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A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery

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Prematurity

Objective: Matrix metalloproteinase-8 (MMP-8) is an enzyme that is released during neutrophil activation. MMP-8 amniotic fluid concentrations are elevated not only in patients with intra-amniotic infection, but also in patients with negative amniotic fluid cultures who deliver preterm neonates. The objective of this study was to determine whether the results of a rapid MMP-8 bedside test predict imminent preterm delivery. This test can be performed in 15 minutes and without laboratory equipment.

Study design: Amniotic fluid was retrieved from 331 patients admitted with increased preterm uterine contractions and intact membranes who met the inclusion criteria. Amniotic fluid was processed for microbial cultures, Gram stain, glucose concentration, and white blood cell count. Amniotic fluid samples were stored, and the MMP-8 rapid test was performed after delivery. End points included spontaneous preterm delivery within 48 hours, 7 days, and 14 days. Diagnostic indices, predictive values, and likelihood ratios were calculated.

Results: The prevalence of spontaneous preterm delivery within 48 hours, 7 days, and 14 days was 11.6% (38/327), 20.2% (66/327), and 24.5% (80/327), respectively (4 patients with

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Conflict of Interest: The test described in this article is the subject of a patent application by the Seoul National University in Seoul, Korea. Dr Bo Yoon, one of the coauthors and a professor at the Seoul National University, is listed as an inventor in this patent.

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augmentation of labor were excluded). A positive MMP-8 rapid test had a positive predictive value of 70% (23/33) for the identification of patients who delivered spontaneously within 48 hours, and 94% (31/33) for patients who were delivered within 7 days and 14 days (likelihood ratios: 17.5 [95% CI, 9-33.9], 61.3 [95% CI, 15.1-250], and 50 [95% CI, 12-196], respectively).
Conclusion: The MMP-8 rapid test can identify patients at risk for preterm delivery within 7 days and 14 days. Moreover, a positive MMP-8 rapid test result can identify patients with intra-amniotic infection/inflammation with a high sensitivity and specificity. This rapid test will give clinicians a fast and accurate assessment of the inflammatory status of the amniotic cavity and allow for better identification of patients at risk for impending preterm delivery.

Intra-amniotic infection/inflammation is causally linked to preterm labor/delivery and fetal injury.[1-5] Intra-amniotic infection (IAI) is a risk factor for impending preterm delivery,[1,2] spontaneous rupture of membranes,[6-8] clinical chorioamnionitis,[2] and adverse short- and long-term neonatal outcome.[2,3,9-12] The traditional method for the detection of infection is isolation of microorganisms from amniotic fluid (AF). However, this requires time, and results may not be available for appropriate patient treatment. Recent evidence has demonstrated that patients with intra-amniotic inflammation, but a negative AF culture, have a similar outcome to patients with a positive AF culture.[13,14] Therefore, the detection of inflammation may be more practical than the detection of infection in patient management. Currently, the diagnosis of intra-amniotic inflammation requires a laboratory test.

Recently, a bedside test has been developed to detect intra-amniotic inflammation that is based on the detection of an elevated concentration of matrix metalloproteinase-8 (MMP-8) in AF. The selection of MMP-8 was based on previous studies performed with standard enzyme-linked immunosorbent assay (ELISA) techniques.[14,15] The configuration of the MMP-8 PTD (preterm delivery) Check test (SK Pharma Co, Ltd, Kyunggi-do, Korea) is similar to a rapid pregnancy test (Figure 1). It requires 20 μL of AF and no laboratory equipment, and the results are available within 15 minutes. This test has a sensitivity of 95% and a specificity of 93% in the detection of intra-amniotic inflammation among patients with spontaneous preterm labor and intact membranes.[16] This study was designed to determine the diagnostic indices, predictive values, efficiency and likelihood ratios of the MMP-8 PTD Check test for the detection of IAI, intra-amniotic inflammation, spontaneous preterm delivery, and severe neonatal morbidity.

Methods

Study population

Patients admitted between January 1998 and October 2003 to the Sotero del Rio Hospital (Santiago, Chile) with the diagnosis of increased uterine contractility and intact membranes were asked to participate in a prospective cohort study that was designed to examine the relationship between clinical, biochemical, and biophysical parameters and the risk of preterm delivery. The inclusion criteria were (1) singleton gestation, (2) a live fetus whose gestational age was between 22 and 35 weeks, (3) cervical dilation of $\leq$3 cm by digital examination, (4) intact membranes, and (5) a signed informed consent that had been approved by the Institutional Review Boards of both the Sotero del Rio Hospital and the National Institute of Child Health and Human Development (NIH/DHHS). The study procedures and clinical management are described in the supplemental material to the article.

Sample collection and MMP-8 PTD Check rapid test

AF was transported to the laboratory in a capped plastic syringe and cultured for aerobic and anaerobic bacteria and genital *Mycoplasmas*. A white blood cell count, glucose concentration, and Gram stain for microorganisms were performed in AF immediately after collection. The remaining sample was stored at −70°C. Details about the MMP-8 PTD Check, MMP-8, and interleukin-6 (IL-6) determinations by ELISA are available in the supplemental material.

Study outcomes

Primary outcome variables were the occurrence of spontaneous preterm delivery (within 48 hours, 7 days, and 14 days of admission), the presence of a positive AF culture, and AF inflammation (defined as an AF IL-6 concentration of $\geq$2.6 ng/mL).[13] Secondary outcomes included spontaneous preterm delivery at $\leq$32 weeks of gestation, at $\leq$34 weeks of gestation, and composite neonatal morbidity (defined as the presence of any of the following conditions: respiratory distress syndrome, suspected or proven neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage, periventricular leukomalacia, and necrotizing enterocolitis). The statistical analysis is described in the supplemental material.

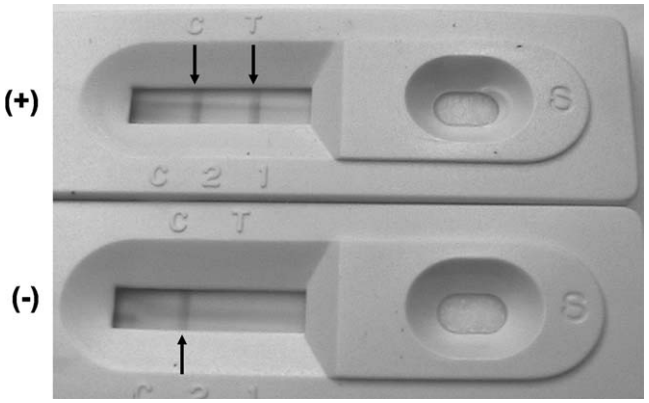

Figure 1 The MMP-8 PTD Check test was considered positive when 2 dark bands were visible, corresponding to the control (*C*) and testing (*T*) channels of the kit (*arrows*).

Results

Study population

Over the study period, 331 patients met the inclusion criteria. An MMP-8 PTD Check test was performed in all AF samples. Table I shows the clinical characteristics of the study population. The prevalence of IAI, intra-amniotic inflammation, and preterm delivery (<37 weeks of gestation) were 7.3% (24/331), 11.5% (38/331), and 41.1% (136/331), respectively. Four patients received augmentation and were excluded from further analysis. The prevalence of spontaneous preterm delivery within 48 hours, 7 days, and 14 days was 11.6% (38/327), 20.2% (66/327), and 24.5% (80/327), respectively. Tocolysis was used in 62.8% (208/331) of the population, and a positive MMP-8 rapid test result was detected in 11% of the cases (36/331). Table II displays the microorganisms that were isolated from the AF of patients with IAI. The most frequent isolate was *Ureaplasma urealyticum*.

MMP-8 rapid test and ELISA agreement

There was nearly perfect agreement[17] between the MMP-8 PTD Check and the results of the AF MMP-8 concentrations as determined by ELISA (Kappa, 0.87; $P < .001$).

MMP-8 PTD Check in the identification of IAI and intra-amniotic inflammation

The efficiency of a positive MMP-8 rapid test result in the identification of IAI and intra-amniotic inflammation was 94% (311/331) and 97% (321/331), respectively. Table III displays the prevalence, diagnostic indices, predictive values, and likelihood ratios of a positive MMP-8 rapid test in the identification of IAI and inflammation in the study population.

Table I Clinical characteristics of the study population

Characteristic	Preterm labor and intact membranes (n = 331)
Maternal age (y)*	25 ± 7
Gestational age at admission (wk)*	32 ± 2
Microbial invasion of the amniotic cavity (%)†	7.3 (24/331)
Gestational age at delivery (wk)*	36 ± 3
Delivery at <37 weeks of gestation (%)†	41.1 (136/331)

* Values expressed as mean ± SD.
† Numbers in parentheses represent the proportion.

Table II Type of microorganisms that were retrieved from AF samples

Microorganism	Positive AF culture (n = 24)*
Ureaplasma urealyticum	45.8 (11/24)
Mycoplasma hominis	29.2 (7/24)
Candida species	12.5 (3/24)
Group B streptococci	8.3 (2/24)
Gram-negative bacilli	4.2 (1/24)
Gram-positive bacilli	4.2 (1/24)
Prevotella species	4.2 (1/24)
Listeria monocytogenes	4.2 (1/24)

Values are given as percentage (proportion).
* Some AF cultures yielded more than 1 microorganism.

MMP-8 PTD Check in the identification of patients at risk for impending preterm delivery

The prevalence, diagnostic indices, predictive values, and likelihood ratios (positive and negative) in the identification of patients delivered within 48 hours, 7 days, 14 days and at <32 and <34 weeks of gestation are displayed in Table IV. The efficiency of a positive MMP-8 rapid test in the identification of patients delivered within 48 hours, 7 days, 14 days and at <32 and <34 weeks of gestation was 92.4% (302/327), 88.7% (290/327), 84.4% (276/327), 88.8% (135/152), and 86% (234/272), respectively.

Results of MMP-8 PTD Check and interval-to-delivery

Survival analysis indicated that patients with a positive MMP-8 rapid test result had a significantly shorter amniocentesis-to-delivery interval than patients with a negative MMP-8 rapid test result (mean, 3 days [95% CI, 1-4 days] vs 41 days [95% CI, 38-44 days]; log rank, $P < .001$; Figure 2). Cox proportional modeling, adjusted for cervical length, tocolysis, antibiotic administration, and gestational age at amniocentesis, indicated that this result remained significant after being controlled for these covariates (hazard ratio: 12.43; 95% CI, 7.5-20.5).

Table III Diagnostic indices, predictive values, and likelihood ratios of MMP-8 PTD Check results for the detection of IAI and inflammation

	Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Intra-amniotic infection*	7.3 (24/331)	83 (20/24)	95 (291/307)	56 (20/36)	99 (291/295)	15.9 (9.6-26.6)	0.2 (0.1-0.3)
Intra-amniotic inflammation†	11.5 (38/331)	84 (32/38)	99 (289/293)	89 (32/36)	98 (289/295)	61.7 (23.1-164.8)	0.2 (0.1-0.4)

* A positive AF culture for microorganisms.
† AF IL-6 concentration, ≥2.6 ng/mL.

Table IV Diagnostic indices, predictive values, and likelihood ratios of MMP-8 PTD Check for the identification of patients with spontaneous preterm delivery within 48 hours, 7 days, 14 days and at <32 and <34 weeks of gestation*

	Prevalence (%)†	Sensitivity (%)†	Specificity (%)†	Positive predictive value (%)†	Negative predictive value (%)†	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Delivery within 48 hours	11.6 (38/327)	61 (23/38)	97 (279/289)	70 (23/33)	95 (279/294)	17.5 (9-33.9)	0.4 (0.2-0.8)
Delivery within 7 days	20.2 (66/327)	47 (31/66)	99 (259/261)	94 (31/33)	88 (259/294)	61.3 (15.1-250)	0.5 (0.1-2.2)
Delivery within 14 days	24.5 (80/327)	39 (31/80)	99 (245/247)	94 (31/33)	83 (245/294)	50 (12-196)	0.6 (0.2-2.5)
Delivery at <32 weeks of gestation	21.1 (32/152)	56 (18/32)	98 (117/120)	86 (18/21)	89 (117/131)	22.5 (7.1-71.7)	0.5 (0.1-1.4)
Delivery <34 weeks of gestation	22.4 (61/272)	44 (27/61)	98 (207/211)	87 (27/31)	86 (207/241)	23.4 (8.5-64.2)	0.6 (0.2-1.6)

* Four patients whose condition required augmentation were excluded from this analysis.
† Numbers in parentheses represent proportions.

MMP-8 PTD Check results and composite neonatal morbidity

Severe neonatal morbidity was present in 4.8% (16/331) of the study population, including respiratory distress syndrome (3% [10/331]), proven neonatal sepsis (1.2% [4/331]), pneumonia (2.4% [8/331]), bronchopulmonary dysplasia (1.5% [5/331]), intraventricular hemorrhage (0.9% [3/331]), periventricular leukomalacia (0.6% [2/331]), and necrotizing enterocolitis (0.6% [2/331]). The prevalence, sensitivity, specificity, positive and negative predictive values, and the likelihood ratios of a positive MMP-8 rapid test in the identification of composite neonatal morbidity are displayed in Table V.

Comment

Principal findings of the study

MMP-8 PTD Check is a sensitive and specific test for the identification of both IAI and inflammation among patients with preterm labor and intact membranes. A patient with a positive MMP-8 PTD Check result is at a substantial risk for spontaneous preterm delivery within 48 hours, 7 days, and 14 days, with likelihood ratios of a positive test ranging from 17 to 61 (Table IV).

A point-of-care test for the detection of intra-amniotic inflammation

Compelling evidence indicates that patients with intra-amniotic inflammation are at greater risk for impending preterm delivery and adverse perinatal outcome than patients without intra-amniotic inflammation.[13,14,18] Moreover, previous studies demonstrated that the pregnancy outcome of women with microbiologically proven IAI is similar to those with intra-amniotic inflammation, but without positive AF cultures for microorganisms.[13,14] Clinical application requires that a method for the detection of intra-amniotic inflammation be easily available to the clinician for 24-hour testing, hence the need for point-of-care testing.

The College of American Pathologists defines *point-of-care testing* as "analytical patient testing activities

Table V Diagnostic indices, predictive values, and likelihood ratios of MMP-8 PTD Check for the identification of composite severe neonatal morbidity

Variable	Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Composite neonatal complications	4.8 (16/331)	63 (10/16)	92 (289/315)	28 (10/36)	98 (289/295)	7.6 (4.5-12.9)	0.4 (0.2-0.7)

provided within the institution, but performed outside the physical facilities of the clinical laboratories. It does not require permanent dedicated space, but instead includes kits and instruments, which are either hand carried or transported to the vicinity of the patient for immediate testing at that site."[19,20] Point-of-care testing is considered important to increase clinical efficiency and improve medical and economic outcomes.[21] The MMP-8 PTD Check fulfills most of the criteria proposed to assess an optimal point-of-care testing,[19] namely: (1) simple testing method, (2) rapid availability of the results (up to 15 minutes), (3) easy interpretation of the results (Figure 1), (4) low maintenance, because the kit can be stored at room temperature, (5) strong correlation with standard laboratory procedures, and (6) low cost, because there is no need for capital equipment and because the market price can be driven by need.

Strength and weakness of the study

Major strengths of the study are: (1) it includes a large cohort of women with preterm labor and intact membranes in a homogeneous population (n = 331 women); (2) the cohort is not contaminated with patients who have a different rate of IAI and intra-amniotic inflammation, such as those with preterm premature rupture of membranes (PROM). Studies with a mixed population (preterm labor with intact membranes and preterm PROM) may require adjustment for the estimation of predictive values that depend on the prevalence of the disease/conditions; and (3) the test was not used in patient treatment. It could be argued that a weakness of this study is that it was conducted with AF which was stored at −70°C. However, validation of the test performed by the biotechnology company before the release of this product indicates substantial agreement between results in fresh and freeze-thawed AF. The optimal treatment of a patient with IAI and/or inflammation and the barriers to clinical research of these particular sets of issues have been discussed elsewhere.[22]

Previous studies of AF MMP-8 concentrations in women with preterm labor and intact membranes

The results of the current study are consistent with previous reports indicating that an elevated AF MMP-8 concentration is associated with impending preterm delivery, IAI, and severe neonatal morbidity.[15,23,24]

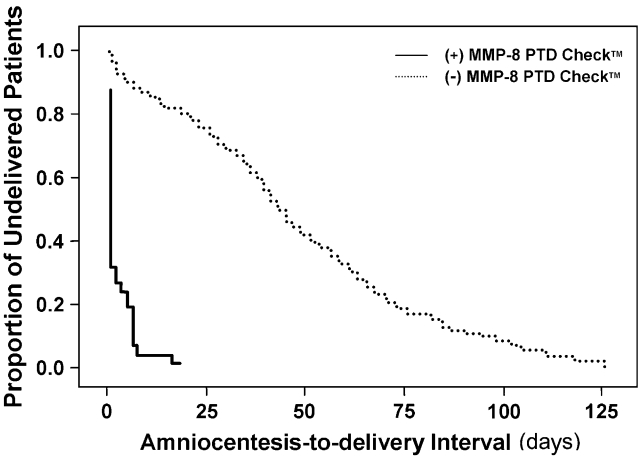

Figure 2 Patients with a positive MMP-8 rapid test result had a significantly shorter amniocentesis-to-delivery interval than patients with a negative MMP-8 rapid test result (mean, 3 days [95% CI, 1-4 days] vs 41 days [95% CI, 38-44 days], respectively; log rank, $P < .001$).

Clinical implication of this study

There is now compelling evidence that fetal exposure to IAI and inflammation is associated with adverse outcomes that include severe neonatal morbidity, perinatal death, and long-term handicap (such as cerebral palsy and chronic lung disease). Thus, the optimal treatment of patients with preterm labor would require knowledge of whether there is IAI and/or inflammation. The MMP-8 PTD Check test can determine the presence of intra-amniotic inflammation within minutes and does not require laboratory equipment. Follow-up clinical trials are required to determine the role of the MMP-8 rapid test for the identification of intra-amniotic inflammation at the time of amniocentesis, as well as to determine whether, based on the rapid test results, treatment with antibiotics and/or anti-inflammatory agents may improve pregnancy outcome.

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