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The antenatal identification of funisitis with a rapid MMP-8 bedside test

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Abstract

AIMS—The purpose of this study was to determine if a bedside test, the MMP-8 PTD Check™, can be of value in the antenatal identification of funisitis. This test can be performed in 15 minutes without any laboratory equipment.

METHODS—The relationship between the presence or absence of funisitis and the results of a MMP-8 PTD Check™ was examined in 139 patients who delivered preterm singleton neonates (gestational age <35 weeks) within 72 hours of amniocentesis. Amniotic fluid (AF) was cultured for aerobic and anaerobic bacteria and for genital mycoplasmas. AF was analyzed for white blood cell (WBC) count, interleukin-6 (IL-6) and a MMP-8 PTD Check™. The IL-6 concentration was also determined in umbilical cord plasma collected at birth. Funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly.

RESULTS—1) Funisitis was present in 27% (38/139) of cases; 2) A positive MMP-8 PTD Check™ had a sensitivity of 97% (37/38), a specificity of 63% (64/101), a positive predictive value of 50% (37/74) and a negative predictive value of 99% (64/65) in the identification of funisitis; 3) Among cases without funisitis, patients with a positive MMP-8 PTD Check™ had a significantly higher median AF IL-6 concentration, AF WBC count, and umbilical cord plasma IL-6 concentration at birth than those with a negative MMP-8 PTD Check™ ($p<0.05$ for each).

CONCLUSIONS—The MMP-8 PTD Check™ is a rapid, simple and sensitive bedside test which allows assessment of the risk of funisitis.

Keywords

bed side test; chorioamnionitis; funisitis; MMP-8; preterm gestation

Introduction

Funisitis is diagnosed in the presence of neutrophil infiltration of the umbilical vessels or Wharton's jelly, and the histologic hallmark of fetal systemic inflammation [13, 23, 24]. Funisitis is temporally the most advanced phase in the ascending intrauterine infection [16], and is associated with an increased risk of neonatal infection-related morbidity[13, 23] and long-term handicap such as cerebral palsy [22].

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Neutrophils in amniotic fluid (AF) are considered to be of fetal origin [17] and can release matrix metalloproteinase-8 (MMP-8), also called neutrophil collagenase, during inflammation[2, 7, 18]. Therefore, an elevated MMP-8 in AF may indicate fetal systemic inflammation.

It is well documented that an elevated concentration of AF MMP-8 is a sensitive and powerful predictor of intra-amniotic infection and/or inflammation [1, 8–10]. Moreover, recent reports have demonstrated that a rapid MMP-8 bedside test is valuable in the identification of intra-amniotic infection and/or inflammation among patients with preterm labor and intact membranes or preterm premature rupture of membranes [6, 12]. However, it has not been assessed yet that a rapid MMP-8 bedside test is valuable in the antenatal identification of funisitis. To this end, the current study was designed to determine the diagnostic performance of a rapid MMP-8 bedside test (MMP-8 PTD Check™; SK Parma Co, Ltd, Kyunggi-do, Korea) in the antenatal identification of funisitis in preterm gestation.

Material and Methods

Study design

The relationship between the presence of funisitis and the results of a MMP-8 PTD Check™ was examined in 139 patients who delivered preterm singleton neonates (gestational age < 35 weeks) within 72 hours of amniocentesis. This period of time was chosen to preserve a meaningful temporal relationship between the results of AF studies and the histologic findings of the umbilical cord obtained at birth. The cohort consisted of patients who delivered at the Seoul National University Hospital between January 1993 and December 1999. At this institution, amniocentesis for retrieval of AF was offered routinely to all patients who were admitted with the diagnosis of preterm labor or preterm premature rupture of membranes. AF was analyzed for microbiologic status and fetal lung maturity. Amniocentesis was also performed to assess fetal lung maturity in patients with pregnancy induced hypertension. This procedure was performed after written informed consent was obtained. The Institutional Review Board approved the collection and use of these samples and information for research purposes. Many of patients in this study were included in our previous studies.

AF and umbilical cord blood

AF was cultured for aerobic and anaerobic bacteria and for genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*), and analyzed for white blood cell (WBC) count. The remaining stored AF was analyzed for interleukin-6 (IL-6) and MMP-8 PTD Check™. IL-6 concentrations in AF were measured with a commercially available enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn., USA). The sensitivity of the test was <1.0 pg/ml. Both intra- and inter-assay coefficients of variation were <10%. Intra-amniotic inflammation (IAI) was defined as an elevated AF IL-6 concentration (≥ 2.6 ng/mL), as previously reported [20]. Umbilical cord blood was collected in ethylene-diaminetetraacetic acid-containing blood collection tubes by venipuncture of the umbilical vein at birth. Samples were then centrifuged, and supernatants were stored in polyprophylene tubes at −70° IL-6 concentrations in umbilical cord plasma were measured with a commercially available enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn., USA). The sensitivity of the test was 0.05 pg/mL. Both intra- and inter-assay coefficients of variation were < 10%.

Rapid MMP-8 bed side test

In 2007, the MMP-8 PTD Check™ was performed with stored AF by one of the authors (C.W.P) who was blinded to the results of the AF studies (i.e., culture results, WBC count,

and IL-6 concentrations, etc.) and pregnancy outcome. In the current study, we mixed 15 ul of AF and 120 ul of buffer (1:8 mixture) which is different from the original test. The cut-off value of the modified test is 20 ng/mL of MMP-8 which is approximately similar to the cut-off value of intra-amniotic inflammation identified and used in our previous reports (23 ng/ml) [14, 19]. The results are available within 15 minutes without any laboratory equipment other than a pipette at the bedside. Details about the test were described in a previous report [12].

Diagnosis of chorioamnionitis and funisitis

Histologic chorioamnionitis was defined in the presence of acute inflammatory changes on examination of a membrane roll and chorionic plate of the placenta; funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly with the use of criteria previously published [20]. Clinical chorioamnionitis was diagnosed according to the definitions previously described in detail [22].

Statistical analysis

The Mann-Whitney U test was used for comparison of continuous variables. Comparisons of proportions were performed with the Fisher's exact test. Logistic regression analysis was used to explore the effect of maternal age, gestational age at delivery, and presence or absence of rupture of membranes at amniocentesis on pregnancy outcome. Statistical significance was defined as a $p < .05$.

Results

Funisitis and MMP-8 PTD Check™

Funisitis was present in 27% (38/139) of cases. The MMP-8 PTD Check™ was positive in 97% (37/38) of cases with funisitis and in 37% (37/101) of cases without funisitis. Table 1 describes the diagnostic indices, predictive values, and likelihood ratios of a MMP-8 PTD Check™ for the identification of funisitis and histologic chorioamnionitis.

Clinical characteristics and pregnancy outcome of study population

Table 2 compares the clinical characteristics as well as gestational age at delivery of the study population according to the results of a MMP-8 PTD Check™ and the presence or absence of funisitis.

Figure 1 shows the frequency of a positive AF culture, intra-amniotic inflammation, histologic chorioamnionitis, and clinical chorioamnionitis according to the results of a MMP-8 PTD Check™ and the presence or absence of funisitis. Patients with funisitis had significantly higher rates of a positive amniotic fluid culture, intra-amniotic inflammation, histologic chorioamnionitis, and clinical chorioamnionitis than those without funisitis but with a positive MMP-8 PTD Check™, and also than those without funisitis and with a negative MMP-8 PTD Check™ (adjusted $p < 0.05$ for each, see Figure 1). Among cases without funisitis, patients with a positive MMP-8 PTD Check™ had significantly higher rates of intra-amniotic inflammation and histologic chorioamnionitis than those with a negative MMP-8 PTD Check™ (adjusted $p<0.001$ for each, see Figure 1). Each p-value was adjusted for maternal age, gestational age at delivery, and presence or absence of rupture of membranes at amniocentesis.

AF inflammation and umbilical cord plasma IL-6

Figure 2 demonstrates AF IL-6 concentration, AF WBC count and umbilical cord plasma IL-6 concentration according to the results of a MMP-8 PTD Check™ and the presence or

absence of funisitis. Patients with funisitis had a significantly higher median AF IL-6 concentration, AF WBC count and umbilical cord plasma IL-6 concentration at birth than those without funisitis ($p < 0.05$ for each, see Figure 2).Moreover, among cases without funisitis, patients with a positive MMP-8 PTD Check™ had a significantly higher median AF IL-6 concentration, AF WBC count and umbilical cord plasma IL-6 concentration at birth than those with a negative MMP-8 PTD Check™ ($p < 0.05$ for each, see Figure 2).

Comment

Principal findings of the study

1) The MMP-8 PTD Check™ was a simple and sensitive test for the antenatal identification of funisitis in preterm gestation; 2) Among patients without funisitis, patients with a positive MMP-8 PTD Check™ had a significantly higher median AF IL-6 concentration, AF WBC count, and umbilical cord plasma IL-6 concentration at birth than those with a negative MMP-8 PTD Check™ .

The significance of a positive MMP-8 PTD Check™ among patients without Funisitis

Patients with a positive MMP-8 PTD Check™ had a significantly higher median AF IL-6 concentration, AF WBC count, and umbilical cord plasma IL-6 concentration at birth than those with a negative MMP-8 PTD Check™ among patients without funisitis in the current study. This is an important observation, because it is consistent with the inference that funisitis is temporally the most advanced phase in ascending intrauterine infection and therefore an intra-amniotic inflammatory response has already occurred before the development of funisitis. Therefore, early detection of intra-amniotic and fetal inflammation by the MMP-8 PTD Check™ in patients without funisitis may be necessary for the prediction of pregnancy outcome and management in patients at risk for preterm delivery.

The relative low positive predictive value and specificity of the MMP-8

PTD Check™ test in the identification of Funisitis; and the significance of a likelihood ratio of a negative test of MMP-8 PTD Check™ MMP-8 PTD Check™ test had a low specificity (63%) and positive predictive value (50%) in the identification of funisitis, but false positive cases had a significantly higher rate of histologic chorioamnionitis and intra-amniotic inflammation than those who were true negative cases. Therefore, although the MMP-8 PPD Check™ test had a low specificity, it was worth performing because this test could categorize patients without funisitis into two groups: those with false positive and true negative groups, which had significantly different rates of adverse pregnancy outcomes such as histologic chorioamnionitis and intra-amniotic inflammation. Moreover, as shown in Table 1, the likelihood ratios of a positive test and a negative test of MMP-8 PPD Check™ for the identification of funisitis were 2.66 (95% CI, 2.05–3.45) and 0.04 (95% CI, 0.01–0.29). The Evidence-Based Medicine Working Group [5] has suggested that a likelihood ratio of less than 0.1 indicates a large and often conclusive decrease in the likelihood of disease. Therefore, the results of the current study suggest that funisitis will rarely occur in patients with a negative MMP-8 PTD Check™ result.

MMP-8 PTD Check™ protocol

We used a cutoff of 23 ng/mL of AF MMP-8 concentration (determined by ELISA) as the definition of intra-amniotic inflammation based upon previous studies [14, 19]. The MMP-8 PTD Check™, however, detects the presence of MMP-8 in human AF with a threshold of 10 ng/mL according to the original test manual, and therefore we modified the rapid test procedure to detect 20 ng/ml of MMP-8, similar to the cut off level of the definition of intra-amniotic inflammation.

A point-of-care test for the antenatal detection of funisitis

As previously reported [12], a MMP-8 rapid test has many of the optimal properties of point-of-care test which include a simple testing method, an inexpensive test to set up, and easy and rapid interpretation of the results. Therefore, in cases of preterm gestation such as preterm labor and intact membranes or preterm rupture of membranes, the antenatal detection of funisitis by means of a MMP-8 rapid test is readily feasible anywhere around the clock.

Strengths and weakness of the study

Strengths of the current study are that a MMP-8 rapid test was not used in the management of the patients and that in addition to AF MMP-8 qualitative assessment utilizing a MMP-8 rapid test, most parameters which could reflect the fetal inflammatory status in all compartments within the amniotic cavity were included, namely: (1) WBC count including neutrophils in AF, which are predominantly of fetal origin [17]; (2) umbilical cord blood IL-6, which has been originally used for the definition of the fetal inflammatory response syndrome [15]; and (3) funisitis, which is the histopathological hallmark of the fetal systemic inflammation [13, 23, 24]. It could be argued that a weakness of this study is that it was conducted with AF which was stored at −70°C. However, we have previously reported that the test underwent extensive validation by the biotechnology company that produces the assay, and that there was substantial agreement between results of AF subjected to freeze-thaw cycles [12]. Also, a criticism of our study could be the relatively small cohort (n=139), we demonstrated the excellent diagnostic performance including a high sensitivity and very low negative likelihood ratio in the antenatal identification of Funisitis. Moreover, there was significant difference in amniotic fluid and fetal inflammatory responses among patients with funisitis, those without funisitis but with a positive MMP-8 PTD Check™, and also those without funisitis and with a negative MMP-8 PTD Check™.

Clinical implication of this study

It is well known that funisitis, and an intra-amniotic and fetal inflammatory response are associated with preterm delivery and severe neonatal morbidity, including early onset neonatal sepsis or long-term handicap such as cerebral palsy [3, 4, 11, 13, 19, 21–23]. In the current study, we demonstrated that the rapid results of the MMP-8 PTD Check™ are valuable in the antenatal identification of funisitis and the identification of patients without funisitis but with an intra-amniotic and fetal inflammatory response. Therefore, the MMP-8 PTD Check™ could be helpful in predicting pregnancy outcome, counseling and management of patients at risk for preterm delivery due to preterm labor and intact membranes or preterm premature rupture of membranes.

Unanswered questions and proposals for future research

Further studies are needed to determine whether treatment with either antibiotics and/or tissue inhibitor of metalloproteinases (TIMP) can achieve negative conversion in cases with a positive MMP-8 rapid test, thereby improving pregnancy and neonatal outcome.

Acknowledgments

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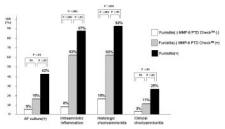

Figure 1.
Frequency of a positive AF culture, intra-amniotic inflammation, hisotologic chorioamnionitis, and clinical chorioamnionitis according to the results of a MMP-8 PTD Check™ and the presence or absence of funisitis. Each p-value was adjusted for maternal age, gestational age at delivery, and the presence or absence of rupture of membranes at amniocentesis.

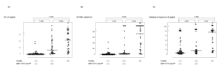

Figure 2.

(A) Amniotic fluid (AF) IL-6 concentrations, (B) AF WBC counts, and (C) IL-6 concentrations in umbilical cord plasma at birth according to the results of the MMP-8 PTD Check™ and the presence or absence of funisitis (AF IL-6: patients without funisitis and with a negative MMP-8 PTD Check™: median, 0.2 ng/mL [range, 0.0008–19.1 ng/mL]; patients without funisitis but with a positive MMP-8 PTD Check™: median, 5.9 ng/mL [range, 0.002–80.2 ng/mL]; Patients with funisitis: median, 26.4 ng/mL [0.08–115.2 ng/mL]; AF WBC: patients without funisitis and with a negative MMP-8 PTD Check™: median, 1 cells/mm^3 [range, 0–210 cells/mm^3]; patients without funisitis but with a positive MMP-8 PTD Check™: median, 3.5 cell/mm^3 [range, 0–1000 cells/mm^3]; Patients with funisitis: median, 360.0 cells/mm^3 [0–3204 cell/mm^3]; Cord plasma IL-6: patients without funisitis and with a negative MMP-8 PTD Check™: median, 3.7 pg/mL [range, 0–370 pg/mL]; patients without funisitis but with a positive MMP-8 PTD Check™: median, 7.6 pg/mL [range, 0–6150 pg/mL]; Patients with funisitis: median, 48.0 pg/mL [3.0–7400 pg/mL]).

Table 1

Diagnostic indices, predictive values, and likelihood ratios of MMP-8 PTD Check™ for the identification of funisitis and histologic chorioamnionitis.

	Prevalence	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Funisitis	27% (38/139)	97% (37/38)	63% (64/101)	50 % (37/74)	99% (64/65)	2.66 (2.05–3.45)	0.04 (0.01–0.29)
Histologic chorioamnionitis	49% (68/139)	84% (57/68)	76% (54/71)	77% (57/74)	83% (54/65)	3.51 (2.28–5.37)	0.21 (0.12–0.37)

Table 2

Clinical characteristics and gestational age at delivery of the study population according to the results of a MMP-8 PTD Check™ and presence or absence of funisitis

	Absence of funisitis				Presence of funisitis	
	Negative MMP-8 PTD Check™ n=64	**P***	**Positive MMP-8 PTD Check™ n=37**	**P†**	**n=38**	**P‡**
Mean maternal age, y (±SD)	29.2±4.4	NS	28.2±3.9	<0.05	30.2±4.2	NS
Parity ≥1	47%	NS	43%	NS	66%	NS
Causes of preterm delivery						
PPROM	22%	NS	24%	<.001	55%	<.005
Preterm labor	25%	<.001	73%	<.05	45%	<.05
Maternal fetal indication	53%	<.001	3%	NS	0%	<.001
Median gestational age at delivery, wk (range)	33.3 (27.3–35.0)	<.001	29.7 (21.4–34.9)	NS	31.6 (23.3–34.9)	<.005

PPROM, preterm premature rupture of membrane

*Comparison between groups 1 and 2;

†Comparison between groups 2 and 3;

‡Comparison between groups 3 and 1.