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A high concentration of fetal fibronectin in cervical secretions increases the risk of intra-amniotic infection and inflammation in patients with preterm labor and intact membranes

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Abstract

Objective: To determine whether the risk of intra-amniotic infection/inflammation and spontaneous preterm delivery (SPTD) varies as a function of the concentration of cervical fetal fibronectin (fFN) in patients with preterm labor and intact membranes.

Methods: This prospective study included 180 patients with preterm labor and intact membranes who had a sample collected for quantitative fFN measurement and underwent amniocentesis. Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas. Intra-amniotic inflammation was defined as an amniotic fluid matrix metalloproteinase-8 concentration >23 ng/mL.

Results: (1) The prevalence of intra-amniotic infection/inflammation and SPTD within 7 days was 32.2% (58/180) and 33.9% (61/178), respectively; (2) The higher the fFN concentration, the greater the risk of intra-amniotic infection/inflammation and SPTD within 7 days ($P < 0.001$, respectively); (3) An fFN concentration 150 ng/mL had a better diagnostic performance than an fFN 50 ng/mL in the identification of intra-amniotic infection/inflammation and SPTD within 7 days; (4) Among the patients with

an fFN <50 ng/mL, intra-amniotic infection/inflammation was identified in 7.6% (6/79) of patients and 66.7% (4/6) delivered within 7 days.

Conclusion: The higher the concentration of fFN, the greater the risk of intra-amniotic infection/inflammation and SPTD in patients with preterm labor and intact membranes.

Keywords: acute chorioamnionitis; acute histologic chorioamnionitis; amniotic fluid infection; amniotic fluid inflammation; biomarker; diagnostic indices; extracellular matrix proteins; fetal fibronectin; funisitis; intra-amniotic infection; intra-amniotic inflammation; microbial invasion of amniotic cavity; prematurity; preterm birth; preterm labor.

Introduction

The term “common pathway of parturition” refers to the anatomical, biochemical and physiologic changes that occur in both term and preterm labor [1, 2]. The features of this pathway could be systemic in nature (i.e. a change in circulating white blood cell count) or focused on the uterus [1]. A major emphasis in the study of parturition has been on the uterine components of the common pathway, which have overt clinical manifestations such as increased myometrial contractility [3], cervical effacement and dilatation [4] and decidual/membrane activation [5, 6].

The process of decidual/membrane activation refers to the cellular and biochemical events that eventually lead to membrane rupture and facilitate the separation of the membranes and placenta from the decidua after delivery [1, 2, 6–8]. In addition, the decidua plays an important role in the mechanisms of parturition by producing uterine agents such as prostaglandin and oxytocin [5, 9–11], which can induce myometrial contractility [12–14].

During pregnancy, the chorioamniotic membranes adhere firmly to the decidua via production of intercellular “cements” which specifically anchor the chorion leave to the decidua. Oncofetal fibronectin [also known as fetal fibronectin (fFN)] is found in amniotic fluid, trophoblast and some malignant cell lines [15]. This moiety can be

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recognized by monoclonal antibody FDC-6: it recognizes an epitope in which α -N-acetylgalactosamine to a threonine residue in a hexapeptide segment (Val-Thr-His-Pro-Gly-This year) located in the type III connecting segment (IIICS) variable region of fibronectin [16, 17].

Lockwood et al. first reported that fFN in cervical secretions and vaginal fluid could be a predictor of spontaneous preterm delivery (SPTD) [16]. The test has been extensively used in clinical obstetrics to assess the risk of preterm delivery, largely because of its high negative predictive value [16, 18–29]. Initially, the fFN test was scored as positive or negative, with a cut-off of 50 ng/mL [16, 18–28, 30, 31]. Subsequently, a quantitative test was developed, and there is evidence that measuring the absolute concentration of fFN is more informative than a categorical value of positive/negative [32–38].

fFN is considered a biochemical marker of membrane/decidual activation, as is the increased expression of insulin growth factor binding protein (IGFBP)-1 [39–52] or α -1 microglobulin (also measurable with a placental alpha microglobulin-1 test in cervical and vaginal fluid) [53–67]. Membrane/decidual activation can be induced by the physiologic events that lead to spontaneous labor and delivery, or by pathologic insults, such as infection or inflammation [1, 2, 5–8].

Intra-amniotic infection is a well-established cause of the “preterm labor syndrome” [1, 2, 68–75], and occurs in about 10% of women with preterm labor and intact membranes [69, 71, 74, 76–85]. Microbial invasion of the amniotic cavity results in intra-amniotic inflammation [2, 69, 71, 73, 75, 86–94], which may extend to the fetus and lead to the fetal inflammatory response syndrome [70, 95–98]. Intra-amniotic infection is a risk factor for impending preterm delivery and adverse neonatal outcomes [69, 71, 76, 86, 99–101]. Intra-amniotic inflammation is present in about 30% of patients with preterm labor and intact membranes and is a risk factor for adverse pregnancy and neonatal outcomes regardless of the presence or absence of intra-amniotic infection [102, 103].

fFN is a major extracellular matrix protein present in the choriodecidual interface [16]. A positive fFN test in cervicovaginal fluid (a cut-off of 50 ng/mL) is a predictor of SPTD in asymptomatic pregnant women in the mid-trimester [16, 21, 22, 24]. In women with an episode of preterm labor, a negative fFN in cervicovaginal fluid has a high negative predictive value, and has been used to select patients who may not need hospitalization or acute treatment with tocolysis or steroids [104–106].

Some investigators [21] have postulated that a positive fFN test is associated with microbial invasion of the amniotic cavity and acute histologic chorioamnionitis.

However, other investigators [28, 107] have reported that a positive fFN test in cervicovaginal fluid is not associated with a positive amniotic fluid culture or acute histologic chorioamnionitis. Therefore, the issue of whether an increased concentration of fFN in cervicovaginal fluid represents a marker of upper genital tract infection in patients with an episode of preterm labor remains unresolved.

Some recent studies have evaluated the usefulness of quantitative fFN testing to identify patients at risk for impending preterm delivery [32–37, 108–118]. This study was conducted to determine if a quantitative cervical fFN test can predict intra-amniotic infection, intra-amniotic inflammation, histologic chorioamnionitis and a short amniocentesis-to-delivery interval in patients with preterm labor and intact membranes.

Materials and methods

Study design and population

The study population consisted of 180 consecutive patients admitted to our institution with the diagnosis of preterm labor and intact membranes who met the following inclusion criteria: (1) singleton pregnancy, (2) gestational age between 19 and 35 weeks, (3) preterm labor defined as the presence of painful regular uterine contractions, with a frequency of at least two every 10 min, requiring hospitalization according to definitions previously described in detail [69, 119] and (4) cervical fFN test performed at the time of amniocentesis. Cervical fFN test and amniocentesis were offered at our institution to all patients admitted with the diagnosis of preterm labor and intact membranes during the study period. Written informed consent was obtained from all patients. We followed the ethical standards for human experimentation established in the Declaration of Helsinki. The Institutional Review Board of our institute approved the collection and use of these samples and information for research purposes. This University Hospital has a Federalwide Assurance with the Office for Human Research Protection (OHRP) of the Department of Health and Human Services (DHHS) of the United States.

Cervical fetal fibronectin

Cervical secretions were collected shortly after admission, before a digital cervical examination and amniocentesis. The fFN concentration in cervical secretions was determined with a commercially available enzyme-linked immunosorbent assay (Adeza Biomedical, Sunnyvale, CA, USA) with a sensitivity of <20 ng/mL as previously reported [120]. For the collection of specimens, a sterile speculum was inserted into the vagina. A Dacron polyester fiber swab was placed in the external cervical os and was left in place for 10 s to achieve saturation. The swabs were then soaked in a polypropylene tube containing 1 mL of buffer and was stored at -20°C until assayed. The Dacron swabs and buffer were provided by the manufacturer (Adeza Biomedical, Sunnyvale, CA, USA). A receiver operating characteristic (ROC) curve analysis was performed to determine the best cut-off of

cervical fFN concentration although a fibronectin concentration of 50 ng/mL was commonly used as a cut-off in qualitative analysis. The cervical fFN results were grouped into three incremental categories using these two cut-offs: <50 ng/mL, 50–149 ng/mL and ≥150 ng/mL. The result of cervical fibronectin measurement was available for the attending physicians.

Amniotic fluid

Amniotic fluid was obtained by transabdominal amniocentesis and was cultured for aerobic and anaerobic bacteria as well as for genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*) and measured for white blood cell count. These results were available for the attending physicians. The remaining fluid was centrifuged and stored in polypropylene tubes at –70°C until assayed. Matrix metalloproteinase-8 (MMP-8) concentrations in amniotic fluid were measured with commercially available enzyme-linked immunosorbent assays (Amersham Pharmacia Biotech, Inc, Little Chalfont, Bucks, UK). The sensitivity of MMP-8 was 0.3 ng/mL. The inter-assay and intra-assay coefficients were less than 10%. Intra-amniotic inflammation was defined as an elevated amniotic fluid MMP-8 concentration (>23 ng/mL) as previously reported [121–130]. The results of MMP-8 were not available for patient management.

Diagnosis of acute histologic chorioamnionitis and funisitis and clinical chorioamnionitis

Acute histologic chorioamnionitis was defined in the presence of acute inflammatory changes on histologic examination of the extraplacental membranes or the chorionic plate of the placenta; acute funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or into Wharton's jelly with the use of criteria previously reported [87, 131–133]. Clinical chorioamnionitis was diagnosed in the presence of a maternal temperature of ≥37.8°C and ≥2 of the following criteria: (1) uterine tenderness; (2) malodorous vaginal discharge; (3) maternal leukocytosis (a white blood cell count of >15,000 cells/mm³); (4) maternal tachycardia (>100 beats/min); and (5) fetal tachycardia (>160 beats/min) [134–145].

Statistical analysis

Continuous variables were compared using the Mann-Whitney *U*-tests and proportions were compared using Fisher's exact test. The Galen and Gambino analysis was used for comparison of sensitivity, specificity and diagnostic accuracy [146]. A ROC curve analysis was performed to determine the relationship between the sensitivity (true-positive rate) and the false-positive rate, and to select the best cut-off values for fFN in the identification of intra-amniotic infection and/or inflammation. Women who did not go into labor spontaneously because they were delivered for maternal or fetal indications were treated as censored observations, with a censoring time equal to the amniocentesis-to-delivery interval. A *P*-value <0.05 was considered as significant. SPSS 22.0 for Windows (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

Clinical characteristics and pregnancy outcomes of the study population

Table 1 displays the clinical characteristics and pregnancy outcomes of the study population. The median gestational age at sampling was 30.8 weeks (interquartile range, 27.5–33.2 weeks). Fifty-six percent of the cases (101/180) had a cervical fFN concentration ≥50 ng/mL. The prevalence of intra-amniotic infection was 7.3% (13/179). *Ureaplasma* species was the most frequently found microorganism isolated from the amniotic cavity (*n*=7). Other isolates included *Mycoplasma hominis* (*n*=2), viridans streptococcal species (*n*=2), *Enterococcus faecalis* (*n*=1), *Corynebacterium* species (*n*=1), *Pseudomonas aeruginosa* (*n*=1), *Candida albicans* (*n*=1) and an unspecified Gram-positive rod (*n*=1). Intra-amniotic inflammation was present in 30% (54/180) of the cases.

The median gestational age at delivery was 35.0 weeks (interquartile range, 32.1–38.4 weeks). The overall prevalence of spontaneous delivery <7 days and 14 days, and preterm delivery <34 and 37 weeks was 33.9% (61/178) and 40.5% (70/173), 34.4% (62/155), and 61.7% (111/177), respectively.

Diagnostic performance of fetal fibronectin in the identification of intra-amniotic infection and/or inflammation and prediction of spontaneous preterm delivery

Figure 1 illustrates the ROC curves for fFN in the identification of intra-amniotic infection and/or inflammation [area under the curve (AUC), 0.81; 95% confidence interval (CI), 0.74–0.88; *P* < 0.001] and identification of the patient who would have an SPTD within 7 days of amniocentesis (AUC, 0.77; 95% CI, 0.69–0.84; *P* < 0.001). The optimal cut-off value for identification of intra-amniotic infection and/or inflammation was 150 ng/mL. The fFN concentration was categorized as <50, 50–149 and ≥150 ng/mL.

Table 2 compares the diagnostic performance of fFN with two different cut-offs (50 ng/mL and 150 ng/mL). A cervical fFN concentration 150 ng/mL had a significantly higher specificity and accuracy in the identification of intra-amniotic infection and/or inflammation and in the identification of the patient who would have an SPTD within 7 days than that of an fFN concentration ≥50 ng/mL (*P* < 0.05 for each). The sensitivity was similar for the two cut-off values.

Table 1: Clinical characteristics of the study population.

Clinical characteristics	Median (interquartile range) or % (n)
Maternal age, years	30 (28–33)
Nulliparity	59.4% (107/180)
Gestational age at amniocentesis, weeks	30.8 (27.5–33.2)
Cervical dilatation, cm	1.5 (0–1.5)
Fibronectin, ng/mL	114.9 (<50–592.9)
Fibronectin >50 ng/mL	56.1% (101/180)
Amniotic fluid analysis	
Positive amniotic fluid culture ^a	7.3% (13/179)
Amniotic fluid matrix metalloproteinase-8 concentration, ng/mL	1.6 (0.5–53.2)
Intra-amniotic inflammation (defined as amniotic fluid matrix metalloproteinase-8 >23 ng/mL)	30% (54/180)
Intra-amniotic infection and/or inflammation	32.2% (58/180)
Gestational age at delivery, weeks	35.0 (32.1–38.4)
Spontaneous preterm delivery	
<34 weeks ^b	38% (57/150)
<37 weeks ^c	60% (99/165)
Interval between amniocentesis and delivery, days	22.2 (2.7–50.3)
<7 days ^d	33.9% (61/178)
<14 days ^d	40.5% (70/173)
Clinical chorioamnionitis	3.3% (6/180)
Acute histologic chorioamnionitis ^e	37.8% (51/135)
Funisitis ^e	13.1% (18/137)

^aOne patient who was not performed amniotic fluid culture was excluded. ^bTwenty-five patients in whom amniocentesis was performed beyond 34 weeks and five women who had delivered intentionally due to maternal-fetal indication before 34 weeks were excluded.

^cFifteen patients who had delivered intentionally due to maternal-fetal indication before 37 weeks were excluded. ^dWomen who had delivered intentionally due to maternal-fetal indication before 7 days ($n = 2$) and 14 days ($n = 7$) were excluded. ^ePlacenta histology was examined in 137 women. Among them, the presence or absence of acute histologic chorioamnionitis was not determined in two women.

Figure 1: Receiver operating characteristic curves for cervical fetal fibronectin.

(A) Identification of intra-amniotic infection and/or inflammation [area under the curve (AUC), 0.81; 95% CI, 0.74–0.88; $P < 0.001$] and (B) identification of spontaneous preterm delivery within 7 days (AUC, 0.77; 95% CI, 0.69–0.84; $P < 0.001$).

Table 3 compares the pregnancy outcomes according to the fFN concentration using a cut-off of 150 ng/mL. Patients with an fFN concentration ≥ 150 ng/mL had a significantly higher rate of an amniocentesis-to-delivery

interval <7 days and 14 days than those with an fFN concentration <150 ng/mL ($P < 0.001$, for each). Even after adjusting for gestational age at amniocentesis, degree of cervical dilatation and presence of intra-amniotic

Table 2: Diagnostic indices of cervical fetal fibronectin in predicting intra-amniotic infection and/or inflammation and spontaneous preterm delivery ≤ 7 days.

Cervical fetal fibronectin	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy	Positive likelihood ratio	Negative likelihood ratio
Intra-amniotic infection and/or inflammation							
≥ 50 ng/mL	89.7 (52/58)	59.8 (73/122)	51.5 (52/101)	92.4 (73/79)	69.4 (125/180)	2.23 (1.77–2.82)	0.17 (0.08–0.37)
≥ 150 ng/mL	86.2 (50/58)	71.3 (87/122) ^a	58.8 (50/85)	91.6 (87/95)	76.1 (137/180) ^a	3.00 (2.23–4.05)	0.19 (0.10–0.37)
Spontaneous preterm delivery ≤ 7 days of amniocentesis ^b							
≥ 50 ng/mL	83.6 (51/61)	59.0 (69/117)	51.5 (51/99)	87.3 (69/79)	67.4 (120/178)	2.04 (1.60–2.60)	0.28 (0.15–0.50)
≥ 150 ng/mL	78.7 (48/61)	70.1 (82/117) ^a	57.8 (48/83)	86.3 (82/95)	73.0 (130/178) ^a	2.63 (1.94–3.57)	0.30 (0.19–0.50)

Data are given as percentage (n) or likelihood ratio (95% confidence interval). ^aP < 0.05 by Galen and Gambino analysis compared to a cut-off of cervical fibronectin <50 ng/mL. ^bWomen who had delivered intentionally due to maternal-fetal indication before 7 days (n = 2) and 14 days (n = 7) were excluded.

Table 3: Clinical characteristics and pregnancy outcomes of the study population according to the cervical fetal fibronectin concentration cut-off of 150 ng/mL.

	Cervical fetal fibronectin, ng/mL		P-value
	≥ 150 (n = 85)	<150 (n = 95)	
Maternal age, years	31 (29–33)	30 (28–33)	0.369
Nulliparity	52.9% (45/85)	65.3% (62/85)	0.098
History of preterm delivery	11.8% (10/85)	12.6% (12/95)	1.000
Gestational age at amniocentesis, weeks	30.3 (27.4–32.9)	31.1 (28.0–33.3)	0.397
Cervical dilatation, cm	1.5 (0.0–3.0)	0.0 (0.0–1.5)	<0.001
Amniotic fluid analysis			
Positive amniotic fluid culture ^a	13.1% (11/84)	2.1% (2/95)	0.007
Amniotic fluid matrix metalloproteinase-8 concentration, ng/mL	27.5 (2.0–347.8)	0.7 (0.3–1.6)	<0.001
Intra-amniotic inflammation (defined as amniotic fluid matrix metalloproteinase-8 >23 ng/mL)	54.1% (46/85)	8.4% (8/95)	<0.001
Intra-amniotic infection and/or inflammation	58.8% (50/85)	8.4% (8/95)	<0.001
Gestational age at delivery, weeks	33.1 (28.7–34.7)	38.0 (35.3–39.3)	<0.001
Use of tocolytics	84.7% (72/85)	84.2% (80/95)	1.000
Spontaneous preterm delivery			
<34 weeks ^b	60.9% (42/69)	18.5% (15/81)	<0.001
<37 weeks ^c	88.0% (66/75)	36.7% (33/90)	<0.001
Interval between amniocentesis and delivery, days			
<7 days ^d	57.8% (48/83)	13.7% (13/95)	<0.001
<14 days ^d	67.1% (53/79)	18.1% (17/94)	<0.001
Clinical chorioamnionitis	3.5% (3/85)	3.2% (3/95)	1.000
Acute histologic chorioamnionitis ^e	52.2% (36/69)	22.7% (15/66)	0.001
Acute histologic chorioamnionitis (delivered ≤ 3 days)	54.3% (19/35)	44.4% (4/9)	0.716
Funisitis ^e	18.3% (13/71)	7.6% (5/66)	0.078
Funisitis (delivered ≤ 3 days)	22.9% (8/35)	22.2% (2/9)	1.000

Data are given as median (interquartile range) or % (n). ^aOne patient who was not performed amniotic fluid culture was excluded.

^bTwenty-five patients in whom amniocentesis was performed beyond 34 weeks and five women who had delivered intentionally due to maternal-fetal indication before 34 weeks were excluded. ^cFifteen patients who had delivered intentionally due to maternal-fetal indication before 37 weeks were excluded. ^dWomen who had delivered intentionally due to maternal-fetal indication before 7 days (n = 2) and 14 days (n = 7) were excluded. ^ePlacenta histology was examined in 137 women. Among them, the presence or absence of acute histologic chorioamnionitis was not determined in two women.

infection/inflammation, an elevated fFN ≥ 150 ng/mL was associated with an increased risk of spontaneous preterm delivery <7 days [relative risk (RR), 3.8; 95% CI, 1.6–9.0] and <14 days (RR, 3.6; 95% CI, 1.5–8.4). Moreover, an

elevated fFN was associated with a higher median amniotic fluid MMP-8 concentration, and higher rates of intra-amniotic infection, intra-amniotic inflammation, acute histologic chorioamnionitis and acute funisitis. However,

Table 4: Risk stratification of intra-amniotic infection and/or inflammation using quantitative cervical fetal fibronectin test.

	Cervical fetal fibronectin, ng/mL			P-value ^a
	<50 (N=79)	50–149 (N=16)	≥150 (N=85)	
Frequency of intra-amniotic infection and/or inflammation	7.6% (6/79)	12.5% (2/16)	58.8% (50/85)	<0.001
Relative risk (95% confidence interval) ^b	Ref	1.9 (0.3–11.4)	16.8 (5.8–48.5)	

^aP-value for linear-by-linear association. ^bAdjusted for gestational age at amniocentesis and cervical dilatation.

among patients who delivered within 3 days of amniocentesis, an elevated fFN was not associated with the presence of acute histologic chorioamnionitis and funisitis.

Quantitative analysis of cervical fetal fibronectin in the prediction of intra-amniotic infection/inflammation

Table 4 shows the frequency of intra-amniotic infection and/or inflammation using the quantitative cervical fFN test. The higher the fFN concentration, the greater the risk of intra-amniotic infection and/or inflammation (fFN concentration <50 ng/mL, 7.6% [6/79]; fFN concentration between 50 and 149 ng/mL, 12.5% [2/16]; fFN concentration ≥150 ng/mL, 58.8% [50/85]; $P<0.001$ by linear-by-linear association). After adjusting for gestational age at amniocentesis and cervical dilatation, fFN concentration between 50 and 149 ng/mL was not significantly associated with the presence of intra-amniotic infection and/or inflammation (RR, 1.9; 95% CI, 0.3–11.5). However, a cervical fluid fFN concentration ≥150 ng/mL was associated with intra-amniotic infection and/or inflammation (RR, 16.8; 95% CI, 5.8–48.5).

Interval to delivery as a function of cervical fetal fibronectin concentration and the presence or absence of intra-amniotic infection, and/or intra-amniotic inflammation

Figure 2 shows the risk of SPTD within 7 days and 14 days of amniocentesis and delivery <34 weeks and <37 weeks. The risk of SPTD increases as cervical fFN concentration increased and intra-amniotic infection and/or inflammation develops. The risk of SPTD <7 days is only 8.2% among women with a cervical fibronectin concentration <50 ng/mL and without intra-amniotic infection/inflammation but 75% in those with a cervical fibronectin

concentration ≥150 ng/mL and intra-amniotic infection and/or inflammation.

After adjusting for gestational age at amniocentesis and cervical dilatation, a cervical fFN concentration ≥150 ng/mL had a significantly higher rate of SPTD of <7 days and 14 days compared to a cervical fFN concentration <50 ng/mL (Table 5). Of note, the cervical fFN concentration was not associated with SPTD at <7 days and 14 days among patients with an intra-amniotic infection and/or inflammation. Of the six women with a cervical fFN concentration <50 ng/mL and intra-amniotic infection and/or inflammation, 67% (four women) delivered within 7 days and 83% (five women) did within 14 days. The risk of preterm delivery within 7 days was similar to that of patients with a cervical fFN concentration between 50 and 149 ng/mL [50% (1/2)], and those with a cervical fFN concentration ≥150 ng/mL [75.0% (36/48)]. In contrast, among women without intra-amniotic infection/inflammation, a cervical fFN concentration ≥150 ng/mL was associated with SPTD <7 days (RR, 4.4) and 14 days (RR, 4.1) even after adjusting for gestational age at amniocentesis and cervical dilatation. Patients with an fFN concentration between 50 and 149 ng/mL has a similar risk of SPTD <7 days and 14 days compared to those with an fFN concentration <50 ng/mL irrespective of the presence of intra-amniotic infection and/or inflammation.

Discussion

Principal findings of the study

- (1) An elevated cervical fFN concentration (≥150 ng/mL) is associated with the presence of intra-amniotic infection and/or inflammation;
- (2) the higher the cervical fFN concentration, the greater the risk of intra-amniotic infection and/or inflammation;
- (3) an fFN concentration ≥150 ng/mL had a better diagnostic performance in the identification of intra-amniotic infection and/or

Figure 2: Frequency of spontaneous preterm delivery according to the results of quantitative cervical fetal fibronectin test (ng/mL) and the presence or absence of intra-amniotic infection and/or inflammation.

Table 5: Risk of spontaneous preterm delivery within 7 and 14 days according to the results of quantitative cervical fetal fibronectin test and the presence or absence of intra-amniotic infection and/or inflammation.

			Cervical fetal fibronectin, ng/mL			P-value ^a
			<50 (N=79)	50–149 (N=16)	≥150 (N=85)	
Spontaneous preterm delivery <7 days (34.3%, 61/178) ^c	Total	Frequency	12.7% (10/79)	18.8% (3/16)	57.8% (48/83)	<0.001
		RR (95% CI) ^b	Ref	1.6 (0.4–6.8)	8.4 (3.7–19.1)	
	Intra-amniotic infection/inflammation (+) (N=56)	Frequency	66.7% (4/6)	50% (1/2)	75.0% (36/48)	0.555
		RR (95% CI) ^b	Ref	0.2 (0.1–5.3)	1.5 (0.2–10.8)	
Spontaneous preterm delivery <14 days (40.5%, 70/173) ^c	Intra-amniotic infection/inflammation (-) (N=122)	Frequency	8.2% (6/73)	14.3% (2/14)	34.3% (12/35)	0.001
		RR (95% CI) ^b	Ref	1.7 (0.3–10.0)	4.4 (1.4–13.7)	
	Total	Frequency	17.9% (14/78)	18.8% (3/16)	67.1% (53/79)	<0.001
		RR (95% CI) ^b	Ref	1.0 (0.2–4.3)	7.4 (3.4–16.3)	
Spontaneous preterm delivery <34 weeks	Intra-amniotic infection/inflammation (+) (N=55)	Frequency	83.3% (5/6)	50% (1/2)	83% (39/47)	0.805
		RR (95% CI) ^b	Ref	0.1 (0.0–2.5)	1.1 (0.1–12.1)	
	Intra-amniotic infection/inflammation (-) (N=118)	Frequency	12.5% (9/72)	14.3% (2/14)	43.8% (14/32)	0.001
		RR (95% CI) ^b	Ref	1.0 (0.2–5.7)	4.1 (1.4–11.9)	

RR, relative risk; CI, confidence interval. ^aP-value for linear-by-linear association. ^bAdjusted for gestational age at amniocentesis and cervical dilatation. ^cPatients who were either lost to follow-up or had a maternal or fetal indication for preterm delivery were excluded.

inflammation and in the prediction of SPTD within 7 days than an fFN concentration ≥ 50 ng/mL; (4) an elevated concentration of fFN (either 50 ng/mL or 150 ng/mL) was associated with SPTD within 7 days and 14 days only in patients without intra-amniotic infection/inflammation;

(5) a low cervical fFN concentration (<50 ng/mL) did not exclude the presence of intra-amniotic infection and/or inflammation as the prevalence of this condition was 7.6% (6/79) and 66.7% (4/6) of these patients delivered prematurely within 7 days of amniocentesis.

Cervical fetal fibronectin concentration as a risk factor for intra-amniotic infection/inflammation

In the current study, a cervical fFN concentration ≥ 150 ng/mL identified intra-amniotic infection and/or inflammation with a sensitivity of 86.2% (50/58) and a specificity of 71.3% (87/122). Among women whose cervical fFN concentration was ≥ 150 ng/mL, 58.8% had intra-amniotic infection and/or inflammation. The risk was 17 times higher than those with a cervical fFN concentration < 50 ng/mL. Therefore, quantitative fFN testing may aid in the assessment of the risk of intra-amniotic infection and/or inflammation in women with preterm labor and intact membranes. For example, if an fFN test is performed for standard clinical indications (i.e. its high negative predictive value for preterm delivery), the quantitative test provides additional information about the risk of intra-amniotic inflammation and/or infection over that of a qualitative test with a cut-off of 50 ng/mL. This could have implications for patient management, such as the decision to offer an amniocentesis for the diagnosis of intra-amniotic infection or inflammation [147]. It is noteworthy that the positive and negative likelihood ratios of fFN concentrations ≥ 150 ng/mL in the identification of intra-amniotic infection and/or inflammation were 3.0 and 0.19, respectively. Therefore, we do not recommend that the decision to perform an amniocentesis be based solely on the results of quantitative cervical fFN concentrations.

Why the cervical fetal fibronectin concentration is elevated in patients with intra-amniotic infection or inflammation?

The mechanisms responsible for the elevated concentration of fFN in patients with intra-amniotic infection or inflammation and preterm labor have not been determined. In the current study, an elevated cervical fFN was associated not only with intra-amniotic infection and/or inflammation, but also with the presence of acute histologic chorioamnionitis and funisitis. It is tempting to speculate that acute inflammation of the chorioamniotic membranes may lead to the production of fFN at the choriondecidual interface, which could descend into cervical secretions and therefore, result in an elevated concentration of this protein.

It is noteworthy that, among patients with an amniocentesis-to-delivery interval < 3 days, the cervical concentration of fFN was not associated with the presence of intra-amniotic infection and/or inflammation or acute

histologic chorioamnionitis. Therefore, the presence of an elevated fFN in cervical secretions is likely to reflect primarily activation of the decidua-membrane component of the common pathway of parturition, which can be induced by infection, inflammation or other pathologic processes leading to spontaneous preterm labor [148].

The findings of our study in the context of what is known

Our results would appear to be at variance with that reported by other investigators [107] who found that a positive cervical fFN (cut-off: 50 ng/mL) was not associated with a positive culture for microorganisms in women with preterm labor with intact membranes. In that study, a positive fFN was predictive of SPTD regardless of the presence of intra-amniotic infection, which is similar to what we have reported herein. However, the focus of that study was the relationship between intra-amniotic infection and a positive or negative cervical fFN [107]. Our study examined the relationship between intra-amniotic inflammation and cervical fFN rather than purely intra-amniotic infection. We previously reported that intra-amniotic inflammation is more common than intra-amniotic infection in patients with preterm labor [102] and preterm PROM [122] and that both has a comparable rate of adverse pregnancy and neonatal outcomes. It is now recognized that intra-amniotic inflammation in the absence of a positive culture is often associated with sterile intra-amniotic inflammation [103, 149, 150] because of danger signals released by cellular stress [151–154].

Fetal fibronectin in asymptomatic patients in the second trimester and the likelihood of preterm delivery and pathologic evidence of intra-amniotic infection or inflammation

In asymptomatic patients in the mid-trimester (23–24 weeks of gestation), Goldenberg et al. [21] reported that patients who had a positive cervicovaginal fFN and delivered preterm had a higher frequency of acute histologic chorioamnionitis than those with a negative fFN. However, histologic examination of the placenta was performed in only 40 patients who delivered before 32 weeks among 2899 patients. We previously reported that in asymptomatic women in the mid-trimester with a positive cervical fFN test, only 5.3% (3/57) had intra-amniotic infection and/or inflammation [120].

Collectively, these findings suggest a high concentration of fFN in cervical secretion represents evidence of the disruption of the chorionic-decidual interface caused by several factors including inflammation, infection and other processes [6, 8].

In the current study, an elevated cervical fFN concentration was associated with preterm delivery at <7 days and <14 days in patients without intra-amniotic infection/inflammation but not in those with intra-amniotic infection/inflammation. One possible explanation is that intra-amniotic infection/inflammation may develop before the disruption of the chorionic-decidual interface, and progress rapidly to SPTD which may cause false-negative fFN results.

Prediction of spontaneous preterm delivery using a quantitative fetal fibronectin test

The identification of patients at low risk of preterm birth among asymptomatic women with an episode of preterm labor is important in clinical practice. fFN has a high negative predictive value for SPTD, and hence, its purported utility to avoid unnecessary admissions, treatment and medical cost [155, 156], although this has been recently questioned [157].

Recently, Bruijn et al. [35] reported that the performance of the quantitative fFN test was equal to the combination of cervical length and qualitative fFN. The authors reported that the risk of SPTD within 7 days was 2% with fFN concentrations <50 ng/mL, 11% with fFN concentrations between 50 and 199 ng/mL, 19% with fFN concentrations between 200 and 499 ng/mL and 36% with fFN concentrations ≥ 500 ng/mL. These findings are consistent with those reported herein that the higher the fFN concentration, the greater the risk of SPTD within 7 days. Other investigators have reported that different fFN concentration cut-off values are useful in distinguishing those patients at risk for preterm birth at <34 weeks' gestation and <14 days of sampling [33, 37].

A strong body of evidence showed that short cervical length is a good predictor of occurrence of spontaneous preterm birth [158–177] and associated with intra-amniotic inflammation [150, 178]. Our view is that a sonographic cervical length is an easy test to perform in labor and delivery units. The results are immediately available and do not require laboratory equipment, and sonography adds other information with prognostic value such as the presence of sludge [179–185] and absence of fetal breathing both of which have been reported to increase the risk of impending preterm delivery [186].

Various cut-offs of fetal fibronectin for prediction of spontaneous preterm delivery

An earlier study defined the concentration of cervicovaginal fFN ≥ 50 ng/mL as a positive result based on ROC curves for predicting SPTD [16]. The value of 50 ng/mL has been universally accepted. We decided to quantify cervical fFN because the absolute concentration may be more predictive than that provided by a single cut-off of 50 ng/mL. Our study shows that quantification is valuable and that fFN concentration ≥ 150 ng/mL has a better diagnostic performance in the identification of intra-amniotic infection and/or inflammation and the prediction of SPTD within 7 days than an fFN concentration ≥ 50 ng/mL.

Some investigators have reported the relationship between SPTD and fFN concentration ≥ 50 ng/mL [18–20, 22]. Goepfert et al. [108] noted that as fFN concentration increased from 20 ng/mL to 300 ng/mL, so did the risk of subsequent SPTD (in patients in the mid-trimester of pregnancy). Lu et al. [187] reported that a concentration of 100 ng/mL was associated with an increased risk of subsequent SPTD and proposed that the use of a single fFN cut-off of 50 ng/mL for defining a positive test in symptomatic women should be reevaluated. Other recent studies reported that the positive predictive value for preterm birth within 7 days and preterm birth <34 weeks of gestation increased by changing the threshold from 50 ng/mL to 200 ng/mL or 500 ng/mL, with a minimal effect on the negative predictive value of this test [33, 37]. Our observations support the conclusions by Goepfert et al. [108], Lu et al. [187], Abbott et al. [33] and Centra et al. [37].

Strengths and limitations

A strength of this study is that a relatively large population of patients was used to examine the relationship between the results of quantitative cervical fFN tests and the presence or absence of intra-amniotic infection/inflammation. A limitation of this study is that we did not measure cervical length or perform serial cervicovaginal fFN measurements.

Clinical implications

Thus far, the clinical utility of fFN has largely depended upon its negative predictive value in predicting impending preterm delivery. However, our observations suggest that quantification of the concentration of cervical fFN may be

useful in identifying patients at increased risk for intra-amniotic infection and/or inflammation and impending preterm delivery. Such quantification is now feasible, and consideration must be given to the potential value of extremely high fFN concentrations in cervicovaginal fluid.

Conclusion

Quantification of fFN in patients with an episode of premature labor has been recommended to identify patients at low risk for preterm delivery. Our observation suggests that the patients with a very high concentration of fFN in cervical secretions are at risk for intra-amniotic inflammation, intra-amniotic infection, impending preterm delivery and acute histologic lesions of placenta such as acute chorioamnionitis (a maternal inflammatory response) and funisitis (a fetal inflammatory response). Therefore, a high concentration of fFN could increase the index of suspicion of intra-amniotic inflammation and favor evaluation of amniotic fluid with amniocentesis.

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