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The clinical significance of a positive Amnisure test in women with preterm labor and intact membranes

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***Objective*: This study was conducted to examine the frequency and clinical significance of a positive Amnisure test in patients with preterm labor and intact membranes by sterile speculum exam. *Study design*: A retrospective cohort study was performed including 90 patients with preterm labor and intact membranes who underwent Amnisure tests prior to amniocentesis (< 72 h); most patients ($n = 64$) also underwent fetal fibronectin (fFN) tests. Amniotic fluid (AF) was cultured for aerobic/anaerobic bacteria and genital mycoplasmas and assayed for matrix metalloproteinase-8. *Results*: (1) the prevalence of a positive Amnisure test was 19% (17/90); (2) patients with a positive Amnisure test had significantly higher rates of adverse pregnancy and neonatal outcomes (e.g., impending preterm delivery, intra-amniotic infection/inflammation, and neonatal morbidity) than those with a negative Amnisure test; (3) a positive test was associated with significantly increased risk of intra-amniotic infection and/or inflammation, delivery within 7, 14, or 28 days and spontaneous preterm birth (< 35 weeks) among patients with a negative fFN test. *Conclusions*: A positive Amnisure test in patients with preterm labor and intact membranes is a risk factor for adverse pregnancy outcome, particularly in patients with a negative fFN test. A positive Amnisure test in patients without symptoms or signs of ROM should not be taken as an indicator that membranes have ruptured.**

Keywords: Adverse pregnancy outcome, infection, intact membranes, intra-amniotic inflammation, prematurity, preterm birth

Introduction

The Amnisure ROM test™ is approved by the Food and Drug Administration (FDA) for the diagnosis of rupture of the fetal membranes (ROM). This test measures the concentration of placental α-microglobulin-1 (PAMG-1) in cervicovaginal fluid. Since this protein is found in concentrations several thousand-fold greater in amniotic fluid (AF) than in cervicovaginal fluid (2,000–25,000 ng/mL versus 0.05–2.0 ng/mL [1–4]), it is an excellent biomarker for AF and, therefore, is useful in detecting ROM (diagnosed when cervicovaginal fluid concentration is > 5.0 ng/mL). Prior studies report that the Amnisure ROM test™ is both highly accurate and specific, with a sensitivity of 99% and a specificity of 88–100% [1–9]. Emerging evidence also suggests that the presence of PAMG-1 in cervicovaginal fluid may be predictive of test-to-delivery duration independent from ROM.

Previously, it was reported that nulliparous women in term labor *without* evidence of ROM who had a positive Amnisure test exhibited a shorter admission-to-delivery interval than patients with a negative Amnisure test [10]. Further, in a separate study [2], three of four patients identified with apparent "false positive" Amnisure ROM test results (presented with spontaneous preterm labor and intact membranes), delivered spontaneously within 72 h and the other within 7 days of the test result. Accordingly, it was hypothesized that the presence of PAMG-1 in cervicovaginal fluid may be independently associated with test-to-delivery duration, spontaneous preterm birth (sPTB) and perinatal morbidity.

Currently, determination of fetal fibronectin (fFN [11–13]) in cervicovaginal fluid and cervical length measurement [14–20] are considered optimal strategies for prediction of time-to-delivery among pregnancies at risk of preterm delivery. Several studies have concluded that fFN further improves the predictive ability of cervical length screening in predicting sPTB [12,21,22] although additional studies are necessary to identify more effective measures.

Prediction of time-to-delivery is clinically important among pregnancies at risk for preterm delivery, particularly in regard to administration of corticosteroids (which have optimal benefit within 24 h to 7 days of administration) [23]. In addition, patients at high risk for preterm birth should deliver in a tertiary care unit. Obstetricians are tasked with predicting time-to-delivery in managing patients at risk for preterm delivery, given the controversy about the use of repeated steroids [24–43].

The current study was undertaken to assess: (1) the clinical significance (risk of intra-amniotic infection/inflammation and/or neonatal morbidity) of a positive Amnisure test among women in preterm labor with intact membranes; and (2) whether this test can accurately predict test-to-delivery duration in patients with preterm labor and intact membranes independently and in combination with fFN.

Materials and methods

Study design and participants

This hypothesis-generating retrospective cohort study included patients who were admitted to Seoul National University

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Hospital between April 2005 and November 2010 and met the following criteria: (1) preterm labor (< 35 weeks of gestation) as determined using standard criteria (including uterine contractility, cervical ripening, and decidual/fetal membrane activation) and no evidence of ROM also determined by standard criteria (including leakage, pooling, nitrazine, and ferning); (2) singleton gestation; and (3) the Amnisure tests were performed before amniocentesis, which was done for the assessment of microbiological status of the amniotic cavity (< 72 h). Patients with preterm labor and clinical ROM were excluded; "clinical ROM" was defined if (1) leakage of AF from the cervical os was seen on speculum examination; or (2) two of the following three signs were present: pooling of AF in the vaginal fornix, a positive nitrazine test, and a positive ferning test.

Amnisure ROM test™

The Amnisure test (PAMG-1 immunoassay, Amnisure ROM test, N-Dia, New York, NY, USA) was performed in patients without clinical ROM prior to amniocentesis, according to the manufacturer's instructions, as described in previous reports [2,10].

Fetal fibronectin test

The fFN test was performed according to methods previously described [44,45]. The fFN concentration was determined with a commercially available enzyme-linked immunosorbent assay (ELISA) (Adeza Biomedical, Sunnyvale, CA, USA) with a sensitivity of < 20 ng/mL.

Amniotic fluid

After obtaining written informed consent from each patient, AF was collected by transabdominal amniocentesis under ultrasonographic guidance, and cultured for aerobic and anaerobic bacteria, as well as genital mycoplasmas (ureaplasmas [*Ureaplasma urealyticum and Ureaplasma parvum*] and *Mycoplasma hominis*). Remaining AF was centrifuged and stored in polypropylene tubes at −70°C until assayed. After delivery, matrix metalloproteinase-8 (MMP-8) was measured in stored AF, because previous studies indicated that it is a sensitive and specific index of inflammation [46,47]. Intra-amniotic inflammation was defined as an elevated AF MMP-8 concentration (> 23 ng/mL). The MMP-8 concentrations were measured with a commercially available ELISA (Amersham Pharmacia Bitech, Inc, Buck, UK). The sensitivity of the test was 0.3 ng/mL. Both intra- and inter-assay coefficients of variation were < 10%.

Diagnosis of significant neonatal morbidity

Significant neonatal morbidity was defined as the presence of any of the following conditions: proven congenital neonatal sepsis, respiratory distress syndrome, early pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade ≥ II), and necrotizing enterocolitis. These conditions were diagnosed according to definitions previously described in detail [48].

Statistical analysis

Proportions were compared with Fisher's exact test, and comparisons of continuous variables between groups were performed with a Mann–Whitney U test. The interval-to-delivery of patients who were delivered for maternal or fetal indications was treated as a censored observation, with a censoring time equal to the test-to-delivery interval; patients who delivered at term (> 37 weeks) were also considered censored observations. Cox proportional hazards modeling was used to compare the test-to-delivery interval between groups defined by Amnisure and fFN test results after adjustment for gestational age at testing, intra-amniotic infection and/or inflammation, and cervical dilatation. Multivariable logistic regression analysis was used to determine whether patients with a positive Amnisure test were more likely to deliver early than patients with a negative test after similarly adjusting for pertinent variables. Findings were compared to fFN test results where possible and stratified models were used to illustrate the predictive ability of Amnisure by fFN test result. A p value < 0.05 was considered significant. Amnisure and fFN screening performance metrics, including prevalence, positive predictive value (PPV), negative predictive value (NPV), positive/negative likelihood ratios, area under the receiver operating characteristic curve (AUC), sensitivity and specificity were also compared separately and stratified by fFN test result.

Human subjects consideration

The Institutional Review Board of the Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance (FWA) with the Office for Human Research Protections (OHRP) of the Department of Health and Human Services (DHHS) of the United States.

Results

During the study period, 96 patients met the inclusion criteria (preterm labor with intact membranes, singleton gestation, and the Amnisure ROM test™ performed within 72 h of amniocentesis). Six patients were lost to follow-up and their delivery outcome was not available, leaving 90 patients included in this study, of which 64 had fFN test results. The Amnisure ROM test™ was positive in 19% (17/90) of patients; of them, none exhibited evidence of leakage of AF from the cervical os or pooling of AF in the vaginal fornix upon speculum examination. Eight patients (47%) with positive Amnisure test results had a positive nitrazine test; among them, four consented to an intra-amniotic dye injection test during the amniocentesis. None exhibited evidence of ROM assessed 6 h after the dye injection.

Characteristics of the study population are presented in Table I. Patients with a positive Amnisure ROM test™ had a greater cervical dilatation than those with a negative test result. Patients with a positive Amnisure test were more likely to experience adverse outcomes (i.e., shorter test-to-delivery duration, preterm delivery, and neonatal morbidity) than those with a negative test (Table II). Of note, patients with a positive Amnisure test had a higher frequency of intra-amniotic infection and/or inflammation than those with a negative test ($p < 0.01$, see Table II). The median AF MMP-8 concentration in patients with a positive Amnisure test was

Table I. Characteristics of study population.

Characteristics	Negative Amnisure ($n = 73$)	Positive Amnisure ($n = 17$)	p value
Maternal age (years)†	30 ± 4	32 ± 3	NS
Nulliparity (n)	41 (56%)	8 (47%)	NS
Gestational age at test (week)†	28.0 ± 4.0	27.1 ± 4.7	NS
Cervical dilatation (cm)†	1 ± 1	2 ± 1	< 0.05
Antenatal corticosteroids (n)	45 (62%)	14 (82%)	NS

Note: †values are given as mean ± standard deviation.

also significantly higher than in patients with a negative test ($p < 0.001$, Figure 1).

Figure 2 displays the test-to-spontaneous preterm delivery interval according to the results of Amnisure and fFN tests. Patients with a positive Amnisure test had a significantly shorter median test-to-delivery interval than those with a negative Amnisure test ($p < 0.01$), and this difference remained significant after adjustment for gestational age at testing, intra-amniotic infection and/or inflammation, and cervical dilatation ($p < 0.05$).

Table II. The result of AF analysis and pregnancy outcomes.

Pregnancy characteristics/ outcomes	Negative Amnisure (n = 73)	Positive Amnisure (n = 17)	*p* value
Positive AF culture (*n*)	1 (1%)	2 (12%)	0.092
Intra-amniotic infection and/or inflammation (*n*)	17 (24%)	10 (59%)	< 0.01[a]
Gestational age at delivery (week) [b]	34.8 ± 4.9	29.8 ± 6.1	< 0.005
Test-to-delivery interval			
≤ 48 h	5 (7%)	7 (41%)	< 0.005[a]
≤ 7 days	13 (18%)	11 (65%)	< 0.001 [a]
≤ 2 week	16 (22%)	13 (77%)	< 0.001 [a]
≤ 4 week	23 (32%)	13 (77%)	< 0.005 [a]
Preterm delivery			
< 35 week	26 (36%)	12 (71%)	< 0.05 [a]
< 37 week	40 (55%)	15 (88%)	< 0.05 [a]
Birth weight (g)[b]	2399 ± 926	1609 ± 981	< 0.01
1-min Apgar score < 7	21/56 (38%)	12/17 (71%)	< 0.05 [a]
5-min Apgar score < 7	8/56 (14%)	9/17 (53%)	< 0.005 [a]
Perinatal death	4 (6%)	2 (12%)	NS
Admission to neonatal intensive care unit[c]	20/62 (32%)	11/15 (73%)	< 0.01 [a]
Significant neonatal morbidity[c]	6/62 (10%)	8/15 (53%)	< 0.005 [a]

Note: [a]significant after adjustment for gestational age at test;
[b]values are given as mean ± standard deviation;
[c]Thirteen infants were excluded from the analysis, because they died shortly after delivery as a result of extreme prematurity or were delivered at another institution, and thus, could not be evaluated with respect to the presence or absence of neonatal complications.

The shortest observed survival time occurred among patients having a negative fFN and positive Amnisure test result.

As indicated in Tables III and IV, the strength of association between the Amnisure and test-to-spontaneous delivery duration was greater than that observed with fFN; in contrast, the magnitude of association between Amnisure and sPTB or intra-amniotic infection/inflammation was less than that of fFN.

While the Amnisure test was not significantly predictive of selected outcomes among patients with a positive fFN test result, it was significantly predictive of spontaneous delivery within 48 h, 7 days and 14 days of test and also of intra-amniotic infection/inflammation and sPTB prior to 35 weeks' gestation in patients with a negative fFN test result, and these results remained significant even after adjustment for gestational age at time of testing

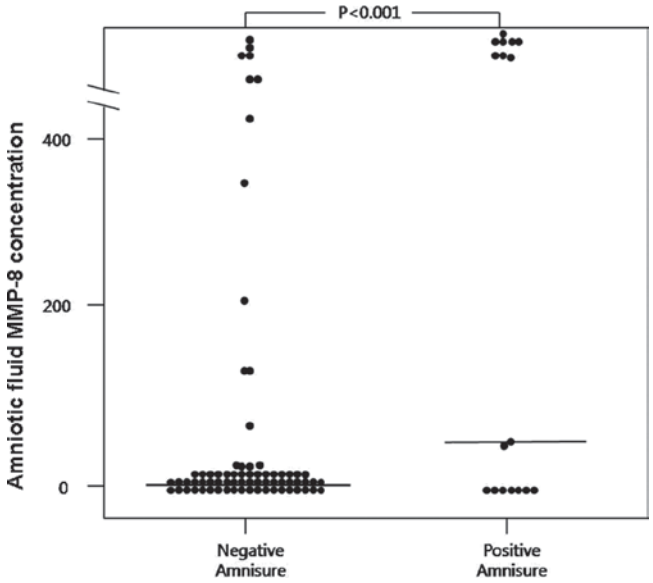

Figure 1. The AF MMP-8 concentrations according to the presence or absence of clinical evidence of ROM and the result of Amnisure test (a negative Amnisure test: median, 1.2 ng/mL [range: 0.3–2109.1 ng/mL]; a positive Amnisure test: median, 49.3 ng/mL [range: 0.3–5304.8 ng/mL]).

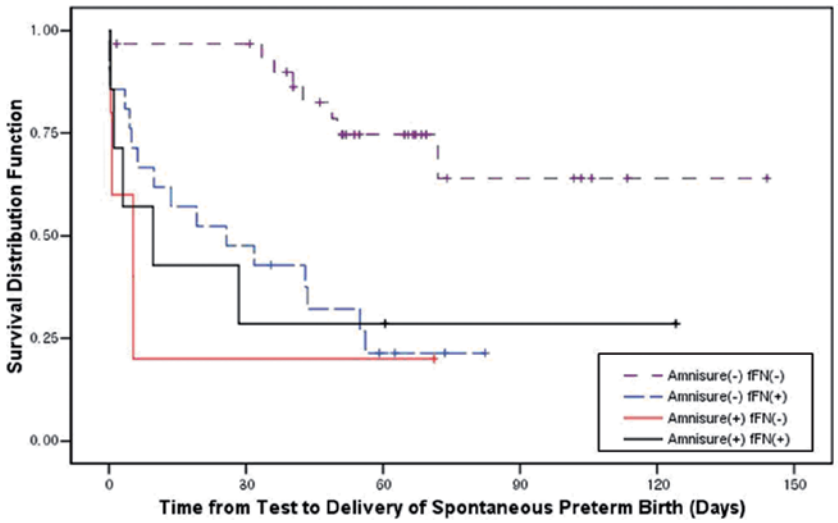

Figure 2. Kaplan–Meier survival distribution function by test result. [The median test-to-delivery duration and hazard ratio (95% confidence interval) for each group adjusted for gestational age at test, cervical dilatation, and intra-amniotic infection and/or inflammation are as follows: Amnisure (+) fFN (+): 9.7 days, 3.8 (1.1–12.8); Amnisure (+) fFN (−): 5.3 days, 5.0 (1.4–18.0); Amnisure (−) fFN (+): 25.7 days, 3.4 (1.3–8.5); Amnisure (−) fFN (−): 53.7 days, 1 (reference).]

Table III. Logistic regression analysis of Amnisure and Fibronectin tests in predicting time-to-spontaneous delivery in patients with preterm labor and intact membranes.

Test result			Test-to-spontaneous delivery duration					
			< 48 h (n = 10)[a]		< 7 days (*n* = 22)[b]		<14 days (*n* = 27)[c]	
			OR	CI	OR	CI	OR	CI
Amnisure test (*n* = 90)		(+)	9.7	2.2–43.3	7.0	2.0–24.1	9.4	2.4–36.4
		(−)	1		1		1	
Fibronectin test (*n* = 64)		(+)	1.8	0.3–9.9	3.2	0.9–11.7	5.2	1.4–19.8
		(−)	1		1		1	
Fibronectin test (+) (*n* = 28)	Aminsure test	(+)	1.6	0.2–15.1	1.4	0.2–8.4	1.7	0.3–9.7
		(−)	1		1		1	
Fibronectin test (−) (*n* = 36)	Aminsure test	(+)	35.6	1.1–>999	365.1	4.0–>999	365.1	4.0–>999
		(−)	1		1		1	

Note: All models adjusted for gestational length at time of test and cervical dilatation except those stratified by fibronectin result due to sample size restrictions. [a]Two patients who delivered preterm within 48 h by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis; [b]two patients who delivered preterm within 7 days by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis; [c]two patients who delivered preterm within 14 days by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis

Table IV. Logistic regression analysis of Amnisure and fibronectin tests in predicting intra-amniotic infection and/or inflammation and sPTB in patients with preterm labor and intact membranes.

			Intra-amniotic infection and/or inflammation (*n* = 27)		Spontaneous preterm delivery			
					< 35 weeks [a] (*n* = 34)		< 37 weeks [b] (*n* = 46)	
Test result			OR	CI	OR	CI	OR	CI
Amnisure test (*n* = 90)		(+)	3.9	1.01–15.1	2.7	0.7–10.3	3.6	0.6–20.6
		(−)	1		1		1	
Fibronectin test (*n* = 64)		(+)	8.5	1.6–46.9	7.3	1.8–29.5	9.8	2.2–43.5
		(−)	1		1		1	
Fibronectin test (+) (*n* = 28)	Aminsure test	(+)	0.5	0.06–5.3	0.4	0.1–2.2	2.3	0.2–28.1
		(−)	1		1		1	
Fibronectin test (−) (*n* = 36)	Aminsure test	(+)	25.2	1.7–363.8	66.7	2.8–>999	10.7	0.97–117.9
		(−)	1		1		1	

Note: All models adjusted for gestational length at time of test and cervical dilatation except those stratified by fibronectin result due to sample size restrictions. [a]Four patients who delivered preterm (before 35 weeks) by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis; [b]nine patients who delivered preterm before 37 weeks by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis.

(due to sample size restrictions the authors were unable to additionally adjust for cervical dilatation).

Tables V and VI report the diagnostic performance metrics for Amnisure and fFN independently and Amnisure stratified by fFN test result. The likelihood ratio of a positive Amnisure test was greater than that of fFN in prediction of spontaneous delivery within 48 h, 7 days and 14 days of test; however, the Amnisure test was less sensitive than fFN determination. Interestingly, the Amnisure test was both highly sensitive and specific in detecting sPTB within 7 and 14 days of test (sensitivity 80%, specificity 97%) among patients with negative fFN tests. Moreover, the likelihood ratio of a positive Amnisure test for spontaneous delivery within 14 days was 365.1, and the likelihood ratio for a negative result was 0.003 in this subgroup: while these estimates are extreme (likely due to our sample size), the statistical and clinical significance are noteworthy.

Table VII describes the clinical course of women with a positive Amnisure test. Most cases delivered after progression of spontaneous preterm labor, and none of these cases were delivered for a suspicion of ROM due to a positive Amnisure test without clinical evidence of ROM.

Discussion

Principal findings of this study

(1) An Amnisure test was positive in nearly one of five patients with preterm labor and intact membranes, and these patients were at greater risk for preterm delivery, intra-amniotic infection/inflammation, and other adverse pregnancy outcomes than patients with negative test results; (2) patients with a positive Amnisure test result had a shorter test-to-delivery interval than those with a negative test; (3) patients with a positive Amnisure test and negative fFN test had the shortest test-to-delivery interval observed in this study; and (4) a positive Amnisure test was significantly predictive of time to delivery, intra-amniotic infection/inflammation, and sPTB among patients with a negative fFN test. These findings are consistent with and extend those of our prior investigations of term pregnancies [2,10].

Mechanism of a positive Amnisure test in preterm labor with intact membranes

Two possible explanations of the mechanism are proposed whereby patients with preterm labor and intact membranes have a positive Amnisure test. First, in both term and preterm labor, the inflammatory process within the chorioamniotic membranes [49–53] may induce weakness or micro-perforations of the membranes through which a small amount of AF can leak. Strong evidence also suggests that intra-amniotic inflammation and infection, which is more prevalent among patients in term and preterm labor than in patients not in labor [49–51,53–73], is associated with the increased bioavailability of enzymes that participate in the degradation of the extracellular matrix of the fetal membranes [46,47,56,68,74–145]. Further, incubation of fetal membranes with pro-inflammatory cytokines leads to biophysical change which favors membrane weakening [146]. Hence, labor and intra-amniotic infection and/or inflammation may lead to microscopic perforation of the amniotic membranes, resulting in microleakage of AF which can be detected by the Amnisure test but not by conventional tests for ROM.

Second, an alternative explanation is that the increased intrauterine pressure during uterine contractions results in transudation of AF through pre-existing pores in the fetal membranes. Mann et al. [147] demonstrated that chorioamniotic membranes of ovine fetuses have pores which allowed passage of molecules of up to 69 kD. Thus, during labor, PAMG-1, which has a molecular weight of only 34 kD, could leak through pores in the fetal membranes explaining the association between positive Amnisure test and time to delivery.

Table V. Amnisure and fibronectin test time-to-delivery screening performance metrics overall and stratified by fibronectin test result

Outcome	Metric	Amnisure test ($n=90$)	Fibronectin test ($n=64$)	Amnisure Among fFN (−) ($n=36$)	Amnisure Among fFN(+) ($n=28$)
Test-to-spontaneous delivery < 48 h[a]	Prevalence	11.4%	12.7%	8.6%	17.9%
	PPV	37.5%	17.9%	40.0%	28.6%
	NPV	94.4%	91.4%	96.7%	85.7%
	LR+*	9.71†	1.79	35.6†	1.62
	LR−*	0.10†	0.56	0.03†	0.62
	Sensitivity	60.0%	62.5%	66.7%	40.0%
	Specificity	87.2%	58.2%	90.6%	78.3%
Test-to-spontaneous delivery < 7 days[b]	Prevalence	25.0%	23.8%	14.3%	35.7%
	PPV	62.5%	35.7%	80.0%	42.9%
	NPV	83.3%	85.7%	96.7%	66.7%
	LR+*	6.97†	3.18	365.1†	1.41
	LR−*	0.14†	0.31	0.003†	0.71
	Sensitivity	45.5%	66.7%	80.0%	30.0%
	Specificity	90.9%	62.5%	96.7%	77.8%
Test-to-spontaneous delivery < 14 days[c]	Prevalence	30.7%	28.6%	14.3%	46.4%
	PPV	75.0%	46.4%	80.0%	57.1%
	NPV	79.2%	85.7%	96.7%	57.1%
	LR+*	9.41†	5.23†	365.1†	1.69
	LR−*	0.11†	0.19†	0.003†	0.59
	Sensitivity	44.4%	72.2%	80.0%	30.8%
	Specificity	93.4%	66.7%	96.7%	80.0%

Note: fFN = fetal Fibronectin; AUC = area under receiver operating characteristic curve;
*adjusted for gestational length at time of test and cervical dilatation except those stratified by fibronectin result due to sample size restrictions;
†significant after adjustment;
[a]two patients who delivered preterm within 48 h by augmentation of labor/cesarean delivery due to maternal-fetal indications were excluded;
[b] two patients who delivered at preterm within 7 days by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded;
[c]two patients who delivered at preterm within 14 days by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded.

Table VI. Amnisure and fibronectin test sPTB screening performance metrics overall and stratified by fibronectin test result.

Outcome	Metric	Amnisure test ($n=90$)	Fibronectin test ($n=64$)	Amnisure Among fFN (−) ($n=36$)	Amnisure Among fFN(+) ($n=28$)
Intra-amniotic infection/and/or inflammation	Prevalence	30.7%	28.1%	16.7%	42.9%
	PPV	58.8%	42.9%	60.0%	28.6%
	NPV	76.1%	83.3%	90.3%	52.4%
	LR+*	3.91†	8.55†	25.2†	0.54
	LR−*	0.26†	0.12†	0.04†	1.84
	Sensitivity	37.0%	66.7%	50.0%	16.7%
	Specificity	88.5%	65.2%	93.3%	68.8%
sPTB <35 weeks[a]	Prevalence	39.5%	39.7%	22.9%	60.7%
	PPV	68.8%	60.7%	80.0%	42.9%
	NPV	67.1%	77.1%	86.7%	33.3%
	LR+*	2.71	7.35†	66.7†	0.38
	LR−*	0.37	0.14†	0.02†	2.62
	Sensitivity	32.4%	68.0%	50.0%	17.6%
	Specificity	90.4%	71.1%	96.3%	63.6%
sPTB <37 weeks[b]	Prevalence	56.8%	54.1%	35.3%	77.8%
	PPV	86.7%	77.8%	80.0%	83.3%
	NPV	50.0%	64.7%	72.4%	23.8%
	LR+*	3.59	9.83†	10.7	2.26
	LR−*	0.28	0.10†	0.1	0.44
	Sensitivity	28.3%	63.6%	33.3%	23.8%
	Specificity	94.3%	78.6%	95.5%	83.3%

Note: [f]FN = fetal Fibronectin;
*adjusted for gestational length at time of test and cervical dilatation except those stratified by fibronectin result due to sample size restrictions;
†significant after adjustment;
[a]four patients who delivered preterm before 35 weeks by augmentation of labor or cesarean delivery due to maternal-fetal indications were excluded from this analysis;
[b]nine patients who were delivered at preterm before 37 weeks by augmentation

Table VII. The clinical course of women with a positive Amnisure test.

Case	GA at test (wks)	GA at delivery (wks)	AF culture	AF MMP-8	Cervical dilatation at test	Delivery mode	Clinical course
1	29.9	29.9	(−)	0.3	0	C/S	Placenta previa and massive vaginal bleeding with labor pain and fetal distress
2	25.9	25.9	(−)	918.4	1	VD	Clinical chorioamnionitis and spontaneous labor progression
3	25.6	25.7	(−)	5304.8	2	VD	Spontaneous labor progression
4	25	25	(−)	7.0	2	C/S	Spontaneous labor progression and breech presentation
5	28.4	28.6	(−)	7.9	2	C/S	Spontaneous labor progression and placenta previa
6	19.3	19.4	(−)	49.3	5	VD	Bag bulging and labor progression
7	26.3	26.6	(+)	1574.3	0	VD	Induction of labor because of suspicion of chorioamnionitis
8	33.6	34	(−)	9.1	1	VD	Spontaneous labor progression
9	26.6	27.1	(−)	1988.9	1	VD	Spontaneous labor progression
10	25	25.7	(−)	744.1	2	C/S	Spontaneous labor progression and breech presentation
11	28.3	29	(−)	900.4	2	C/S	Spontaneous labor progression and previous C/S
12	21.9	23	(+)	3522.4	3	VD	Spontaneous labor progression
13	33.9	35.3	(−)	3.7	2	VD	Spontaneous ROM at 35 + 1 weeks and induction of labor
14	31	35	(−)	43.5	2	VD	Spontaneous ROM at 35weeks and subsequent spontaneous labor progression
15	32.7	41.3	(−)	0.5	0	VD	Term delivery
16	30.3	40.1	(−)	0.8	1	VD	Term delivery
17	17.4	35.1	(−)	1815.6	1	VD	Induction of labor at 35 weeks because of suspicion of chorioamnionitis

Note: GA: gestational age, AF: amniotic fluid, MMP-8: matrix metalloproteinase-8, NA: not available, VD: vaginal delivery, C/S: cesarean section, ROM: rupture of membranes.

Erdemoglu et al [148]. evaluated the clinical significance of insulin-like growth factor binding protein-1 in cervicovaginal fluid among patients with suspected premature rupture of membranes (PROM) (patients with a history of PROM but no clinical evidence of PROM by speculum examination) and demonstrated that only a positive test (detecting insulin-like growth factor binding protein-1 in cervicovaginal fluid) was associated with delivery within 7 days. This study also supports that microscopic ROM may occur in patients at risk for preterm delivery.

Limitations

Potential limitations include the sample size and missing fFN data ($n = 26$). Patients missing fFN tests were no more likely to experience any of the study outcomes, nor did they differ with respect to descriptive characteristics from the patients having fFN determinations (meaning it is unlikely that missing data introduced differential bias in this study). Further, it might be argued that the association between a positive Amnisure test and perinatal morbidity may be attributed to the suspicion of ROM in the study population (confounding by indication). However, 10 out of 11 cases with a positive Amnisure test who delivered within 7 days of test (case nos. 1–11 in Table IV) were delivered because of spontaneous progression of labor despite tocolysis administration. The single remaining case delivered after induction of labor because of suspicion of chorioamnionitis and not suspicion of clinical ROM.

Conclusion

This study provides evidence that: (1) a positive Amnisure test increases the risk for intra-amniotic infection/inflammation, spontaneous preterm delivery and neonatal morbidity in patients with preterm labor and intact membranes; and (2) the Amnisure test may also be clinically useful as a second tier test following fFN determination in predicting time-to-delivery and perinatal morbidity.

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