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Comparison of rapid MMP-8 and interleukin-6 point-of-care tests to identify intra-amniotic inflammation/infection and impending preterm delivery in patients with preterm labor and intact membranes

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Abstract

Objective—Among patients presenting with preterm labor and intact membranes, those with intra-amniotic inflammation have adverse obstetrical and neonatal outcomes. The diagnosis of intra-amniotic inflammation can easily be made by detecting an elevated concentration of the cytokine interleukin (IL)-6 or the enzyme neutrophil collagenase, also known as matrix metalloproteinase (MMP)-8. The diagnostic performances of MMP-8 and IL-6 enzyme-linked immunosorbent assay tests are similar. Recently, a rapid test has become available for point-of-care determination of either MMP-8 or IL-6. The objectives of this study were to compare the diagnostic indices and predictive values between the rapid MMP-8 and IL-6 tests for the identification of intra-amniotic inflammation in patients with preterm labor and intact membranes.

Materials and Methods—We performed a retrospective cohort study including 124 women with singleton pregnancies who presented with symptoms of preterm labor and underwent transabdominal amniocentesis for the evaluation of microbial invasion of the amniotic cavity (MIAC). MIAC was defined according to amniotic fluid culture results (aerobic and anaerobic bacteria as well as genital *Mycoplasmas*). Amniotic fluid white blood cell (WBC) counts were determined using a hemocytometer chamber. An elevated amniotic fluid MMP-8 concentration

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was assessed using Yoon's MMP-8 Check® (cutoff: 10ng/mL). An elevated amniotic fluid IL-6 concentration was scored when there was a positive result for the lateral flow-based immunoassay (cutoff of 745 pg/mL and 1000 pg/mL). In order to objectively compare rapid MMP-8 and rapid IL-6 tests to identify intra-amniotic inflammation, an amniotic fluid WBC count of 50 cells/mm³ was used to define intra-amniotic inflammation.

Results—1) The rapid tests had the same sensitivity for the detection of intra-amniotic inflammation [85.7% (18/21) for all]; 2) the specificity of the rapid MMP-8 test was higher than that of the rapid IL-6 test (cut-off: 745 pg/mL) for the identification of intra-amniotic inflammation [72.8% (75/103) vs. 64.1% (66/103); $p<0.05$]; and 3) there were no differences in the sensitivity and specificity between the rapid MMP-8 test and the rapid IL-6 test (cut-off: 1000 pg/mL) in the identification of intra-amniotic inflammation. Of 13 patients with discrepant results between the rapid MMP-8 and rapid IL-6 tests, two had a positive MMP-8 but a negative rapid IL-6 test, and both delivered preterm — one within 24 hours, and the other within 10 days — and both had acute histologic chorioamnionitis. On the other hand, there were 11 patients with a positive rapid IL-6 but a negative rapid MMP-8 result: 10 delivered preterm, 3 had acute histologic chorioamnionitis, and 1 had subacute chorionitis.

Conclusion—We conclude that the rapid MMP-8 test has a better specificity than the rapid IL-6 (cut-off: 745 pg/mL) assay for the detection of intra-amniotic infection. Moreover, we observed that among patients who were not identified as having intra-amniotic infection or inflammation by the standard cultivation technique and amniotic fluid WBC count, those who had a positive rapid MMP-8 test delivered preterm and had acute histologic chorioamnionitis.

Keywords

amniocentesis; biomarker; chorioamnionitis; funisitis; immunoassay; microbial invasion of the amniotic cavity (MIAC); point-of-care test; pregnancy; prematurity; preterm birth

Introduction

Intra-amniotic inflammation occurs in up to one-third of pregnancies with preterm labor (1–12), and its presence is a risk factor for adverse perinatal outcomes, including early preterm birth (7, 8, 10, 11, 13–24), substantial neonatal morbidity (6, 10, 11, 24–40), clinical and acute histologic chorioamnionitis (7, 8, 10, 11, 41–49), and funisitis (7, 8, 34, 46, 48, 50–53). Importantly, pregnant women with intra-amniotic inflammation, regardless of the presence or absence of microorganisms in the amniotic cavity, have similar outcomes (7, 8, 11, 45). Sterile intra-amniotic inflammation (intra-amniotic inflammation without microorganisms detectable with cultivation or molecular techniques) is associated with adverse outcomes in the context of preterm labor with intact membranes (11, 45), preterm pre-labor rupture of the membranes (preterm PROM) (8, 46), asymptomatic patients with a short cervix (54), and clinical chorioamnionitis at term (55). Thus, intra-amniotic inflammation is a frequent and important disorder. Rapid and accurate identification may be helpful in guiding clinical management to minimize potential adverse outcomes for both the mother and fetus/neonate.

Previous studies have reported that amniotic fluid matrix metalloproteinase (MMP)-8 (10, 56–73) and interleukin (IL)-6 (6, 7, 24, 26, 33–35, 74–108) have diagnostic and prognostic value in the identification of intra-amniotic inflammation, imminent spontaneous preterm delivery, acute inflammatory lesions of the placenta, and adverse neonatal outcomes such as cerebral palsy. In addition, an elevated MMP-8 concentration in the cervical fluid has been linked to cervical ripening (109) and, when found in the vaginal fluid, bacterial vaginosis (110, 111).

Amniotic fluid MMP-8 (58, 64, 65) and IL-6 (77–79, 82) concentrations perform better than an amniotic fluid white blood cell (WBC) count, a glucose concentration, and a Gram stain for the identification of intra-amniotic inflammation/infection (102). Moreover, the diagnostic performance is similar to proteomic markers; for this reason, this complex platform (surface-enhanced laser-capture ionization mass spectrometry) is no longer attractive and has largely been abandoned (102). However, the results of conventional laboratory [enzyme-linked immunosorbent assay (ELISA)] tests can take several hours and are often not available in time to inform clinical decisions.

Point-of-care (POC) tests have been widely used in both adult (112) and pediatric medicine, including the diagnosis of neonates (113, 114) as the tests are simple to perform, provide rapid, easy-to-interpret results, require low maintenance, and are cost-effective. The POC tests also strongly correlate with standard laboratory procedures (115–118). In addition, the rapid IL-6 test can be used to identify intra-amniotic inflammation in both fresh and stored amniotic fluid samples (106). We previously reported that the results of rapid MMP-8 (64) and IL-6 (105) POC tests correlated well with those derived from ELISA MMP-8 or IL-6 tests, respectively. Currently, rapid MMP-8 (64–67, 69, 70) and IL-6 (103, 105–108, 119, 120) tests are available and provide results within 15–20 minutes without the need for sophisticated laboratory equipment. However, there has not been a comparison of the rapid MMP-8 and IL-6 tests. Therefore, the objectives of this study were to compare the diagnostic performance of the rapid MMP-8 and IL-6 tests to identify intra-amniotic inflammation in patients with preterm labor and intact membranes.

Materials and Methods

A retrospective cohort study was conducted that included 124 patients who had an episode of preterm labor with intact membranes and underwent an amniocentesis for the diagnosis of intra-amniotic infection or intra-amniotic inflammation. The samples of these patients were stored in the Bank of Biological Materials of Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) (Detroit, MI). The inclusion criteria were: 1) singleton gestation; 2) transabdominal amniocentesis performed between 20 and 34 weeks of gestation with microbiologic studies; and 3) a live-born fetus with available neonatal outcomes. Patients were excluded from the study if they had placenta previa or if their fetus had a chromosomal or structural anomaly.

Patients of this study comprise a subset from a previous study (107). Women with the diagnosis of preterm labor and intact membranes were offered amniocentesis for the

identification of microorganisms in the amniotic cavity. Excess amniotic fluid not used for clinical tests was retained for research purposes. All patients provided written informed consent, and the use of biological specimens and clinical data for research purposes was approved by the Institutional Review Boards of Wayne State University and NICHD.

The clinical definitions, amniotic fluid processing, amniotic fluid analysis for microbiologic studies, and inflammatory responses, including WBC counts (121), glucose concentrations (122), Gram stains (123), and rapid IL-6 (105, 107, 108) and ELISA IL-6 tests (74–76, 79, 81, 84, 102), have been described in previous reports. Microbial invasion of the amniotic cavity (MIAC) is defined as the presence of microorganisms in the amniotic cavity detected by cultivation techniques. Intra-amniotic infection (also called “microbial-associated intra-amniotic inflammation”) is characterized by the combination of MIAC and intra-amniotic inflammation. We used an amniotic fluid WBC count 50 cells/mm^3 to define intra-amniotic inflammation (118) as the reference for the comparison of the diagnostic performances of the rapid MMP-8 and IL-6 POC tests. A positive test for IL-6 ELISA in amniotic fluid was defined as an IL-6 concentration 2.6 ng/mL (7, 11, 45, 105). The comparison used two cut-offs values for the positive amniotic fluid rapid IL-6 test: 1) 745 pg/mL , the cut-off value based on the result of the ROC (receiver operator characteristic) curve in our study population (105, 107, 108); and 2) 1000 pg/mL , the cut-off value previously used by another group (103), which is the cut-off value employed in the semi-quantitative rapid IL-6 assay (124).

Analysis of amniotic fluid samples for rapid MMP-8 concentration

Amniotic fluid was processed and the unused fluid was centrifuged for 10 minutes at 2000 g at 4°C , aliquoted and pipetted, and then stored at -70°C until assayed. After thawing the stored amniotic fluid, the MMP-8 rapid test (Yoon’s MMP-8 Check®; OBMed Co., Ltd., Seoul, Republic of Korea) was performed by personnel blinded to the clinical information. The rapid MMP-8 test is a qualitative immunochromatographic test that detects the presence of MMP-8 in the amniotic fluid with a threshold of 10 ng/mL . The rapid MMP-8 concentration was determined by immunoassays obtained from Yoon’s MMP-8 Check® (64–66, 70). This test can be performed at the patient’s bedside, using a pipette, and requires the addition of $25 \mu\text{L}$ of amniotic fluid and $75 \mu\text{L}$ (three drops) of buffer to the test window; however, for this study, these tests were performed in the OBMed Co., Ltd. laboratory after the amniotic fluid thawed. The test is considered to be positive when two lines are present [i.e., one at the control (C) line and one at the test (T) line]. The presence of the control line (C) only indicates a negative result. An invalid result is defined when the control line (C) is absent [Figure 1 in Reference (64)]. The results become available within 20 minutes. When the results were equivocal (showing very weak bands), the test was repeated using $12.5 \mu\text{L}$ of amniotic fluid and $75 \mu\text{L}$ (three drops) of buffer (64–66, 70).

A rapid IL-6 test was performed based on the lateral flow-based immunoassay as described in previous reports (105, 107, 108). The cut-off value for the detection of intra-amniotic inflammation was determined according to the ROC curve results previously described (107).

Study outcomes

The primary objective of the study was to compare the diagnostic performances of rapid MMP-8 and IL-6 POC tests for the detection of intra-amniotic inflammation. The secondary objectives were to compare the diagnostic performances of these kits to detect other outcomes, including intra-amniotic infection and impending preterm delivery.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of arithmetic data distributions. Sensitivity, specificity, accuracy, a positive likelihood ratio, and a negative likelihood ratio were calculated for the identification of each outcome. A modified t-test for correlated samples, as described by Galen and Gambino (125), was used to compare sensitivity and specificity. The Kaplan-Meier method was used to generate the survival curves that compare the gestational age at delivery between groups of patients with positive and negative rapid test results. The gestational age at delivery of patients who had a spontaneous delivery was treated as a censored observation. The log rank test was employed for the analysis. Statistical analysis was performed using SPSS 19 (IBM Corp, Armonk, NY, USA). A p-value <0.05 was considered statistically significant.

Results

Characteristics of the study population

A total of 124 women who underwent preterm labor and had intact membranes were included in this study. Demographic characteristics of the study cohort are presented in Table 1. The frequency of MIAC was 17.7% (22/124), intra-amniotic inflammation was 16.9% (21/124), MIAC and/or intra-amniotic inflammation was 25.0% (31/124), and preterm delivery was 73.4% (91/124) (Table 1). The most frequent microorganism isolated in the amniotic cavity was *Ureaplasma* spp. (18.2%; 4/22).

The prevalence of spontaneous delivery within 48 hours and 7 days after amniocentesis was 39.5% (49/124) and 44.4% (55/124), respectively. Approximately 22% (27/124) and 34% (42/124) of patients delivered spontaneously between 28 and 32 weeks of gestation, respectively (Table 1).

Diagnostic performance of rapid MMP-8 and rapid IL-6 tests (using a cut-off value of 745 pg/mL) for the identification of MIAC and intra-amniotic inflammation

The prevalence, diagnostic indices, predictive values, and likelihood ratios of a positive rapid MMP-8 test and a positive rapid IL-6 test for the identification intra-amniotic inflammation are depicted in Table 2. The rapid MMP-8 test had higher specificity and accuracy compared to the rapid IL-6 test in the identification of intra-amniotic inflammation [specificity: 72.8% (75/103) vs. 64.1% (66/103), $p < 0.05$; accuracy: 75% (93/124) vs. 67.7% (84/124)] (Table 2). There were no significant differences in the sensitivity between the rapid MMP-8 and rapid IL-6 tests for the identification of intra-amniotic inflammation (Table 2).

Comparisons between the diagnostic performance of the rapid MMP-8 and IL-6 tests for the identification of MIAC and intra-amniotic infection indicated the following: 1) the rapid

MMP-8 test had higher specificity and accuracy than the rapid IL-6 test for the diagnosis of MIAC [specificity: 74.5% (76/102) vs. 65.7% (67/102), $p < 0.05$; accuracy: 77.4% (96/124) vs. 70.2% (87/124)]; 2) intra-amniotic infection [specificity: 69.6% (78/112) vs. 61.6% (69/112), $p < 0.05$; accuracy: 72.6% (90/124) vs. 65.3% (81/124)]; and 3) no significant differences in the sensitivity between the rapid MMP-8 and rapid IL-6 tests for the identification of MIAC and intra-amniotic infection were found (Table 3).

Rapid MMP-8 and IL-6 tests and amniocentesis-to-delivery interval

The performance of rapid MMP-8 and rapid IL-6 tests for the identification of patients at risk for impending preterm delivery is shown in Table 4. The rapid MMP-8 and IL-6 tests had equivalent diagnostic indices to identify patients who delivered spontaneously within 48 hours or 7 days after admission with a preterm labor episode. In addition, these rapid tests were comparable in their ability to identify the patient who will deliver < 28 weeks and < 32 weeks of gestation. The rapid MMP-8 test had a positive likelihood ratio of almost 10, while the rapid IL-6 test had a positive likelihood ratio of 6 to identify patients who had an early spontaneous preterm delivery (< 32 weeks of gestation).

Characteristics of 22 patients with bacteria in the amniotic fluid (MIAC)

Table 5 demonstrates the results of rapid MMP-8, rapid IL-6, and ELISA IL-6 tests in 22 patients with MIAC. In 2 patients, both the rapid MMP-8 and rapid IL-6 tests failed to identify MIAC (patient numbers 21 and 22 in Table 5). Both patients had an ELISA IL-6 concentration < 2.6 ng/mL and a low amniotic fluid WBC count. Interestingly, *Ureaplasma* spp. was detected in the amniotic cavity of both patients. One patient delivered at term and did not have acute inflammatory lesions of the placenta (patient number 21), while the other patient delivered spontaneously at 34 3/7 weeks of gestation with acute subchorionitis/chorionitis (patient number 22, Table 5).

Comparison between the rapid MMP-8 and rapid IL-6 tests (using a cut-off value of 1000 pg/mL)

Table 6 shows the prevalence, diagnostic indices, predictive values, and likelihood ratios of a positive rapid MMP-8 test and a positive rapid IL-6 test (cut-off value: 1000 pg/mL) for the identification intra-amniotic inflammation (defined as a WBC count > 50 cells/mm³). The two POC tests had comparable diagnostic indices (sensitivity and specificity) for the identification of intra-amniotic inflammation [rapid MMP-8 vs. rapid IL-6: sensitivity: 85.7% (18/21) vs. 85.7% (18/21); specificity: 72.8% (75/103) vs. 68.9% (71/103); accuracy: 75% (93/124) vs. 71.8% (89/124)] (Table 6). Comparison of the two POC tests regarding the secondary outcomes (MIAC, intra-amniotic infection and impending preterm delivery within 48 hours or 7 days after admission, spontaneous preterm delivery < 28 and < 32 weeks of gestation) was not statistically significant (data presented in the Supplementary Table).

The relationship between the results of the rapid MMP-8 and IL-6 tests and gestational age at delivery

Patients with a positive amniotic fluid rapid MMP-8 test had a significantly shorter median gestational age at delivery than those with a negative test result [median 28.1 weeks, (95%

CI: 25.6–30.6) vs. median 36.6 weeks (95% CI: 35.7–37.5), $p < 0.0001$] (Figure 1A). Interestingly, all patients with a positive rapid MMP-8 result delivered before 37 weeks of gestation. Patients with a positive rapid IL-6 test (cut-off value: 745 pg/mL) result also had a significantly shorter median gestational age at delivery than those with a negative test [median 28.9 weeks (95% CI: 25.2–32.6) vs. median 37.3 weeks (95% CI: 36.6–38), $p < 0.0001$] (Figure 1B). Patients with a positive rapid IL-6 test (cut-off value: 1000 pg/mL) result also had a significantly shorter median gestational age at delivery than those with a negative test [median 28.1 weeks (95% CI: 25.9–30.3) vs. median 38 weeks (95% CI: 36.6–39.5), $p < 0.0001$] (Figure 1C).

Characteristics of patients with discrepant results between the rapid MMP-8 and rapid IL-6 tests

Table 7 includes patients with discrepant results between the two rapid tests, who had an amniotic fluid WBC count < 50 cells/mm³ and no microorganisms identified. Two patients had a positive rapid MMP-8 test but a negative rapid IL-6 test. Both patients had acute inflammatory lesions of the placenta consistent with histologic chorioamnionitis and delivered preterm. There were 11 patients with a positive rapid IL-6 but a negative rapid MMP-8 result; of these, 10 delivered preterm, 3 had acute histologic chorioamnionitis, and 1 had subacute chorionitis.

Discussion

Principal findings of the study: 1) the rapid MMP-8 and IL-6 tests have similar sensitivity in the detection of intra-amniotic inflammation (using both cut-off values: 745 pg/mL and 1000 pg/mL); and 2) the specificity of the rapid MMP-8 test for the identification of intra-amniotic inflammation was significantly better than that of the rapid IL-6 test (cut-off value: 745 pg/mL).

The diagnosis of intra-amniotic inflammation and intra-amniotic infection

Under normal circumstances, the amniotic cavity contains very few white blood cells and low concentrations of pro-inflammatory cytokines. In the presence of bacteria and its products, such as endotoxin (126, 127), the number of white blood cells increases as well as the concentration of cytokines. For many years, we have used the concentration of IL-6 to assess the presence and magnitude of intra-amniotic inflammation (7). Recently, we characterized the behavior of the cytokine network in the amniotic fluid of patients in preterm labor according to the presence or absence of intra-amniotic inflammation (in the presence or absence of microorganisms) (128).

Given the importance of neutrophils in the generation of the intra-amniotic inflammatory response, we studied neutrophil products, such as defensins (129, 130) and matrix-degrading enzymes, and have been impressed with the diagnostic performance of the latter in the identification of intra-amniotic inflammation. Indeed, the performance of MMP-8 concentrations in the prediction of adverse pregnancy outcome is similar to that we previously reported with IL-6 determinations (7, 79).

MMP-8 in the amniotic fluid: a marker of inflammation

MMP-8, also known as collagenase-2 or neutrophil collagenase, is a member of the MMP family (131, 132). It is stored as an inactive pro-enzyme in secondary granules of mature neutrophils (133). Since neutrophils are the first cells to arrive at the site of inflammation, MMP-8 is present at the initial stages of the inflammatory process (133). MMP-8 is not specific to neutrophils, as it can be secreted by a variety of inflammatory cells, e.g., macrophages (134–136), plasma cells (137), and T cells (138); mesenchymal cells, or smooth muscle cells (134); epithelial cells (135, 139–142); endothelial cells (143); and malignant cells (133, 144). Also, MMP-8 must be activated before it can exert its biological activities; examples of such activators include cathepsin G (145), chymotrypsin (145), MMP-3 (146), MMP-7 (147), MMP-10 (148), and some bacterial proteases (149).

MMP-8 has multiple biological activities, and several substrates for this enzyme have been identified: collagen (150, 151), laminin-5 (152), fibronectin (153), chemokines (154), lipopolysaccharide-induced CXC chemokine (LIX) (155), monokine induced by gamma interferon (MIG) or CXCL9 (155), interferon gamma-induced protein (IP)-10 or CXCL10 (156), and monocyte chemotactic protein (MCP)-1 (156). Experimental studies with animal models that have a gene deletion for MMP-8 have shown that this enzyme is a central mediator in both acute and chronic inflammation (131, 133, 157, 158).

MMP-8 plays a role in preterm labor and delivery

MMP-8 concentrations are detectable in the amniotic fluid of patients with normal pregnancy as well as preterm delivery (10, 56–58, 62, 63, 68). Moreover, amniotic fluid concentrations of MMP-8 are higher in patients with spontaneous labor at term compared to those at term without labor (56). We consider the increased bioavailability of MMP-8 in spontaneous labor as indicative of activation of the common pathway of parturition (3, 109, 159, 160). MMP-8 can induce degradation of collagen types I, II, and III (133, 151, 161), thus favoring membrane rupture (56, 57, 162–164).

In the context of intra-amniotic inflammation in patients with preterm labor and preterm PROM, MMP-8 can serve as a biomarker to increase the index of suspicion for the presence of bacteria in the amniotic fluid (MIAC), by virtue of detecting the inflammatory response induced by microbial products (56–58, 61). MMP-8 probably reflects a fetal inflammatory response type I (60, 66), given that neutrophils in the amniotic cavity are largely considered to be of fetal origin (48, 165).

Amniotic fluid concentrations of MMP-8 may also be useful in detecting imminent spontaneous preterm delivery (57, 58, 63, 68, 70), MIAC (67), funisitis (60, 66, 69), and neonates at risk for an adverse outcome such as cerebral palsy (odds ratio (OR) 6.0; 95% CI 1.1–33) (62). An elevation of amniotic fluid MMP-8 concentration >23 ng/mL in the midtrimester of pregnancy is a predictor of spontaneous preterm delivery <32 weeks of gestation (OR 68.4; 95% CI 7.8–599.1) (59).

IL-6 has been considered a marker of the acute phase response to injury and infection (166). Although administration of IL-6 to pregnant mice does not result in preterm labor and delivery, recent studies indicate that IL-6 is important in controlling the timing of parturition

in mice (167). We previously reported its value in the diagnosis of intra-amniotic inflammation and intra-amniotic infection using conventional ELISAs and POC tests (105, 107, 108).

A point-of-care test for the diagnosis of intra-amniotic inflammation in the amniotic fluid

The nature of obstetrical complications is such that it requires the results of diagnostic and prognostic tests to be quickly available in time for clinical decision-making. In some cases, 8 hours (the typical turnaround time for an ELISA test) is too long for some important management decisions, such as whether to administer tocolytic agents, steroids, or antibiotics, and other interventions. The time lapse is one reason why modern medicine is moving away from laboratory-based tests to POC tests (115–118). Therefore, having developed methods for the diagnosis of intra-amniotic infection and intra-amniotic inflammation, we are now focused on the implementation of such POC tests in clinical obstetrics.

The amniotic fluid MMP-8 and IL-6 (semi-quantitative) POC tests (124) can be performed rapidly at the bedside, and the results are available within 20 minutes without the need for laboratory equipment. These kits were reported to be reliable and to have a diagnostic performance suitable for clinical use (64–67, 69, 70, 103, 105–108, 119, 120). Evidence in support of this view includes the following: 1) our group reported that a positive amniotic fluid rapid MMP-8 test has positive predictive values of 70% and 94% for the identification of patients who had spontaneous preterm delivery within 48 hours or 7 days after admission, respectively (64). This test also has a positive likelihood ratio of 61.7 for the identification of intra-amniotic inflammation in patients who have preterm labor with intact membranes (64). In addition, the amniotic fluid rapid MMP-8 test has a high predictive value for the identification of intra-amniotic inflammation in patients with preterm PROM (65) and funisitis (66, 69). Moreover, 42% of patients with spontaneous preterm delivery <30 weeks of gestation could be identified by a rapid MMP-8 bedside test at the time of their mid-trimester genetic amniocentesis (70); 2) the use of rapid IL-6 for the detection of intra-amniotic inflammation in patients with preterm labor and preterm PROM was previously described (103, 107, 108). Kacerovsky et al, using the quantitative rapid IL-6 kit (cut-off value: 1000 pg/mL), reported a sensitivity of 50%, specificity of 95%, positive predictive value of 82%, negative predictive value of 81%, and likelihood ratio of 8.4 for the detection of MIAC, and a sensitivity of 60%, specificity of 94%, positive predictive value of 75%, negative predictive value of 88%, and likelihood ratio of 9.4 for the detection of MIAC and histologic chorioamnionitis (103).

The key question addressed in this study is the comparison between the rapid MMP-8 test and the quantitative rapid IL-6 (using both cut-off values for this assay). When the cut-off value for rapid IL-6 was set at 745 pg/mL, the rapid MMP-8 test had better specificity for the identification of intra-amniotic inflammation. Both tests performed similarly in the identification of impending preterm delivery. When the cut-off value (1000 pg/mL) for an amniotic fluid IL-6 concentration was used, there were no differences in the diagnostic indices between the rapid MMP-8 and IL-6 tests. However, an important observation of this study was that all patients who had a positive rapid MMP-8 test delivered preterm even if the

results of the amniotic fluid culture, WBC count, and glucose concentration failed to indicate intra-amniotic infection or intra-amniotic inflammation. Moreover, the placentas of these patients demonstrated acute histologic chorioamnionitis, supporting the results of the rapid MMP-8 test (that showed evidence of intra-amniotic inflammation).

Our group and others have been able to validate the use of rapid MMP-8 kit in diverse ethnic populations, yielding similar diagnostic indices among studies (64). The rapid IL-6 kit (cut-off value: 1000 pg/mL) was previously validated in Caucasian populations, and we were able to validate this index in an African-American population. Collectively, our findings as well as previous reports support a generalized use of both POC tests—the rapid MMP-8 test and the quantitative rapid IL-6 test—for the identification of intra-amniotic inflammation in patients presenting with preterm parturition. One of the clinical expectations of the POC test is that the procedure will be simple and require no additional processing; this is addressed by the simple-to-perform rapid MMP-8 test. In contrast, the quantitative IL-6 rapid test requires a special reader that limits the utilization of this assay and increases its cost. There is an additional rapid IL-6 test, which is semi-quantitative and does not require an additional reader, thereby transforming it to a simple bedside test similar to the rapid MMP-8 kit (124). However, thus far, this kit was not validated for the detection of intra-amniotic inflammation.

Conclusion

We conclude that the rapid MMP-8 test has a better specificity than the rapid IL-6 (cut-off value: 745 pg/mL) assay for the detection of intra-amniotic inflammation. Moreover, we observed that among patients who were not identified by standard cultivation techniques and an amniotic fluid WBC count as having intra-amniotic infection or intra-amniotic inflammation, those with a positive MMP-8 rapid test delivered preterm and had acute histologic chorioamnionitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Kaplan-Meier survival curve of gestational age at delivery (weeks) according to the rapid MMP-8 and rapid IL-6 test results. Patients whose labor was induced were censored. **A.** The median (IQR) gestational age at delivery (weeks) of women with positive amniotic fluid rapid MMP-8 tests was significantly shorter than that of patients with negative results [median 28.1 weeks, (IQR: 25.6–30.6) vs. median 36.6 weeks (IQR: 35.7–37.5), $p < 0.0001$]. **B.** Patients with a positive rapid IL-6 test result (cut-off value: 745 ng/mL) also had a significantly shorter median (IQR) gestational age at delivery than those with a negative test [median 28.9 weeks, (IQR: 25.2–32.6) vs. median 37.3 weeks, (IQR: 36.6–38), $p < 0.0001$]. **C.** Patients with a positive rapid IL-6 test (cut-off value: 1000 pg/mL) result also had a significantly shorter median gestational age at delivery than those with a negative test [median 28.1 weeks (95% CI: 25.9–30.3) vs. median 38 weeks (95% CI 36.6–39.5), $p < 0.0001$].

Table 1

Clinical characteristics of the study population

Characteristics	Median (interquartile range) or percent (n=124)
Maternal age (years)	24 (20–29)
Body mass index (kg/m ²)	23.8 (20.8–30.1)
Nulliparity	33.1% (41/124)
Gestational age at amniocentesis (weeks)	30.9 (27.0–32.4)
Birthweight (grams)	2155 (1155–2695)
Preterm delivery (<37 weeks of gestation)	73.4% (91/124)
Interval from amniocentesis to delivery (days)	10 (1–37)
Spontaneous delivery within two days after amniocentesis (%)	39.5% (49/124)
Spontaneous delivery within seven days after amniocentesis (%)	44.4% (55/124)
Spontaneous delivery <28 weeks of gestation (%)	21.8% (27/124)
Spontaneous delivery <32 weeks of gestation (%)	33.9% (42/124)
Microbial invasion of the amniotic cavity (MIAC) identified by cultivation (%)	17.7% (22/124)
Amniotic fluid white blood cell count ≥ 50 cells/mm ³ (intra-amniotic inflammation)	16.9% (21/124)
MIAC and/or intra-amniotic inflammation	25.0% (31/124)
Acute histologic chorioamnionitis (%) *	57.4% (27/47)
Acute funisitis (%) *	36.2% (17/47)
Acute inflammatory lesions of the placenta (%) *	57.4% (27/47)

Data are presented as median (interquartile range) or % (n).

Acute inflammatory lesions of the placenta include acute histologic chorioamnionitis and/or acute funisitis.

* Included only patients who had an interval from amniocentesis to delivery ≥ 3days (n=52). Among these patients, placental histology reports were not available for 5 patients, and placental histopathology reports were not available for 12/124 patients.

Table 2

Diagnostic performance of rapid MMP-8, rapid IL-6 and ELISA IL-6 tests for the identification of intra-amniotic inflammation

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		Rapid IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation identified by an amniotic fluid white blood cell count 50 cells/mm ³ [16.9% (21/124)]	Sensitivity	85.7% (18/21)	63.6–96.9	85.7% (18/21)	63.6–96.9
	Specificity *	72.8% (75/103)	63.2–81.1	64.1% (66/103)	54.0–73.3
	Positive Likelihood Ratio	3.2	2.2–4.5	2.4	1.8–3.2
	Negative Likelihood Ratio	0.2	0.07–0.6	0.2	0.1–0.6
	Positive predictive value	39.1% (18/46)	25.1–54.6	32.7% (18/55)	20.7–46.7
	Negative predictive value	96.2% (75/78)	89.2–99.2	95.7% (66/69)	87.8–99.1
	Accuracy	75% (93/124)	66–82	67.7% (84/124)	59–76

CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; IL: interleukin; MMP: matrix metalloproteinase

* P-values <0.05 between the rapid MMP-8 and rapid IL-6 tests by a modified paired t-test using the Galen & Gambino method.

Diagnostic performance of the rapid MMP-8 and rapid IL-6 (cut-off value: 745 pg/mL) tests for the identification of microbial invasion of the amniotic cavity (MIAC) and intra-amniotic infection

Table 3

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		Rapid IL-6	
		% (n)	95% CI	% (n)	95% CI
Microbial invasion of the amniotic cavity (MIAC) identified by culture [17.7% (22/124)]	Sensitivity	90.9% (20/22)	70.8–98.9	90.9% (20/22)	70.8–98.9
	Specificity *	74.5% (76/102)	64.9–82.6	65.7% (67/102)	55.8–75.2
	Positive Likelihood Ratio	3.6	2.5–5.1	2.7	1.9–3.6
	Negative Likelihood Ratio	0.12	0.03–0.5	0.14	0.04–0.5
	Positive predictive value	43.5% (20/46)	28.9–58.9	36.4% (20/55)	23.8–50.4
	Negative predictive value	97.4% (75/77)	91.0–99.7	97.1% (67/69)	89.9–99.7
	Accuracy	77.4% (96/124)	0.69–0.84	70.2% (87/124)	0.61–0.78
	Sensitivity	100% (12/12)	73.5–100	100% (12/12)	73.5–100
Intra-amniotic infection (microbial-associated intra-amniotic inflammation) [9.7% (12/124)]	Specificity *	69.6% (78/112)	60.2–78.0	61.6% (69/112)	51.9–70.6
	Positive Likelihood Ratio	3.3	2.5–4.4	2.6	2.1–3.3
	Negative Likelihood Ratio	0		0	
	Positive predictive value	26.1% (12/46)	14.3–41.1	21.8% (12/55)	11.8–35.0
	Negative predictive value	100% (78/78)	95.4–100	100% (69/69)	94.8–100
	Accuracy	72.6% (90/124)	0.64–0.80	65.3% (81/124)	0.56–0.74

CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; IL: interleukin; MMP: matrix metalloproteinase.

* P-values <0.05 between the rapid MMP-8 and rapid IL-6 tests by a modified paired t-test using the Galen & Gambino method.

Diagnostic performance of the rapid MMP-8 and rapid IL-6 (cut-off value: 745 pg/mL) tests for the identification of patients with spontaneous preterm delivery

Table 4

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		Rapid IL-6	
		% (n)	95% CI	% (n)	95% CI
Spontaneous delivery within 2 days after amniocentesis [39.5% (49/124)]	Sensitivity	71.4% (35/49)	56.7–83.4	79.6% (39/49)	65.7–89.8
	Specificity	85.3% (64/75)	75.3–92.4	78.7% (59/75)	67.7–87.3
	Positive Likelihood Ratio	4.9	2.7–8.7	3.7	2.4–5.9
	Negative Likelihood Ratio	0.3	0.2–0.5	0.3	0.2–0.5
	Positive predictive value	76.1% (35/46)	61.2–87.4	70.9% (39/55)	57.1–82.4
	Negative predictive value	82.1% (64/78)	71.7–89.8	85.5% (59/69)	74.9–92.8
Spontaneous delivery within 7 days after amniocentesis [44.4% (55/124)]	Accuracy	79.8% (99/124)	0.72–0.87	79.0% (98/124)	0.71–0.86
	Sensitivity	69.1% (38/55)	55.2–80.9	80% (44/55)	67.0–89.6
	Specificity	88.4% (61/69)	78.4–94.9	84.1% (58/69)	73.2–91.8
	Positive Likelihood Ratio	5.9	3.0–11.7	5.0	2.9–8.8
	Negative Likelihood Ratio	0.35	0.2–0.5	0.2	0.1–0.4
	Positive predictive value	82.6% (38/46)	68.6–91.2	80% (44/55)	67.0–89.6
Spontaneous delivery <28 weeks of gestation [21.8% (27/124)]*	Negative predictive value	78.2% (61/78)	67.4–86.8	84.1% (58/69)	73.2–91.8
	Accuracy	79.8% (99/124)	0.72–0.87	82.3% (102/124)	0.74–0.86
	Sensitivity	81.5% (22/27)	61.9–93.7	88.9% (24/27)	70.8–97.7
	Specificity	87.5% (7/8)	47.4–99.7	75% (6/8)	34.9–96.8
	Positive Likelihood Ratio	6.5	1.0–41.1	3.6	1.1–11.9
	Negative Likelihood Ratio	0.2	0.1–0.5	0.2	0.1–0.5
Spontaneous delivery <32 weeks of gestation [33.9% (42/124)]**	Positive predictive value	95.7% (22/23)	78.1–99.9	92.3% (24/26)	74.9–99.1
	Negative predictive value	58.3% (7/12)	27.7–84.8	66.7% (6/9)	29.9–92.5
	Accuracy	82.9% (29/35)	0.66–0.93	85.7% (30/35)	0.70–0.95
	Sensitivity	73.8% (31/42)	57.9–86.1	80.9% (34/42)	65.9–91.4
	Specificity	92.3% (36/39)	79.1–98.4	87.2% (34/39)	72.6–95.7
	Positive Likelihood Ratio	9.6	3.2–28.9	6.3	2.8–14.5
	Negative Likelihood Ratio	0.3	0.2–0.5	0.2	0.1–0.4

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		Rapid IL-6	
		% (n)	95% CI	% (n)	95% CI
	Positive predictive value	91.2% (31/34)	76.3–98.1	87.2% (34/39)	72.6–95.7
	Negative predictive value	76.6% (36/47)	61.9–87.7	80.9% (34/42)	65.9–91.4
	Accuracy	82.7% (67/81)	0.73–0.90	83.9% (68/81)	0.74–0.91

CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; IL: interleukin; MMP: matrix metalloproteinase.

*The analysis was performed only for patients who had an amniocentesis <28 weeks of gestation (n= 35).

**The analysis was performed only for patients who had an amniocentesis <32 weeks of gestation (n= 81).

Clinical characteristics, amniotic fluid inflammatory response, and acute inflammatory placental lesions in patients with microbial invasion of the amniotic cavity (MIAC) using cultivation techniques

Table 5

No.	Organisms	GA at delivery (weeks)	AF glucose (mg/dl)	AF WBC count (cell/mm ³)	Rapid MMP-8 (cut-off: 10 ng/ml)	Rapid IL-6 (pg/ml)	ELISA IL-6 (ng/ml)	Acute histologic chorioamnionitis	Acute funisitis
1	<i>Ureaplasma urealyticum</i>	28 ⁺⁶	13	180	Positive	10,000	9.4	Acute chorioamnionitis	No
2	<i>Streptococcus agalactiae</i>	25 ⁺²	19	5	Positive	8208	248.9	Acute chorioamnionitis	Umbilical arteritis
3	<i>Mobiluncus</i> spp.	32 ⁺⁰	10	570	Positive	8144	76.9	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
4	<i>Haemophilus influenza</i>	30 ⁺⁶	10	40	Positive	6467	92.1	Necrotizing chorioamnionitis	Necrotizing funisitis
5	<i>Fusobacterium</i> spp., Gram-negative bacilli	21 ⁺⁶	19	1564	Positive	6228	317.7	Subchorionic microabscesses	Necrotizing funisitis
6	Gram-negative bacilli	21 ⁺¹	20	66	Positive	5934	242.7	Subacute chorioamnionitis	Umbilical arteritis
7	Gram-positive cocci	22 ⁺⁵	10	125	Positive	5540	470.6	Necrotizing chorioamnionitis	Umbilical arteritis
8	<i>Bacteroides</i> spp., <i>Mobiluncus</i> spp., <i>Clostridium sporogenes</i>	22 ⁺⁴	10	295	Positive	4748	517.8	Necrotizing chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
9	<i>Ureaplasma urealyticum</i>	33 ⁺⁰	10	500	Positive	4628	85.9	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
10	<i>Mycoplasma hominis</i>	33 ⁺⁰	1	420	Positive	4613	172.3	N/A	N/A
11	<i>Candida albicans</i>	26 ⁺³	10	2160	Positive	4448	201.3	Necrotizing chorioamnionitis	No
12	<i>Staphylococcus capitis</i>	28 ⁺⁶	20	24	Positive	4374	360.5	Subacute chorioamnionitis	Umbilical arteritis
13	<i>Candida albicans</i>	32 ⁺⁴	10	1292	Positive	4252	96.3	Acute chorioamnionitis	Necrotizing funisitis
14	<i>Streptococcus agalactiae</i>	25 ⁺⁰	10	4	Positive	3575	93.6	Necrotizing chorioamnionitis	No
15	<i>Candida albicans</i> , <i>Lactobacillus</i> spp.	33 ⁺¹	10	43	Positive	3554	200.6	Acute subchorionitis/chorionitis	No
16	<i>Prevotella</i> spp., <i>Enterococcus faecalis</i>	25 ⁺¹	10	1	Positive	3208	52.6	No	No
17	Gram-negative bacilli	26 ⁺⁵	N/A	610	Positive	10000	1.8	N/A	N/A
18	<i>Streptococcus</i> spp., <i>Genella morbillorum</i>	31 ⁺⁶	10	1920	Positive	6796	0.7	Necrotizing chorioamnionitis	Umbilical arteritis
19	<i>Streptococcus anginosus</i> , <i>Streptococcus mitis</i>	22 ⁺⁶	10	10	Negative	7246	73.3	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
20	<i>Fusobacterium</i> spp., Gram-negative bacilli	28 ⁺¹	1	22	Negative	3996	301.4	Acute chorioamnionitis	Umbilical arteritis
21	<i>Ureaplasma urealyticum</i>	39 ⁺²	19	2	Negative	155	1.3	No	No
22	<i>Ureaplasma urealyticum</i>	34 ⁺³	24	0	Negative	60	0.2	Acute subchorionitis/chorionitis	No

AF: amniotic fluid; ELISA: enzyme-linked immunosorbent assay; GA: gestational age; N/A: not available; WBC, white blood cell.

Acute subchorionitis/chorionitis: acute histologic chorioamnionitis, stage 1; acute chorioamnionitis: acute histologic chorioamnionitis, stage 2; necrotizing chorioamnionitis and subacute chorioamnionitis: acute histologic chorioamnionitis, stage 3; subchorionic micro-abscesses: severe acute histologic chorioamnionitis; umbilical phlebitis/chorionic vasculitis: acute funisitis, stage 1; umbilical arteritis: acute funisitis, stage 2; and necrotizing funisitis: acute funisitis, stage 3.

Table 6

Diagnostic performance of the rapid MMP-8 and rapid IL-6 tests (cut-off value: 1000 pg/mL) for the identification of intra-amniotic inflammation

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		Rapid IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation identified by an amniotic fluid white blood cell count 50 cells/mm ³ [16.9% (21/124)]	Sensitivity	85.7% (18/21)	63.6–96.9	85.7% (18/21)	62.6–96.2
	Specificity	72.8% (75/103)	63.2–81.1	68.9% (71/103)	58.9–77.5
	Positive likelihood ratio	3.2	2.2–4.5	2.8	1.97–3.86
	Negative likelihood ratio	0.2	0.07–0.6	0.2	0.07–0.59
	Positive predictive value	39.1% (18/46)	25.1–54.6	36 % (18/50)	22.92–50.81
	Negative predictive value	96.2% (75/78)	89.2–99.2	95.95% (71/74)	88.61–99.16
	Accuracy	75% (93/124)	66.4–82.3	71.8% (89/124)	62.99–79.48

CI: confidence interval; IL: interleukin; MMP: matrix metalloproteinase.

A positive rapid IL-6 test is defined by a cut-off value of 1000 pg/mL.

Clinical characteristics, amniotic fluid inflammatory response, and acute inflammatory placental lesions in patients with discrepant results between the rapid MMP-8 and rapid IL-6 tests.

Table 7

No.	Rapid MMP-8 test	Rapid IL-6 test	GA at amniocentesis (weeks)	GA at delivery (weeks)	Amniotic fluid culture	AF glucose (mg/dL)	AF WBC count (cell/mm ³)	ELISA IL-6 (ng/mL)	Acute histologic chorioamnionitis	Acute funisitis
1.	Positive	Negative	33 ⁺³	34 ⁺⁶	-	24	1	2.4	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
2.	Positive	Negative	29 ⁺⁵	29 ⁺⁵	-	40	0	17.9	Acute subchorionitis/chorionitis	No
3.	Negative	Positive	32 ⁺²	32 ⁺⁴	-	19	2	2.6	N/A	N/A
4.	Negative	Positive	31 ⁺⁵	32	-	26	0	2.2	No	No
5.	Negative	Positive	27 ⁺⁵	28 ⁺¹	-	35	3	3.4	Acute subchorionitis/chorionitis	No
6.	Negative	Positive	32 ⁺¹	32 ⁺¹	-	15	1	7.1	No	No
7.	Negative	Positive	32 ⁺²	33 ⁺³	-	31	2	2.8	No	No
8.	Negative	Positive	23 ⁺⁴	23 ⁺⁴	-	21	0	2.8	No	No
9.	Negative	Positive	33 ⁺⁶	35 ⁺⁵	-	26	3	2.7	Acute subchorionitis/chorionitis	No
10.	Negative	Positive	20 ⁺¹	20 ⁺⁶	-	28	2	1.6	Subacute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
11.	Negative	Positive	31 ⁺⁵	40	-	19	0	2.0	No	No
12.	Negative	Positive	30 ⁺³	31 ⁺⁶	-	49	1	3.4	Acute chorioamnionitis	Umbilical arteritis
13.	Negative	Positive	32 ⁺⁶	33	-	45	0	2.6	N/A	N/A

AF: amniotic fluid; ELISA: enzyme-linked immunosorbent assay; GA: gestational age; IL: interleukin; MMP: matrix metalloproteinase; N/A: not available; WBC: white blood cell count.

Acute subchorionitis/chorionitis: acute histologic chorioamnionitis, stage 1; acute chorioamnionitis: cute histologic chorioamnionitis, stage 2; necrotizing chorioamnionitis and subacute chorioamnionitis: acute histologic chorioamnionitis, stage 3; subchorionic microabscesses: severe acute histologic chorioamnionitis; umbilical phlebitis/chorionic vasculitis: acute funisitis, stage 1; umbilical arteritis: acute funisitis, stage 2; and necrotizing funisitis: acute funisitis, stage 3.

The positive rapid MMP-8 test result is defined as a rapid MMP-8 concentration >10 ng/mL; the positive rapid IL-6 test result is defined as a rapid IL-6 concentration 745 pg/mL.