Original Research

Quantitative Fetal Fibronectin at 18 Weeks of Gestation to Predict Preterm Birth in Asymptomatic High-Risk Women

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OBJECTIVE: To compare quantitative fetal fibronectin measurement from 18 to 21 weeks of gestation to measurement at 22–27 weeks of gestation for the prediction of spontaneous preterm birth.

METHODS: In a prospective cohort study, we studied the accuracy of cervicovaginal fluid quantitative fetal fibronectin concentrations measured between 18 0/7 weeks of gestation and 21 6/7 weeks of gestation in high-risk asymptomatic women to predict spontaneous preterm birth before 34 weeks of gestation. Predefined fibronectin thresholds were 10 or greater, 50 or greater, and 200 ng/mL or greater. Diagnostic accuracy of the early test (n=898) was compared with the standard test performed between 22 0/7 and 27 6/7 weeks of gestation (n=691) in the same cohort. Subgroup analysis was performed according to cervical length measurement.

RESULTS: Of 898 women, 8.7% delivered spontaneously before 34 weeks of gestation. Only 3.8% of the women with concentrations less than 10 ng/mL (65% of test results) delivered before 34 weeks of gestation. A concentration threshold of 10 ng/mL measured at 18 and 22 weeks of gestation had comparably high sensitivity (early 0.71, 95% confidence interval 0.60–0.81; standard 0.76, 0.63–0.87) and negative predictive value (early 0.96, 0.94–0.98; standard 0.97, 0.95–0.99) for delivery before 34 weeks of gestation. Specificity was also comparable (early 0.69, 0.65–0.72; standard 0.70, 0.66–0.74). A threshold of 200 ng/mL had high specificity (early 0.96, 0.94–0.98; standard 0.96, 0.94–0.97) with lower sensitivity (early 0.26, 0.17–0.37; standard 0.35, 0.22–0.49). Consideration of cervical length strengthened prediction.

CONCLUSION: Quantitative cervicovaginal fetal fibronectin measured from 18 to 21 weeks of gestation has similar predictive value as measurement at 22–27 weeks of gestation for prediction of spontaneous preterm birth. Low fibronectin concentrations are associated with spontaneous preterm birthrates approaching population background levels.

(Obstet Gynecol 2016;0:1–9)
DOI: 10.1097/AOG.0000000000001240

Measurement of cervicovaginal fetal fibronectin at 22–35 weeks of gestation has been established as the best predictive biomarker for spontaneous preterm birth in asymptomatic and symptomatic women,[1–5] predominantly on the basis of its high negative predictive value. The gestational testing window, based on the presumed physiologic presence of fibronectin in 17% of vaginal secretions at earlier gestations,[6] has

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Supported by Tommy's Charity No. 1060508; National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St. Thomas' National Health Service Foundation Trust and King's College London; with minority financial and equipment assistance from Hologic USA (Marlborough, Massachusetts). Natasha L. Hezelgrave is funded by a NIHR Doctoral Research Fellowship (DRF-2013-06-171).

Presented at the 16th annual conference of the British Maternal and Fetal Medicine Society, April 25–26, 2013, Dublin, Ireland.

The authors thank James T. Kurtzman (Loma Linda University, California) and Manju Chandiramani (Imperial College London) for their assistance in protocol development and Jenny Carter, Ruth Cate (Kings College London), and Christine Adamson (West Middlesex University Hospital, London) for their assistance with trial management and participant recruitment.

The views expressed are those of the authors and not necessarily those of Tommy's, the National Health Service, the NIHR, or the Department of Health.

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Financial Disclosure
Drs. Hezelgrave, Abbott, and Shennan received financial assistance to provide educational talks on preterm birth from Hologic, USA, and they also received funding for research paid to their institution. Dr. Shennan has been an unpaid member of the Hologic European Perinatal Advisory Board since December 2013. The other authors did not report any potential conflicts of interest.

ISSN: 0029-7844/16

become clinically embedded. Few studies have investigated earlier testing, although there appears to be an association with elevated fetal fibronectin concentrations from 13 weeks of gestation onward and spontaneous preterm birth.[7]

There has also been a reliance on the commonly used fibronectin bedside test based on a qualitative positive or negative result using a threshold of 50 ng/mL.[5] However, fetal fibronectin concentrations, quantified using enzyme-linked immunosorbent assay, do exhibit a linear correlation with the risk of spontaneous preterm birth.[8] Indeed, extending these observations, we recently reported improved prediction using a rapid bedside quantitative fetal fibronectin test.[9] Review of the fibronectin thresholds for an early pregnancy test would therefore also seem appropriate; if fibronectin concentrations are higher in earlier gestations, prediction may require the use of different thresholds.

We hypothesized that quantitative measurement of fetal fibronectin may allow risk stratification at earlier gestations. The primary aim was to compare the diagnostic accuracy of a bedside quantitative system measurement of cervicovaginal fluid fetal fibronectin in high-risk asymptomatic women between 18 and 21 6/7 weeks of gestation compared with the standard 22- to 27 6/7-week gestational window for prediction of spontaneous preterm birth before 34 weeks of gestation. We also explored the relationship of quantitative fetal fibronectin with ultrasonographic cervical length.

MATERIALS AND METHODS

This prospective masked observational cohort study was undertaken in two prematurity surveillance antenatal clinics in London between October 2010 and February 2012 as part of the Evaluation of Quantitative Fetal Fibronectin in Prediction of Preterm Birth study. Women were eligible if they were between 18 and 21 6/7 weeks of gestation with a singleton pregnancy and were at high risk of spontaneous preterm birth (one or more of: previous spontaneous preterm birth or premature preterm rupture of membranes, previous late miscarriage [16–23 6/7 weeks of gestation], previous invasive cervical surgery [eg, loop excision or cone biopsy], uterine abnormality, or a cervical length less than 25 mm in the current pregnancy). Women with a history of sexual intercourse within the previous 24 hours, vaginal bleeding, or rupture of membranes were excluded from the study as a result of a known interference with fibronectin measurement[10] as were women symptomatic of preterm birth. Written informed consent was obtained from all participants and baseline demographic characteristics, risk factors, and obstetric and gynecologic history were entered onto an online secure study-specific database (www.medscinet.net/PTBstudies). Gestational ages were confirmed by standard first-trimester ultrasound scans. Ethical approval was obtained from the South East London Research Ethics Committee (ethics committee approval number: 10/HO806/68).

During sterile speculum examination, a polyester swab was inserted into the posterior fornix of the vagina (10 seconds) to collect a sample of cervicovaginal secretions. The swab was placed into the test buffer solution and tested immediately. One aliquot (200 microliters) of this solution was analyzed with the conventional qualitative TLi Rapid analyzer and another was analyzed with the quantitative Rapid 10Q analyzer according to the manufacturer's instructions (briefly, one aliquot of the sample is placed on a test cassette and inserted into the analyzer; a result is displayed electronically after 10 minutes). All clinicians were trained in the use of the fibronectin analyzer and the two tests were run concurrently. Categorical TLi data (negative or positive) were provided to clinicians, but the 10Q results remained masked to the patient and clinician (a random result code was generated by the 10Q analyzer) until all outcome data collection had been collected. Thresholds of 10 (lower limit of test), 50 (previous standard),[5] and 200 ng/mL (based on existing literature)[9,11] were predefined. As a result of the small number of women with concentrations 500 ng/mL or greater, absolute and relative risks were calculated in all those 200 ng/mL or greater, but predictive statistics were explored for use of 500 ng/mL or greater as an additional threshold. The reliability of the Rapid 10Q analyzer has previously been reported.[11] Cervical length measurement (in mm) was taken using transvaginal ultrasonography performed by trained staff, directly after the fibronectin swab was taken, and the mean of three values used in the analysis. Women were routinely offered a cervical cerclage if the cervical length dropped below 25 mm before 24 weeks of gestation.

Enrolled women attending the prematurity surveillance clinic for a repeat visit during the standard fibronectin testing window (22–27 6/7 weeks of gestation) underwent repeat quantitative fetal fibronectin testing (as described previously). If a woman was tested on multiple occasions, the first visit between 18 and 21 6/7 weeks of gestation ("early test") and the first visit between 22 and 27 6/7 weeks of gestation ("standard test") was chosen. Pregnancy outcome details were obtained from case note review by trained research midwives. Women were considered to have

spontaneous preterm birth (reference standard) if they had spontaneous onset of labor or experienced preterm rupture of membranes and delivered prematurely regardless of mode of 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=9 less than 30 weeks of gestation, n=14 less than 34 weeks of gestation, n=27 less than 37 weeks of gestation).

Statistical analysis was performed using Stata 11.2. Results of quantitative fetal fibronectin in the pregrouped categories were used to calculate spontaneous preterm birth rates. Fisher exact test was used to determine statistical significance between the fibronectin categories and preterm birth rate. Each predefined threshold for fibronectin was used to establish sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio, and relative risk for spontaneous preterm birth at before 34 weeks of gestation (primary outcome) and before 30 and 37 weeks of gestation as well as delivery within 4 and 8 weeks from testing. The performance of both tests was directly compared using binomial regression with a log link adjusting for repeated testing for some women using robust standard errors. Receiver operating characteristic (ROC) curves were created for each endpoint. Binomial regression with a log link was used to estimate risk ratios for early delivery in women with raised quantitative fetal fibronectin compared with those falling into the lowest category (less than 10 ng/mL).

The utility of quantitative fetal fibronectin was also assessed following stratification by the presence and absence of a short cervix (less than 25 mm) as well as the utility of cervical length measurement after stratification by quantitative fetal fibronectin category. A composite score was developed by using logistic regression for the continuous variables of cervical length and the log of fetal fibronectin concentration to predict prematurity before 34 weeks of gestation. The composite score was compared with two continuous measurements using ROC curve analysis.[12] This study is reported in accordance with Standards for the Reporting of Diagnostic Accuracy studies guidelines (http://www.stard-statement.org).

RESULTS

A consecutive series of 968 women meeting the eligibility criteria were recruited (Fig. 1). Of consented women, 49 were excluded after application of the exclusion criteria, 20 had no outcome data available, and 1 had an "invalid" quantitative fetal fibronectin test. Data (fibronectin concentration and outcome data) were obtained from 898 asymptomatic high-risk women with singleton pregnancies who underwent "early" quantitative fibronectin testing between 18 and 21 6/7 weeks of gestation (mean±standard deviation 19 3/7±1.07).

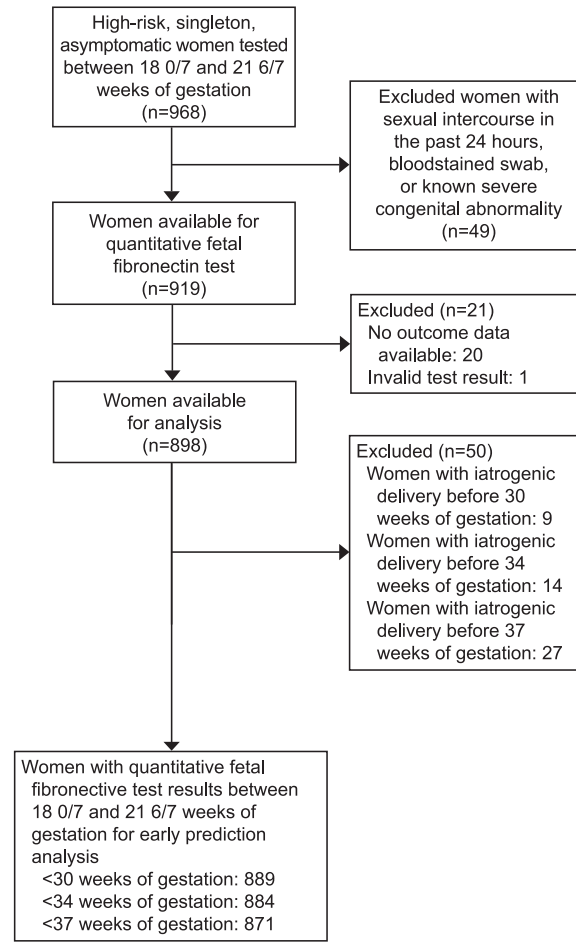

Fig. 1. Flow diagram of participants through the study.
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Of these women, 691 women underwent subsequent quantitative fetal fibronectin measurement in the standard testing window of 22–27 6/7 weeks of gestation (23 1/7±0.98 weeks; 193 did not reattend and 14 miscarried before their second test). Obstetric and demographic characteristics for the included study participants are shown in Table 1. As expected, there was a higher proportion of women with a previous late miscarriage in the whole cohort compared with women who had both an early and standard test (P=.04). There were no significant differences between the two cohorts in the other demographic characteristics. There were no adverse events related to the test.

Table 1. Demographic and Obstetric Characteristics of High-Risk Asymptomatic Women Tested for Cervicovaginal Fluid Fetal Fibronectin Concentrations From 18 Weeks of Gestation (n=898)

Characteristic	Value	
	18–21 6/7-Week Test (n=898)	22–27 6/7-Week Test (n=691)
Age (y)	33±5.2	33±5.1
BMI (kg/m^2)	25.8±5.7	25.8±5.6
Ethnicity		
White	482 (54)	367 (53)
Black	289 (32)	227 (33)
Other	127 (14)	97 (14)
Previous preterm birth	318 (35)	241 (35)
Previous PROM	138 (15)	99 (14)
Previous 2nd-trimester miscarriage	239 (27)	196 (28)
Previous cervical surgery	461 (46)	309 (45)
Incidental short cervix (less than 25 mm)	22 (2)	17 (3)
Uterine abnormality	2 (0.2)	2 (0.3)
Smoking history		
Current	47 (5)	35 (5)
Exsmoker	185 (20)	137 (20)
Never	666 (74)	519 (75)

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

Overall, in all women having an "early" fibronectin test, the proportion of women with preterm birth (spontaneous preterm birth or preterm prelabor rupture of membranes with preterm delivery) was 147 of 871 (16.9%) at less than 37 weeks of gestation, 77 of 884 (8.7%) at less than 34 weeks of gestation, and 45 of 889 (5.0%) at less than 30 weeks of gestation. As expected, women with a previous preterm birth, late miscarriage, or preterm prelabor rupture of fetal membranes had a higher subsequent spontaneous preterm birth rate than women with another risk factor but with no history of spontaneous pregnancy loss before 37 weeks of gestation (invasive cervical surgery, uterine anomaly, or incidental finding of a short cervix); after exclusion of iatrogenic preterm deliveries, women with a history of spontaneous pregnancy loss had a spontaneous preterm birth rate of 115 of 492 (23.4%) at less than 37 weeks of gestation, 61 of 503 (12.1%) at less than 34 weeks of gestation, and 36 of 508 (7.1%) at less than 30 weeks of gestation compared with 32 of 379 (8.4%), 16 of 381 (4.2%), and 9 of 381 (2.4%), respectively, for women with no history of spontaneous preterm pregnancy loss.

After initial recruitment, 18 of 898 (2%) women had a late miscarriage before 24 weeks of gestation, 18 of 895 (2.0%) delivered spontaneously within 4 weeks of testing, and 35 of 892 (3.9%) within 8 weeks of early fibronectin testing. The mean and median gestation at delivery was 38 1/7 (±4.1) weeks and 39 1/7 weeks (interquartile range 37 5/7–40 2/7), respectively.

The number of women and proportion of preterm deliveries assigned to each of the prespecified fibronectin categories is shown in Table 2. Approximately 65% of women (587/898) undertaking the early test had fibronectin concentrations of less than 10 ng/mL, compared with more than 80% (759/898) with concentrations of less than 50 ng/mL (the traditional cutoff for a "negative" qualitative test). The proportion of women experiencing preterm birth rose with increasing fibronectin concentrations at both 18 and 22 weeks of gestation for all gestational endpoints ($P<.001$ by Fisher exact test).

A greater proportion of women with fibronectin concentration of less than 10 ng/mL at 18 weeks of gestation delivered before 30 and 34 weeks of gestation compared with women with this result at 22 weeks of gestation, although the absolute risk remained low (only 2.3% and 3.8% of women whose early test was less than 10 ng/mL delivered before 30 and 34 weeks of gestation, respectively, compared with 1.3% and 2.9% of women with fibronectin less than 10 ng/mL tested from 22 weeks of gestation; Table 2). Only 0.3% of women (2/385) whose early test was categorized as less than 10 ng/mL delivered within 4 weeks of testing compared with 0.7% (3/457) women tested at 22 weeks of gestation, and only 10 of 582 (1.7%) of early women in this less than 10 ng/mL category delivered within 8 weeks of testing compared with 6 of 453 (1.6%) women undergoing standard testing. Conversely, 7 of 52 (13.4%) and 9 of 52 (17.3%) women whose early test was categorized into the greater than 200 ng/mL category delivered within 4 and 8 weeks of testing at 18 weeks of gestation, respectively. In comparison, a higher proportion of women with fibronectin concentrations greater than 200 ng/mL when tested from 22 weeks of gestation delivered within 4 weeks (11/45 [24.4%]) and 8 weeks (15/44 [34.1%]) of gestation, respectively.

The diagnostic accuracy of quantitative fetal fibronectin for predicting spontaneous preterm birth at less than 34 weeks (primary outcome) and less than 30 weeks (predefined secondary outcome) at both gestational testing points with the use of prespecified thresholds of less than 10, less than 50, less than 200, and less than 500 ng/mL is shown in Table 3 and Appendix 1, available online at http://links.lww.com/AOG/A752. A fibronectin concentration threshold of 200 ng/mL

Table 2. Spontaneous Preterm Birth in Asymptomatic High-Risk Women According to Quantitative Cervicovaginal Fluid Fetal Fibronectin Categories for Both the Early (18–21 6/7 Weeks of Gestation) and Standard Tests (22–27 6/7 Weeks of Gestation)

			Spontaneous Preterm Birth (Week of Gestation)*		
qfFN Category (ng/mL)	Test	n (%)	Less Than 30	Less Than 34	Less Than 37
Less than 10	Early	587 (65.4)	13 (2.3, 1.0–3.5)	22 (3.8, 2.2–5.4)	64 (11.3, 8.7–13.9)
	Standard	458 (66.3)	6 (1.3, 0.2–2.4)	13 (2.9, 1.3–4.4)	40 (9.0, 6.3–11.6)
10–49	Early	172 (20.0)	11 (6.4, 2.7–10.1)	22 (12.9, 7.8–18.0)	38 (22.5, 16.1–28.8)
	Standard	119 (17.2)	4 (3.4, 0.6–6.7)	12 (10.2, 4.6–15.7)	24 (20.5, 13.1–27.3)
50–199	Early	87 (10.0)	7 (8.1, 2.2–14.0)	13 (15.1, 7.4–22.8)	23 (27.6, 17.4–36.7)
	Standard	69 (10.0)	3 (4.4, −0.6 to 9.3)	11 (15.9, 7.1–24.8)	19 (27.9, 17.0–38.9)
200 or greater	Early	52 (7.0)	14 (26.9, 14.5–39.4)	20 (39.2, 25.3–53.1)	22 (43.1, 29.1–57.2)
	Standard	45 (6.5)	14 (31.1, 17.0–45.2)	19 (43.2, 27.9–58.4)	24 (55.8, 40.3–71.3)
All	Early	898 (100)	45 (5.1, 3.6–6.4)	77 (8.7, 6.8–10.6)	147 (16.9, 14.4–19.4)
	Standard	691 (100)	27 (3.9, 2.5–5.4)	55 (8.1, 6.0–10.1)	107 (15.9, 13.1–18.6)

qfFN, quantitative cervicovaginal fluid fetal fibronectin.
Data are n (%, 95% confidence interval) unless otherwise specified.
* Women with iatrogenic deliveries before the gestation of analysis were excluded (early test: n=9 at less than 30 weeks of gestation, n=14 at less than 34 weeks of gestation, and n=27 at less than 37 weeks of gestation; standard test: n=5 at less than 30 weeks of gestation, n=8 at less than 34 weeks of gestation, and n=17 at less than 37 weeks of gestation).

or greater had high specificity at both gestational testing windows for prediction of spontaneous preterm birth at less than 34 and less than 30 weeks of gestation with poor, but comparable, sensitivity. A fibronectin concentration threshold of 10 ng/mL at the early test had high sensitivity and NPV at both the early and standard gestational testing windows for prediction of spontaneous preterm birth at less than 34 and 30 weeks of gestation. Receiver operating characteristic curves for prediction of these outcomes were comparable (Fig. 2).

Appendices 3 and 4 show the predictive statistics for spontaneous delivery within 4 and 8 weeks of testing. Thresholds were comparable for prediction aside from the sensitivity of the 200-ng/mL threshold for prediction of delivery within 8 weeks, which was

Table 3. Prediction of Spontaneous Preterm Birth at Less Than 34 Weeks of Gestation According to Cervicovaginal Fluid Fetal Fibronectin Concentration for Both the Early (18–21 6/7 Weeks of Gestation) and Standard Tests (22–27 6/7 Weeks of Gestation)

		Fetal Fibronectin Threshold (ng/mL)			
Predictive Variable	Test	10 or Greater	50 or Greater	200 or Greater	500 or Greater
Sensitivity*	Early	71.4 (60.0–81.2)	42.9 (31.6–54.6)	26.0 (16.6–37.2)	6.5 (2.1–14.5)
	Standard	76.4 (63.0–86.8)	54.5 (40.6–68.0)	34.5 (22.2–48.6)	9.1 (3.0–20.0)
Specificity*	Early	68.9 (65.4–72.0)	87.1 (84.6–89.3)	96.2 (94.6–97.4)	99.5 (98.6–99.9)
	Standard	69.9 (66.1–73.5)	86.8 (83.9–89.3)	96.0 (94.2–97.4)	99.7 (98.3–100.0)
PPV	Early	17.9 (13.8–22.7)	24.1 (17.2–32.1)	39.2 (25.8–53.9)	62.5 (24.5–91.5)
	Standard	18.2 (13.4–23.8)	26.5 (8.7–35.7)	43.2 (28.3–59.0)	62.5 (24.5–91.5)
NPV	Early	96.2 (94.3–97.6)	94.1 (92.2–95.7)	93.2 (91.2–94.8)	91.8 (89.8–93.5)
	Standard	97.1 (95.1–98.5)	95.6 (93.6–97.1)	94.4 (92.3–96.0)	92.6 (90.4–94.5)
Positive LR	Early	2.3 (1.92–2.7)	3.3 (2.4–4.6)	6.8 (4.06–11.3)	17.5 (4.3–71.7)
	Standard	2.5 (2.1–3.1)	4.1 (3.0–5.7)	8.7 (5.1–14.7)	19.0 (4.7–77.5)
Negative LR	Early	0.4 (0.29–0.6)	0.7 (0.5–0.8)	0.8 (0.7–0.9)	0.94 (0.88–1.0)
	Standard	0.3 (0.2–0.6)	0.5 (0.4–0.7)	0.7 (0.6–0.8)	0.91 (0.84–1.0)
ROC area	Early	0.74 (0.67–0.80)			
	Standard	0.79 (0.73–0.86)			

PPV, positive predictive value; NPV, negative predictive value; ROC, receiver operating curve; LR, likelihood ratio.
Data are % (95% confidence interval). The numbers of events are given in Table 2.
* Sensitivity and specificity comparisons (risk difference) for the early vs the standard test at each fibronectin threshold are not statistically significant ($P>.05$).

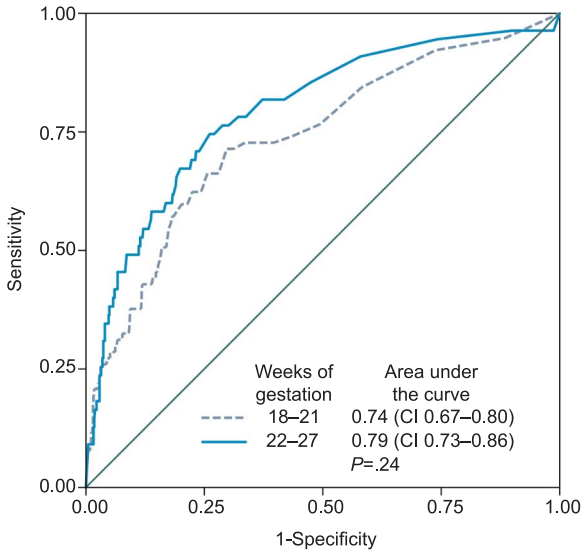

Fig. 2. Receiver operating characteristic curve to illustrate the performance of quantitative cervicovaginal fluid fetal fibronectin test at 18 0/7 to 21 6/7 weeks of gestation (early test) and 22 0/7 to 27 6/7 weeks of gestation (standard test) for prediction of spontaneous preterm birth at less than 34 weeks of gestation. CI, confidence interval.

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lower in the early test compared with the standard test, 25.7% compared with 46.9% (P=.04). Consideration of fibronectin concentration as a continuous variable gave comparable ROC curve areas for prediction of spontaneous delivery within 4 and 8 weeks of testing (Appendices 2 and 3, available online at http://links.lww.com/AOG/A752).

The relative risk of spontaneous preterm birth at less than 34 weeks of gestation was strongly associated with fetal fibronectin concentration at both testing time points (Appendix 4, available online at http://links.lww.com/AOG/A752). For both the early and standard tests, there was a noticeable increase in relative risk between the less than 10 ng/mL and 10 ng/mL or greater groups with risk plateauing between 10 and 199 ng/mL and then increasing again to 200 ng/mL or greater.

Of all women tested at 18 weeks of gestation, 877 women had a concurrent ultrasonographic transvaginal cervical length measurement and 10% (88/877) were found to have a cervical length measurement of less than 25 mm. Table 4 illustrates the proportion of women who delivered prematurely when analyzed according to cervical length measurement (above and below 25 mm) and quantitative fibronectin category. After exclusion of iatrogenic deliveries, 31%, 38%, and 51% of women with a cervical length of less than 25 mm delivered spontaneously before 30, 34, and 37 weeks of gestation, respectively, compared with 2%, 5%, and 13% of those with a cervical length longer than or equal to 25 mm (Table 4). Prediction of spontaneous preterm birth using cervical length as a continuous variable for prediction of preterm birth before 34 weeks of gestation had a comparable ROC curve to prediction using quantitative fetal fibronectin alone; cervical length had an area

Table 4. Proportion of Women With Spontaneous Preterm Birth When Analyzed According to Cervical Length Measurement (Above and Below 25 mm) and Quantitative Cervicovaginal Fluid Fetal Fibronectin Category Measured Between 18 and 21 6/7 Weeks of Gestation

Cervical Length (mm) and qfFN Category (ng/mL)	n	Spontaneous Preterm Birth (Week of Gestation)*		
		Less Than 30	Less Than 34	Less Than 37
Cervix 25 mm or greater				
qfFN less than 10	543	9 (1.7)	17 (3.2)	54 (10.3)
qfFN 10–49	147	4 (2.7)	12 (8.3)	24 (16.7)
qfFN 50–200	66	0 (0)	5 (7.6)	12 (18.5)
qfFN 200 or greater	33	4 (12.0)	7 (21.9)	9 (28.1)
Total	789	17 (2.2)	41 (5.3)	99 (12.9)
Cervix less than 25 mm				
qfFN less than 10	29	3 (11.1)	4 (14.8)	8 (29.6)
qfFN 10–49	22	6 (27.3)	8 (36.4)	12 (54.6)
qfFN 50–199	20	7 (36.8)	8 (42.1)	11 (57.9)
qfFN 200 or greater	17	10 (58.8)	12 (70.6)	12 (75.0)
Total	88	26 (30.6)	32 (37.6)	43 (50.6)

qfFN, quantitative fetal fibronectin.
Data are n (%) unless otherwise specified.
* Women with iatrogenic deliveries before the gestation of analysis were excluded (n=9 at less than 30 weeks of gestation, n=14 at less than 34 weeks of gestation, and n=25 at less than 37 weeks of gestation).

under the curve of 0.77 (0.70–0.83); fibronectin in this cohort had an area under the curve of 0.74 (0.67–0.80) (χ^2 test P=.61).

A combination of cervical length and quantitative fetal fibronectin performed between 18 and 21 6/7 weeks of gestation predicted spontaneous preterm birth before 34 weeks of gestation with ROC area under the curve of 0.80 (0.74–0.86). This was superior to fetal fibronectin alone (χ^2=0.02), but not cervical length alone (χ^2=0.16). However, fibronectin discriminated risk among those with a short cervix. For those women with available cervical length data, the PPV for fibronectin concentration 200 ng/mL or greater for spontaneous preterm birth at less than 34 weeks of gestation was 39% (19/49). The PPV increased to 71% if the cervical length was less than 25 mm and reduced to 22% if the cervical length was 25 mm or greater (χ^2 P=.001). For women with fibronectin concentrations less than 10 ng/mL, the spontaneous preterm birth prevalence of 4% (21/562) was increased to 15% (4/27) if a short cervix was diagnosed concurrently and reduced to 3% (17/535) if the cervix was "normal" (χ^2 test P=.002). Conversely, for women with a short cervix, the PPV for spontaneous preterm birth was increased from 38% (32/85) to 71% (12/17) if the fibronectin concentration was 200 ng/mL or greater and reduced to 15% (4/27) if the concentration was less than 10 ng/mL (χ^2 test P=.001).

Figure 3 demonstrates the survival curve an outcome of gestation at delivery for those women who were found to have a cervical length of less than 25 mm and the relationship to quantitative fetal fibronectin concentrations measured between 18 and 21 6/7 weeks of gestation.

DISCUSSION

This study describes the utility of quantitative fetal fibronectin measurement in cervicovaginal fluid before the previously defined standard of 22 weeks of gestation or more to estimate the risk of preterm birth in high-risk asymptomatic women. Risk of preterm birth at less than 34 weeks of gestation was significantly related to the fetal fibronectin concentration at 18–21 6/7 weeks of gestation and there was clear discriminative value to using alternative risk thresholds of less than 10 ng/mL to define "low risk" and greater than 200 ng/mL to define higher risk.

When considering overall benefit and harm, a test with a high sensitivity is desirable. A fibronectin threshold of 10 ng/mL from 18 weeks of gestation had a relatively high sensitivity and clinically relevant NPV in differentiating those high-risk women who were unlikely to deliver prematurely from those who would benefit from further surveillance. A concentration less than 10 ng/mL conferred a less than 4% risk of preterm birth at less than 34 weeks of gestation, similar to the background prevalence of 3.3% in our hospital population. The observation that more than 60% of tests in our study fell into this category demonstrates the potential to reassure most high-risk women of a likely positive outcome. Although specificity is lower, decisions for intervention and other management could be tailored for women with higher concentrations. Although there is still potential for "false-positives" at higher concentrations, nearly 40%

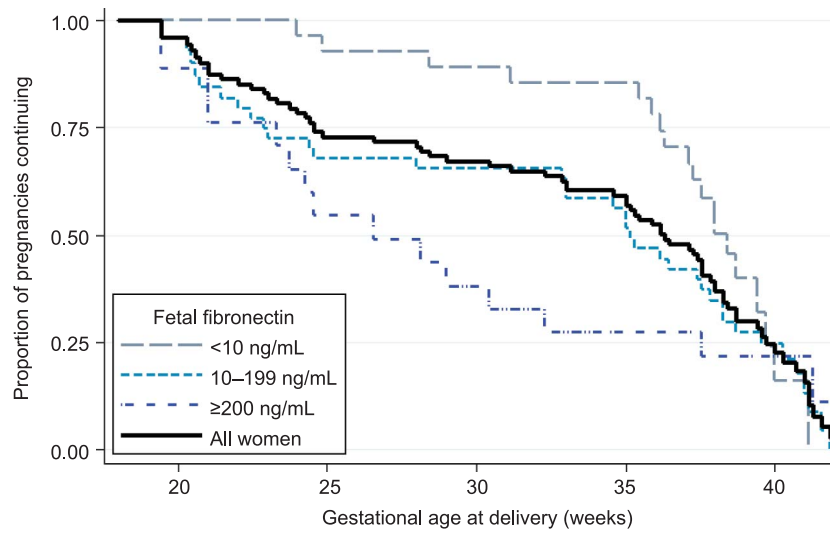

Fig. 3. Kaplan-Meier survival curve demonstrating outcome according to quantitative cervicovaginal fluid fetal fibronectin concentration categories for women who had a short cervix (less than 25 mm) (n=88).

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of women 200 ng/mL or greater delivered prematurely before 34 weeks of gestation, demonstrating that the test can identify women more likely to benefit from surveillance with cervical length measurement, intervention, and appropriate counseling.

Risk of preterm birth rose with fibronectin concentrations; thus, clinical risk discrimination can be enhanced by interrogating concentration rather than just using the traditional 50-ng/mL low-risk threshold, previously determined as the optimal balance of sensitivity and specificity.[7] For example, 13% of women classified as "negative" by the qualitative test (but who had an early result between 10 and 49 ng/mL) delivered at less than 34 weeks of gestation. Moreover, 10% of those in this fibronectin category with a normal cervical length delivered before 34 weeks of gestation and would have been incorrectly labeled as "low risk." This important shift in the lower threshold from 50 to 10 ng/mL is supported by data published as part of the whole Evaluation of Quantitative Fetal Fibronectin in Prediction of Preterm Birth cohort study using the standard 22-week test.[9] Thus, a new threshold definition can be used to identify "lower risk women" from a high-risk cohort. This change in threshold also highlights that women with early concentrations greater than 10 ng/mL may require continued surveillance during the critical gestational period where interventions to prolong pregnancy or improve preterm neonatal outcome may be of value. Repeat testing after 22 weeks of gestation will continue to inform management, particularly if levels subsequently fall below 10 ng/mL.

Prediction of preterm birth before 34 weeks of gestation using cervicovaginal fetal fibronectin or cervical length was comparable, but combined measurements were synergistic and could identify women who would have been incorrectly classified as "low risk" had only one or other screening tests been utilized. Although the sample size when stratified by cervical length measurement was small, limiting interpretation, a combined approach appears to offer the most accurate prediction if resources permit, although at 18 weeks of gestation, a test result of less than 10 ng/mL would provide reassurance in the absence of scanning availability.

In terms of generalizability, the preterm birth rate was comparable with that in published high-risk studies.[13] The population was of varied ethnic origin and the test can be considered transferable to a broad high-risk population. Positive predictive value is likely to be lower and NPV higher if the test were to be used in lower risk women.

A limitation was that women with a history of pregnancy loss or early preterm birth were routinely offered cerclage if a short cervix was detected (progesterone not routinely prescribed at the time of study). Cerclage use may have influenced outcome[14] so it is possible that intervention slightly altered predictive ability, but ethically studies can no longer be undertaken in the current setting without providing some intervention. Furthermore, clinicians were not blinded to the quantitative result, which may have affected management. The number of women with concentrations 500 ng/mL or greater (upper limit of detection) was small (n=8), so caution is required for interpretation of predictive statistics in this group. The statistical comparison of the tests at different gestations does not allow for the outcome of women who delivered between the two tests time points and should therefore not be used as the basis of deciding whether to use an early test as well, or instead of, a later test.

Quantitative cervicovaginal fetal fibronectin measurement at 18–21 6/7 weeks of gestation has potential to discriminate between high-risk women who do not need intensive surveillance from those who require further management. The application of this earlier test has potential to guide intervention (eg, cerclage or progesterone) before cervical shortening is detected, and future trials comparing the use of intervention guided by test result are warranted.

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