37 (0.27–0.51)	0.60 (0.50–0.72)	0.74 (0.65–0.84)	0.91 (0.85–0.97)
ROC area	0.78 (0.73–0.84)			

PPV, positive predictive value; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating curve.
Data are % (95% confidence interval) unless otherwise specified.
* All comparisons for each gestational endpoint are statistically significant ($P<.01$) except 10–49 ng/mL compared with 50–199 ng/mL and 200–499 ng/mL compared with 500 ng/mL or greater ($P>.1$ for all gestational endpoints).

For prediction of delivery at less than 30 and less than 34 weeks of gestation, the quantitative fetal fibronectin test was more accurate than the TLI_{IQ} test, as illustrated by the ROC curve in Figure 2 (n=1,428 women with both TL_{IQ} and quantitative results). Quantitative fetal fibronectin was a good predictor for delivery at less than 34 weeks of gestation (area under the curve [AUC] 0.78, 0.73–0.84) and less than 30 weeks (AUC 0.81, 0.76–0.88), but with modest prediction for spontaneous preterm birth at less than 37 weeks of gestation (AUC 0.70, 0.66–0.75). In comparison, the AUC for the TLI_{IQ} test on the same women for delivery at less than 34 weeks of gestation was 0.68 (0.63–0.73, $P<.001$ by DeLong, DeLong, Clarke-Pearson test for correlated ROC curves).[15]

The RR of spontaneous preterm birth was associated with a higher fetal fibronectin concentration for all gestational endpoints when compared with fetal fibronectin concentration less than 10 ng/mL (Table 5). In particular, when compared with less than 10 ng/mL, the RR of spontaneous preterm birth for women with fetal fibronectin of 10–49 ng/mL (negative TLI_{IQ}) was 4.0 (2.4–6.7, $P<.01$). Furthermore, the RR of spontaneous preterm birth at less than 34 weeks of gestation in women with high fetal fibronectin concentration (200 ng/mL or greater) was significantly greater when compared with women with modest levels (50–199 ng/mL; 2.5 [1.5–4.2], $P<.01$).

Only 4 of 998 (0.4%) and 11 of 998 (1.1%) women with fetal fibronectin concentration of less than 10 ng/mL delivered within 4 and 8 weeks of testing, respectively, compared with 16 of 78 (20.5%) and 23 of 78 (29.5%) of women with concentrations greater than 200 ng/mL ($P<.001$). The AUC for quantitative fetal fibronectin prediction of spontaneous preterm birth within 4 and 8 weeks of testing was 0.88 (0.81–0.95) and 0.83 (0.77–0.89), respectively.

Of the cohort included and used for analysis of quantitative fetal fibronectin, 1,132 women had paired ultrasonographic transvaginal cervical length measurements. Nine hundred forty-one (83.1%) women had a cervical length greater than 25 mm, 125 (11.0%)

Table 4. Prediction of Spontaneous Preterm Birth Before 30 Weeks of Gestation According to Quantitative Fetal Fibronectin Threshold (n=1,441)

	Fetal Fibronectin Threshold (ng/mL)			
Predictive Variable	10 or Greater	50 or Greater	200 or Greater	500 or Greater
Sensitivity	77.8 (62.9–88.8)	60.0 (44.3–74.3)	46.7 (31.7–62.1)	17.8 (8.0–32.1)
Specificity	70.5 (68.0–72.9)	87.7 (85.5–89.4)	95.9 (94.7–96.9)	99.1 (98.4–99.5)
PPV	7.8 (5.5–10.7)	13.6 (9.1–19.1)	26.9 (17.5–38.2)	38.1 (18.1–61.6)
NPV	99.0 (98.2–99.5)	98.6 (97.7–99.1)	98.2 (97.4–98.9)	97.4 (96.4–98.2)
Positive likelihood ratio	2.64 (2.21–3.14)	4.9 (3.7–6.4)	11.4 (7.6–17.1)	19.1 (8.3–43.8)
Negative likelihood ratio	0.32 (0.18–0.55)	0.5 (0.3–0.7)	0.6 (0.4–0.7)	0.83 (0.72–0.95)
ROC	0.81 (0.73–0.89)			

PPV, positive predictive value; NPV, negative predictive value; ROC, receiver operating curve.
Data are % (95% confidence interval) unless otherwise specified.

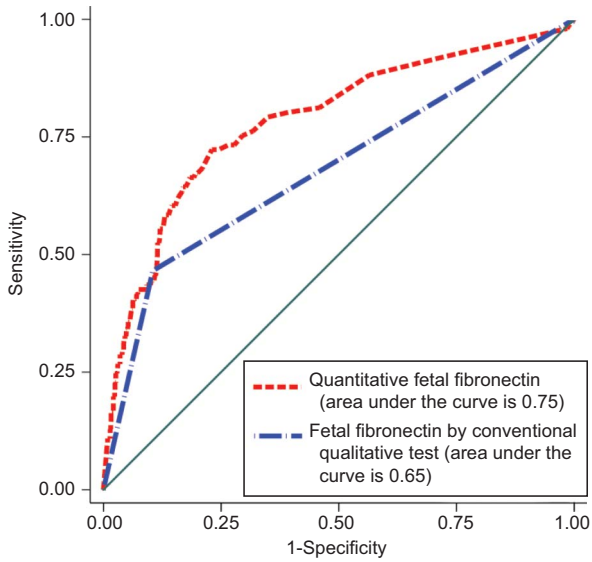

Fig. 2. Receiver operating characteristic curve to illustrate the performance of quantitative fetal fibronectin test for prediction of spontaneous preterm birth at less than 34 weeks of gestation compared with the conventional qualitative test.

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a cervical length 15–24.9 mm, and 66 (5.8%) less than 15 mm. Of the women with a short cervical length of less than 25 mm, 32 of 191 (17%), 56 of 191 (28%), and 81 of 191 (42%) delivered spontaneously before 30, 34, and 37 weeks of gestation, respectively, compared with 6 of 941 (0.6%), 32 of 941 (3.4%), and 85 of 941 (9.0%) of those without cervical shortening at the time of testing. Prediction of spontaneous preterm birth using cervical length measurements as a continuous variable had a comparable test characteristic to the use of quantitative fetal fibronectin; spontaneous preterm birth at less than 37 weeks of gestation had an AUC 0.75 (0.70–0.80), spontaneous preterm birth at less than 34 weeks of gestation an AUC 0.82 (0.77–0.87), spontaneous preterm birth at less than 30 weeks of gestation an AUC 0.90 (0.85–0.96), within 4 weeks AUC 0.94 (0.88–0.98), and 8 weeks AUC 0.86 (0.80–0.92).

Table 6 illustrates the proportion of women who delivered prematurely, stratified according to cervical length measurement and quantitative fetal fibronectin category. For women with a short cervix (less than 25 mm) on ultrasound scan, the PPV for spontaneous preterm birth at less than 34 weeks of gestation increased from 9.5% for those with low cervicovaginal fluid quantitative fetal fibronectin concentrations less than 10 ng/mL compared with 55% for those with concentrations greater than 200 ng/mL (χ^2 test $P \leq .001$). This was even more pronounced for women with the shortest cervical length (less than 15 mm, n=66); in this group, the risk of spontaneous preterm birth at less than 34 weeks of gestation was 18% (3/16) for women with quantitative fetal fibronectin less than 10 ng/mL compared with 78% (21/27) for those with quantitative fetal fibronectin greater than 200 ng/mL (χ^2 test $P \leq .001$). Conversely, for those women with high quantitative fetal fibronectin concentrations (greater than 200 ng/mL), the PPV for spontaneous preterm birth at less than 34 weeks of gestation was increased from 3.5% if the cervix was found to be long (25 mm or greater) to 55.1% with a concurrently diagnosed short cervix (less than 25 mm) (χ^2 test $P \leq .001$).

In this group of women, a combination of cervical length and quantitative fetal fibronectin (both continuous variables) predicted spontaneous preterm birth at less than 34 weeks of gestation with ROC AUC of 0.84 (0.79–0.89). This was superior to fetal fibronectin alone (0.79, 0.74–0.85, $P < .01$).

Figure 3 demonstrates the survival curve and outcome of gestation at delivery for those women with short cervical length measurements (less than 15 mm) and the relationship to quantitative fetal fibronectin concentration.

Table 5. Relative Risk of Spontaneous Preterm Birth According to Quantitative Fetal Fibronectin Concentration

	Spontaneous Preterm Birth		
qfFN Category (ng/mL)	Less than 30 Wk of Gestation	Less Than 34 Wk of Gestation	Less Than 37 Wk of Gestation
Less than 10	1	1	1
10–49	3.2 (1.3–8.0)	4.0 (2.5–6.4)	2.5 (1.8–3.4)
50–199	4.9 (1.8–13.3)	5.5 (3.3–9.1)	3.3 (2.3–4.7)
200–499	22.7 (10.4–49.5)	10.1 (6.2–16.6)	5.7 (4.0–8.0)
Greater than 500	37.9 (16.6–86.2)	15.6 (9.2–26.5)	6.3 (4.0–9.9)

qfFN, quantitative fetal fibronectin.
Data are relative risk (95% confidence interval).

Table 6. Proportion of Women With Spontaneous Preterm Birth When Analyzed According to Cervical Length Measurement (above and below 25 mm) and Quantitative Fetal Fibronectin Category (ng/mL)

		Spontaneous Preterm Birth		
Cervical Length and qfFN Category	n*	Less Than 30 Wk of Gestation	34 Wk of Gestation	37 Wk of Gestation
Cervix 25 mm or greater				
fFN less than 10	678	4 (0.6)	12 (1.8)	46 (6.9)
fFN 10–199	236	1 (0.4)	19 (8.1)	36 (15.5)
fFN 200 or greater	27	1 (3.6)	1 (3.5)	3 (11.5)
Total	941	6 (0.6)	32 (3.4)	85 (9.0)
Cervix less than 25 mm				
fFN less than 10	71	3 (4.1)	7 (9.5)	14 (19.2)
fFN 10–199	74	10 (12.8)	22 (28.2)	33 (43.4)
fFN 200 or greater	46	19 (38.7)	27 (55.1)	34 (69.3)
Total	191	32 (15.9)	56 (27.9)	81 (40.9)

fFN, fetal fibronectin.
Data are n (%) unless otherwise specified.
* Women with iatrogenic deliveries before the gestation of analysis were excluded (n=5 less than 30 weeks of gestation, n=11 less than 34 weeks of gestation, n=30 less than 37 weeks of gestation).

DISCUSSION

This study demonstrates the diagnostic accuracy of increasing concentrations of quantitative fetal fibronectin for prediction of spontaneous preterm birth in high-risk asymptomatic women. In women traditionally classified as "positive" (greater than 50 ng/mL), the RR of spontaneous preterm birth at less than 34 weeks of gestation is 2.5 times greater if a fetal fibronectin concentration greater than 200 ng/mL is detected compared with a moderate concentration of 50–199 ng/mL. In women classified as "negative" (less than 50 ng/mL), those with relatively low concentrations (10–49 ng/mL) are still four times more likely to deliver spontaneously than women with the lowest fibronectin (less than 10 ng/mL). Indeed, the risk of spontaneous preterm birth in high-risk women with fetal fibronectin less than 10 ng/mL is no greater than a general U.K. obstetric population (3.3%).[15]

A threshold of 10 ng/mL has sufficiently high sensitivity and NPV to identify which women at high risk are unlikely to deliver preterm and therefore can be treated as low risk. Because the majority (70%) of tests performed fell below this threshold, this indicates a unique opportunity to provide reassurance for most high-risk women; this potentially could reduce health care costs as women return to low-risk care pathways. Although at this threshold specificity would be low, decisions requiring high specificity such as therapeutic interventions could still be withheld unless a higher threshold is reached. Altering the high-risk threshold

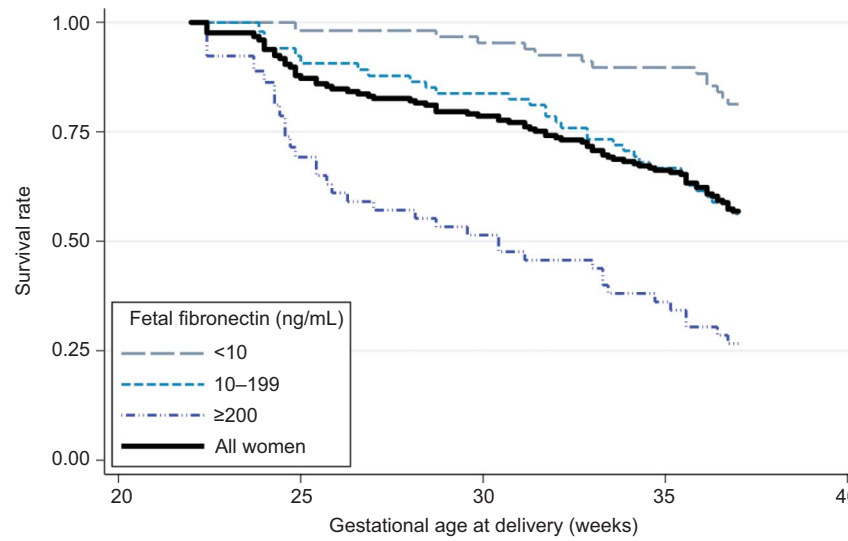

Fig. 3. Kaplan-Meier survival curve demonstrating outcome according to quantitative fetal fibronectin concentration for those with a short cervix (less than 15 mm) (n=66).

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to 200 ng/mL or 500 ng/mL consistently increased the PPV (with minimal effect on the NPV) for delivery before 34 weeks of gestation. Although sensitivity is low (for example, one may not discharge a woman from continued surveillance below this threshold), higher risk therapeutic interventions could be potentially targeted to this group.

Quantitative fetal fibronectin test results also strengthen cervical length testing, more accurately discriminating those women with a short cervix destined to deliver early from those who will not; less than 10% compared with 55% of women with a short cervix and fibronectin concentration of less than 10 and greater than 200 ng/mL, respectively, delivered spontaneously at less than 34 weeks of gestation. Also, in contrast to measurement of cervical length, because quantitative fetal fibronectin can be performed with little training, it may prove useful and cheaper as a standalone test.

The traditional positive–negative cutoff of 50 ng/mL was originally assigned[10] as a balance between optimal sensitivity and specificity and subsequently supported by our research demonstrating that fetal fibronectin greater than 50 ng/mL predicted spontaneous delivery at less than 34 weeks of gestation in high-risk women (AUC 0.64[9]). However, we now show improved prediction with quantification (AUC 0.78).

The association between increasing cervicovaginal fetal fibronectin and progressive risk is biologically plausible because raised fetal fibronectin indicates disruption of the fetal-maternal interface. If as hypothesized, proteolytic degradation of fetal fibronectin is the result of activation of matrix metalloproteinases released from immune cells after infection or inflammation,[16] the more substantial the infectious or inflammatory insult, the greater the fetal fibronectin release with corresponding risk of delivery.

Our data support previous reports on quantification of fetal fibronectin in asymptomatic high-risk[12] and low-risk women.[11] These were retrospective laboratory-based studies with measurements using time-consuming and expensive enzyme-linked immunosorbent assays, but Evaluation of a Quantitative Instrument for the Prediction of Preterm Birth is the first prospective study using measurements by the bedside rapid analyzer, which can be readily implemented within the clinical environment. The preterm birth rate seen within our high-risk cohort is comparable with that in countries with high-prevalence preterm birth rates, so the results may be generalizable. Furthermore, the sensitivity and specificity of this test are consistent across different populations studied.[17]

The major strength is that these data were from a prospectively collected, masked, adequately powered data set. The number of women with markedly elevated levels (greater than 200 ng/mL) is the largest, to date, seen in any published study on quantitative fetal fibronectin. Clinicians were not, however, blinded to the TLI_{IQ} result, which may have affected management (eg, cerclage for short cervix) and outcome. Of note, it was not standard practice in the United Kingdom to routinely prescribe progesterone to high-risk women; 5% of women received progesterone as part of a placebo randomised trial in the United Kingdom (study number: ISRCTN14568373, data currently blinded).[18]

The next stage is to evaluate whether these findings can influence management to optimize outcome. Interventions including cerclage and progesterone have a limited evidence base and criteria for use are still debated. Quantitative fetal fibronectin measurement may have the potential to identify who will benefit from intervention. This area will require further work with appropriately designed trials but may see quantitative fetal fibronectin incorporated into clinical pathways where certain thresholds dictate particular courses of management.

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