Published in final edited form as:

J Matern Fetal Neonatal Med. 2018 January ; 31(2): 228–244. doi:10.1080/14767058.2017.1281904.

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

Piya Chaemsaithong[1,2], **Roberto Romero**[1,3,4,5], **Nikolina Docheva**[1,2], **Noppadol Chaiyasit**[1,2], **Gaurav Bhatti**[1,2], **Percy Pacora**[1,2], **Sonia S. Hassan**[1,2], **Lami Yeo**[1,2], and **Offer Erez**[1,2]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, Michigan, USA

[2]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

[3]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

[4]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, Michigan, USA

[5]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

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

Correspondence: Piya Chaemsaithong, MD, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, pchaemsa@link.cuhk.edu.hk. Roberto Romero, MD, D. Med. Sci, Perinatology Research Branch, NICHD/NIH/DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone: (313) 993-2700, Fax: (313) 993-2694, prbchiefstaff@med.wayne.edu.

Presented at the 12th World Congress of Perinatal Medicine, November 3–6, 2015, Madrid, Spain, as a poster presentation.

Conflict of Interest Statement: The authors declare no conflicts of interest.

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/mm^3 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 μL of amniotic fluid and 75 μL (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 μL of amniotic fluid and 75 μL (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/mm^3). 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/mm^3 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

This research was supported, in part, by the Perinatology Research Branch, Program for Perinatal Research and Obstetrics, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH/DHHS); and, in part, with Federal funds from NICHD/NIH/DHHS under Contract No. HHSN275201300006C.

References

1. Kara M, Ozden S, Arioglu P, Cetin A. The significance of amniotic fluid interleukin-6 levels in preterm labour. Aust N Z J Obstet Gynaecol. 1998; 38:403–406. [PubMed: 9890219]
2. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. Semin Fetal Neonatal Med. 2012; 17:12–19. [PubMed: 21944863]
3. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014; 345:760–765. [PubMed: 25124429]
4. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One. 2008; 3:e3056. [PubMed: 18725970]

5. Romero R, Mazor M, Munoz H, et al. The preterm labor syndrome. Anne N Y Acad Sci. 1994; 734:414–429.
6. Cobo T, Kacerovsky M, Jacobsson B. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol. 2014; 211:708.
7. Yoon BH, Romero R, Moon JB, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001; 185:1130–1136. [PubMed: 11717646]
8. Shim SS, Romero R, Hong JS, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol. 2004; 191:1339–1345. [PubMed: 15507963]
9. Romero R, Gomez R, Chaiworapongsa T, et al. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol. 2001; 15:41–56. [PubMed: 11520399]
10. Park CW, Yoon BH, Kim SM, et al. The frequency and clinical significance of intra-amniotic inflammation defined as an elevated amniotic fluid matrix metalloproteinase-8 in patients with preterm labor and low amniotic fluid white blood cell counts. Obst Gynecol Sci. 2013; 56:167–175. [PubMed: 24327997]
11. Romero R, Miranda J, Chaiworapongsa T, et al. Prevalence and clinical significance of sterile intraamniotic inflammation in patients with preterm labor and intact membranes. Am J Reprod Immunol. 2014; 72:458–474. [PubMed: 25078709]
12. Musilova I, Kutova R, Pliskova L, et al. Intraamniotic inflammation in women with preterm prelabor rupture of membranes. PLoS One. 2015; 10:e0133929. [PubMed: 26208287]
13. Romero R, Mazor M, Wu YK, et al. Infection in the pathogenesis of preterm labor. Semin Perinatol. 1988; 12:262–279. [PubMed: 3065940]
14. Romero R, Mazor M. Infection and preterm labor. Clin Obstet Gynecol. 1988; 31:553–584. [PubMed: 3066544]
15. Romero R, Sirtori M, Oyarzun E, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol. 1989; 161:817–824. [PubMed: 2675611]
16. Gibbs RS, Romero R, Hillier SL, et al. A review of premature birth and subclinical infection. Am J Obstet Gynecol. 1992; 166:1515–1528. [PubMed: 1595807]
17. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000; 342:1500–1507. [PubMed: 10816189]
18. Romero R, Espinoza J, Chaiworapongsa T, Kalache K. Infection and prematurity and the role of preventive strategies. Semin Neonatol. 2002; 7:259–274. [PubMed: 12401296]
19. Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev. 2002; 8:3–13. [PubMed: 11921380]
20. Romero R, Espinoza J, Goncalves LF, et al. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med. 2006; 11:317–326. [PubMed: 16839830]
21. Romero R, Espinoza J, Goncalves LF, et al. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007; 25:21–39. [PubMed: 17205421]
22. Vrachnis N, Vitoratos N, Iliodromiti Z, et al. Intrauterine inflammation and preterm delivery. Ann N Y Acad Sci. 2010; 1205:118–122. [PubMed: 20840262]
23. Cobo T, Palacio M, Martinez-Terron M, et al. Clinical and inflammatory markers in amniotic fluid as predictors of adverse outcomes in preterm premature rupture of membranes. Am J Obstet Gynecol. 2011; 205:126.e1–e8. [PubMed: 21621184]
24. Gervasi MT, Romero R, Bracalente G, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. J Perinat Med. 2012; 40:329–343. [PubMed: 22752762]
25. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol. 1992; 79:351–357. [PubMed: 1738513]

26. Yoon BH, Romero R, Park JS, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol. 2000; 182:675–681. [PubMed: 10739529]
27. Hitti J, Tarczy-Hornoch P, Murphy J, et al. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. Obstet Gynecol. 2001; 98:1080–1088. [PubMed: 11755557]
28. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? where? why? Lancet. 2005; 365:891–900. [PubMed: 15752534]
29. Lee J, Oh KJ, Yang HJ, et al. The importance of intraamniotic inflammation in the subsequent development of atypical chronic lung disease. J Matern Fetal Neonatal Med. 2009; 22:917–923. [PubMed: 19718578]
30. Kramer BW, Kallapur S, Newnham J, Jobe AH. Prenatal inflammation and lung development. Semin Fetal Neonatal Med. 2009; 14:2–7. [PubMed: 18845493]
31. Jobe AH, Kallapur SG. Chorioamnionitis, surfactant, and lung disease in very low birth weight infants. J Pediatr. 2010; 156:3–4. [PubMed: 20006756]
32. Hofer N, Kothari R, Morris N, et al. The fetal inflammatory response syndrome is a risk factor for morbidity in preterm neonates. Am J Obstet Gynecol. 2013; 209:542e1–e11. [PubMed: 23994220]
33. Kacerovsky M, Musilova I, Andrys C, et al. Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. Am J Obstet Gynecol. 2014; 210:325e1–e10. [PubMed: 24184182]
34. Combs CA, Gravett M, Garite TJ, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol. 2014; 210:125e1–e15. [PubMed: 24274987]
35. Combs CA, Gravett M, Garite TJ. Reply: To PMID 24274987. Am J Obstet Gynecol. 2014; 211:708–709.
36. Strunk T, Inder T, Wang X, et al. Infection-induced inflammation and cerebral injury in preterm infants. Lancet Infect Dis. 2014; 14:751–762. [PubMed: 24877996]
37. Bastek JA, Weber AL, McShea MA, et al. Prenatal inflammation is associated with adverse neonatal outcomes. Am J Obstet Gynecol. 2014; 210:450.e1–e10. [PubMed: 24361788]
38. Manuck TA, Varner MW. Neonatal and early childhood outcomes following early vs later preterm premature rupture of membranes. Am J Obstet Gynecol. 2014; 211:308e1–e6. [PubMed: 24858202]
39. Manuck TA, Sheng X, Yoder BA, Varner MW. Correlation between initial neonatal and early childhood outcomes following preterm birth. Am J Obstet Gynecol. 2014; 210:426e1–e9. [PubMed: 24793722]
40. Manuck TA, Rice MM, Bailit JL, Grobman WA, Reddy UM, Wapner RJ, et al. Preterm neonatal morbidity and mortality by gestational age: a contemporary cohort. Am J Obstet Gynecol. 2016; 215:103e1–e14. [PubMed: 26772790]
41. Romero R, Salafia CM, Athanassiadis AP, et al. The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology. Am J Obstet Gynecol. 1992; 166:1382–1388. [PubMed: 1595794]
42. Hillier SL, Witkin SS, Krohn MA, et al. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. Obstet Gynecol. 1993; 81:941–948. [PubMed: 8497360]
43. Odibo AO, Rodis JF, Sanders MM, et al. Relationship of amniotic fluid markers of intra-amniotic infection with histopathology in cases of preterm labor with intact membranes. J Perinatol. 1999; 19:407–412. [PubMed: 10685269]
44. Park CW, Yoon BH, Park JS, Jun JK. An elevated maternal serum C-reactive protein in the context of intra-amniotic inflammation is an indicator that the development of amnionitis, an intense fetal and AF inflammatory response are likely in patients with preterm labor: clinical implications. J Matern Fetal Neonatal Med. 2013; 26:847–853. [PubMed: 23484918]
45. Romero R, Miranda J, Chaiworapongsa T, et al. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. Am J Reprod Immunol. 2014; 71:330–358. [PubMed: 24417618]

46. Romero R, Miranda J, Chaemsaithong P, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med. 2015; 28:1394–1409. [PubMed: 25190175]
47. Kim SM, Romero R, Park JW, et al. The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis in preterm gestation. J Matern Fetal Neonatal Med. 2015; 28:1500–1509. [PubMed: 25184305]
48. Kim CJ, Romero R, Chaemsaithong P, et al. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol. 2015; 213:S29–S52. [PubMed: 26428501]
49. Park CW, Park JS, Moon KC, Jun JK, Yoon BH. Preterm labor and preterm premature rupture of membranes have a different pattern in the involved compartments of acute histologoic chorioamnionitis and/or funisitis: patho-physiologic implication related to different clinical manifestations. Pathol Int. 2016; 66:325–332. [PubMed: 27090052]
50. Lee SE, Romero R, Kim CJ, et al. Funisitis in term pregnancy is associated with microbial invasion of the amniotic cavity and intra-amniotic inflammation. J Matern Fetal Neonatal Med. 2006; 19:693–697. [PubMed: 17127492]
51. Kacerovsky MVF, Kutova R, Pliskova L, et al. Cervical microbiota in women with preterm prelabor rupture of membranes. PLoS One. 2015; 10:e0126884. [PubMed: 25993616]
52. Park CWKS, Park JS, Jun JK, Yoon BH. Fetal, amniotic and maternal inflammatory responses in early stage of ascending intrauterine infection, inflammation restricted to chorio-decidua, in preterm gestation. J Matern Fetal Neonatal Med. 2014; 27:98–105. [PubMed: 23691922]
53. Revello RAM, Dudzik D, Abehsera D, Bartha JL. Differential amniotic fluid cytokine profile in women with chorioamnionitis with and without funisitis. J Matern Fetal Neonatal Med. 2016; 29:2161–2165. [PubMed: 26372455]
54. Romero R, Miranda J, Chaiworapongsa T, et al. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. J Matern Fetal Neonatal Med. 2014:1–17.
55. Romero R, Miranda J, Kusanovic JP, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. J Perinat Med. 2015; 43:19–36. [PubMed: 25720095]
56. Maymon E, Romero R, Pacora P, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol. 2000; 183:94–99. [PubMed: 10920315]
57. Maymon E, Romero R, Chaiworapongsa T, et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. Am J Obstet Gynecol. 2001; 185:1143–1148. [PubMed: 11717648]
58. Maymon E, Romero R, Chaiworapongsa T, et al. Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. Am J Obstet Gynecol. 2001; 185:1149–1155. [PubMed: 11717649]
59. Yoon BH, Oh SY, Romero R, et al. An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol. 2001; 185:1162–1167. [PubMed: 11717651]
60. Park JS, Romero R, Yoon BH, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. Am J Obstet Gynecol. 2001; 185:1156–1161. [PubMed: 11717650]
61. Angus SR, Segel SY, Hsu CD, et al. Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. Am J Obstet Gynecol. 2001; 185:1232–1238. [PubMed: 11717662]
62. Moon JB, Kim JC, Yoon BH, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. J Perinat Med. 2002; 30:301–306. [PubMed: 12235718]
63. Biggio JR Jr, Ramsey PS, Cliver SP, et al. Midtrimester amniotic fluid matrix metalloproteinase-8 (MMP-8) levels above the 90th percentile are a marker for subsequent preterm premature rupture of membranes. Am J Obstet Gynecol. 2005; 192:109–113. [PubMed: 15672011]

64. Nien JK, Yoon BH, Espinoza J, et al. A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. Am J Obstet Gynecol. 2006; 195:1025–1030. [PubMed: 17000236]
65. Kim KW, Romero R, Park HS, et al. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. Am J Obstet Gynecol. 2007; 197:292e1–e5. [PubMed: 17826425]
66. Park CW, Lee SM, Park JS, et al. The antenatal identification of funisitis with a rapid MMP-8 bedside test. J Perinat Med. 2008; 36:497–502. [PubMed: 19127606]
67. Lee SJ, Won HS, Kim MN, et al. Diagnostic value of the matrix metalloproteinase-8 rapid test for detecting microbial invasion of the amniotic cavity. Eur J Clin Microbiol Infect Dis. 2008; 27:1257–1260. [PubMed: 18566842]
68. Kim A, Lee ES, Shin JC, Kim HY. Identification of biomarkers for preterm delivery in mid-trimester amniotic fluid. Placenta. 2013; 34:873–878. [PubMed: 23953866]
69. Park HS, Kim SA. Abstract No 322: The value of the genedia MMP-8 rapid test for diagnosing intraamniotic infection/inflammation and predicting adverse pregnancy outcomes in women with preterm premature rupture of membranes. Am J Obstet Gynecol. 2015; 212:S174.
70. Kim SM, Romero R, Lee J, et al. About one-half of early spontaneous preterm deliveries can be identified by a rapid matrix metalloproteinase-8 (MMP-8) bedside test at the time of mid-trimester genetic amniocentesis. J Matern Fetal Neonatal Med. 2016; 29:2414–2421. [PubMed: 26643648]
71. Chaemsaithong, P., Romero, R., Docheva, N., et al. A rapid point-of-care test (MMP-8) for the identification of intra-amniotic inflammation and impending preterm delivery. Abstract presented at 12th World Congress of Perinatal Medicine; 3rd–6th November, 2015; Madrid, Spain.
72. Chaemsaithong, P., Romero, R., Chaiyasit, N., et al. Rapid MMP-8 as a point-of-care test in the identification of intra-amniotic inflammation in patients with preterm PROM. Abstract presented at 12th World Congress of Perinatal Medicine; 3rd–6th November, 2015; Madrid, Spain.
73. Chaiyasit, N., Chaemsaithong, P., Romero, R., et al. A rapid MMP-8 test for the identification of intra-amniotic inflammation/infection in patients with clinical chorioamnionitis at term: a solution at last!. Abstract presented at 12th World Congress of Perinatal Medicine; 3rd–6th November, 2015; Madrid, Spain.
74. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection J Clin Investig. 1990; 85:1392–1400. [PubMed: 2332497]
75. Santhanam U, Avila C, Romero R, et al. Cytokines in normal and abnormal parturition: elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with intrauterine infection. Cytokine. 1991; 3:155–163. [PubMed: 1888885]
76. Romero R, Sepulveda W, Kenney JS, et al. Interleukin 6 determination in the detection of microbial invasion of the amniotic cavity. Ciba Found Symp. 1992; 167:205–220. Discussion: 20–23. [PubMed: 1425014]
77. Romero R, Yoon BH, Mazor M, et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and gram stain in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 1993; 169:805–816. [PubMed: 7694461]
78. Romero R, Yoon BH, Mazor M, et al. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and gram stain in the detection of microbial invasion in patients with preterm premature rupture of membranes. Am J Obstet Gynecol. 1993; 169:839–851. [PubMed: 7694463]
79. Romero R, Yoon BH, Kenney JS, et al. Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. Am J Reprod Immunol. 1993; 30:167–183. [PubMed: 8311926]
80. Greig PC, Ernest JM, Teot L, et al. Amniotic fluid interleukin-6 levels correlate with histologic chorioamnionitis and amniotic fluid cultures in patients in premature labor with intact membranes. Am J Obstet Gynecol. 1993; 169:1035–1044. [PubMed: 8238116]
81. Gomez R, Romero R, Galasso M, et al. The value of amniotic fluid interleukin-6, white blood cell count, and gram stain in the diagnosis of microbial invasion of the amniotic cavity in patients at term. Am J Reprod Immunol. 1994; 32:200–210. [PubMed: 7533501]

82. Coultrip LL, Lien JM, Gomez R, et al. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. Am J Obstet Gynecol. 1994; 171:901–911. [PubMed: 7943100]
83. Dudley DJ, Hunter C, Mitchell MD, Varner MW. Clinical value of amniotic fluid interleukin-6 determinations in the management of preterm labour. Br J Obstet Gynaecol. 1994; 101:592–597. [PubMed: 8043537]
84. Yoon BH, Romero R, Kim CJ, et al. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. Am J Obstet Gynecol. 1995; 172:960–970. [PubMed: 7892891]
85. Negishi H, Yamada H, Mikuni M, et al. Correlation between cytokine levels of amniotic fluid and histological chorioamnionitis in preterm delivery. J Perinat Med. 1996; 24:633–639. [PubMed: 9120746]
86. Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol. 1997; 177:19–26. [PubMed: 9240577]
87. Yoon BH, Romero R, Jun JK, et al. Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. Am J Obstet Gynecol. 1997; 177:825–830. [PubMed: 9369827]
88. Wenstrom KD, Andrews WW, Hauth JC, et al. Elevated second-trimester amniotic fluid interleukin-6 levels predict preterm delivery. Am J Obstet Gynecol. 1998; 178:546–550. [PubMed: 9539524]
89. Baud O, Emilie D, Pelletier E, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestation: histological associations and neonatal outcome. Br J Obstet Gynaecol. 1999; 106:72–77. [PubMed: 10426263]
90. Yoon BH, Park CW, Chaiworapongsa T. Intrauterine infection and the development of cerebral palsy. BJOG. 2003; 110:124–127. [PubMed: 12763129]
91. Jacobsson B, Mattsby-Baltzer I, Andersch B, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. Acta Obstet Gynecol Scand. 2003; 82:423–431. [PubMed: 12752072]
92. Jacobsson B, Mattsby-Baltzer I, Hagberg H. Interleukin-6 and interleukin-8 in cervical and amniotic fluid: relationship to microbial invasion of the chorioamniotic membranes. BJOG. 2005; 112:719–724. [PubMed: 15924526]
93. Holst RM, Laurini R, Jacobsson B, et al. Expression of cytokines and chemokines in cervical and amniotic fluid: relationship to histological chorioamnionitis. J Matern Fetal Neonatal Med. 2007; 20:885–893. [PubMed: 18050018]
94. Menon R, Camargo MC, Thorsen P, et al. Amniotic fluid interleukin-6 increase is an indicator of spontaneous preterm birth in white but not black Americans. Am J Obstet Gynecol. 2008; 198:77e1–e7. [PubMed: 18166313]
95. Cobo T, Palacio M, Navarro-Sastre A, et al. Predictive value of combined amniotic fluid proteomic biomarkers and interleukin-6 in preterm labor with intact membranes. Am J Obstet Gynecol. 2009; 200:499e1–e6. [PubMed: 19375569]
96. Massaro G, Scaravilli G, Simeone S, et al. Interleukin-6 and Mycoplasma hominis as markers of preterm birth and related brain damage: our experience. J Matern Fetal Neonatal Med. 2009; 22:1063–1067. [PubMed: 19900045]
97. Thomakos N, Daskalakis G, Papapanagiotou A, et al. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: relationship to intra-amniotic microbial invasion and preterm delivery. Eur J Obstet Gynecol Reproduct Biol. 2010; 148:147–151.
98. Marconi C, de Andrade Ramos BR, Peracoli JC, et al. Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. Am J Reprod Immunol. 2011; 65:549–556. [PubMed: 21214658]
99. Cobo T, Kacerovsky M, Palacio M, et al. A prediction model of histological chorioamnionitis and funisitis in preterm prelabor rupture of membranes: analyses of multiple proteins in the amniotic fluid. J Matern Fetal Neonatal Med. 2012; 25:1995–2001. [PubMed: 22372866]

100. Cobo T, Kacerovsky M, Holst RM, et al. Intra-amniotic inflammation predicts microbial invasion of the amniotic cavity but not spontaneous preterm delivery in preterm prelabor membrane rupture. Acta Obstet Gynecol Scand. 2012; 91:930–935. [PubMed: 22524241]
101. Bogavac M, Brkic S, Simin N, Celic D. Mid-pregnancy interleukins levels in serum and amniotic fluid as predictors of preterm delivery. J Matern Fetal Neonatal Med. 2012; [Epub ahead of print]. doi: 10.3109/14767058.2012.722709
102. Romero R, Kadar N, Miranda J, et al. The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not superior to amniotic fluid interleukin-6. J Matern Fetal Neonatal Med. 2014; 27:757–769. [PubMed: 24028673]
103. Kacerovsky M, Musilova I, Hornychova H, et al. Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes. Am J Obstet Gynecol. 2014; 211:385e1–e9. [PubMed: 24705131]
104. Cobo T, Jacobsson B, Kacerovsky M, et al. Systemic and local inflammatory response in women with preterm prelabor rupture of membranes. PloS One. 2014; 9:e85277. [PubMed: 24465522]
105. Chaemsaithong P, Romero R, Korzeniewski SJ, et al. A point of care test for the determination of amniotic fluid interleukin-6 and the chemokine CXCL-10/IP-10. J Matern Fetal Neonatal Med. 2015; 28:1510–1519. [PubMed: 25182862]
106. Kacerovsky M, Musilova I, Stepan M, et al. Detection of intraamniotic inflammation in fresh and processed amniotic fluid samples with the interleukin-6 point of care test. Am J Obstet Gynecol. 2015; 213:435–436. [PubMed: 26003057]
107. Chaemsaithong P, Romero R, Korzeniewski SJ, et al. A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. J Matern Fetal Neonatal Med. 2016; 29:349–359. [PubMed: 25758618]
108. Chaemsaithong P, Romero R, Korzeniewski SJ, et al. A point of care test for interleukin-6 in amniotic fluid in preterm prelabor rupture of membranes: a step toward the early treatment of acute intra-amniotic inflammation/infection. J Matern Fetal Neonatal Med. 2016; 29:360–367. [PubMed: 25758620]
109. Sennstrom MB, Brauner A, Bystrom B, et al. Matrix metalloproteinase-8 correlates with the cervical ripening process in humans. Acta Obstet Gynecol Scand. 2003; 82:904–911. [PubMed: 12956839]
110. Diaz-Cueto L, Cuica-Flores A, Ziga-Cordero F, et al. Vaginal matrix metalloproteinase levels in pregnant women with bacterial vaginosis. J Soc Gynecol Investig. 2006; 13:430–434.
111. Rahkonen L, Rutanen EM, Unkila-Kallio L, et al. Factors affecting matrix metalloproteinase-8 levels in the vaginal and cervical fluids in the first and second trimester of pregnancy. Hum Reprod. 2009; 24:2693–2702. [PubMed: 19654111]
112. Schefold JC, Hasper D, von Haehling S, et al. Interleukin-6 serum level assessment using a new qualitative point-of-care test in sepsis: a comparison with ELISA measurements. Clin Biochem. 2008; 41:893–898. [PubMed: 18395522]
113. Meem M, Modak JK, Mortuza R, et al. Biomarkers for diagnosis of neonatal infections: a systematic analysis of their potential as a point-of-care diagnostics. J Global Health. 2011; 1:201–209.
114. Batfalsky A, Lohr A, Heussen N, et al. Diagnostic value of an interleukin-6 bedside test in term and preterm neonates at the time of clinical suspicion of early and late-onset bacterial infection. Neonatology. 2012; 102:37–44. [PubMed: 22507910]
115. Gutierres SL, Welty TE. Point-of-care testing: an introduction. Ann Pharmacother. 2004; 38:119–125. [PubMed: 14742805]
116. Kost GJ, Tran NK. Point-of-care testing and cardiac biomarkers: the standard of care and vision for chest pain centers. Cardiol Clin. 2005; 23:467–490. [PubMed: 16278118]
117. Lewandrowski K. Point-of-care testing: an overview and a look to the future (circa 2009, United States). Clin Lab Med. 2009; 29:421–432. [PubMed: 19840677]
118. Pfafflin A, Schleicher E. Inflammation markers in point-of-care testing (POCT). Anal Bioanal Chem. 2009; 393:1473–1480. [PubMed: 19104782]

119. Vousden N, Chandiramani M, Seed P, Shennan A. Interleukin-6 bedside testing in women at high risk of preterm birth. J Matern Fetal Neonatal Med. 2011; 24:1301–1304. [PubMed: 21381876]
120. Berthiaume M, Rousseau E, Rola-Pleszczynski M, Pasquier JC. Rapid evaluation of the absence of inflammation after rupture of membranes. J Matern Fetal Neonatal Med. 2014; 27:865–869. [PubMed: 23947432]
121. Romero R, Quintero R, Nores J, et al. Amniotic fluid white blood cell count: a rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery. Am J Obstet Gynecol. 1991; 165:821–830. [PubMed: 1951538]
122. Romero R, Jimenez C, Lohda AK, et al. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. Am J Obstet Gynecol. 1990; 163:968–974. [PubMed: 1698338]
123. Romero R, Emamian M, Quintero R, et al. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. Am J Obstet Gynecol. 1988; 159:114–119. [PubMed: 2456013]
124. http://milenia-biotec.de/immunology/mileniar-quickline-il-6/?L=1. 2016.
125. Galen, RS., Gambino, SR. Beyond normality: the predictive value and efficiency of medical diagnoses. New York: John Wiley & Sons; 1975. Evaluation of laboratory tests: comparing sensitivity and specificity data; p. 131-240.
126. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor: the detection of endotoxin in amniotic fluid. Am J Obstet Gynecol. 1987; 157:815–819. [PubMed: 2445204]
127. Romero R, Roslansky P, Oyarzun E, et al. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am J Obstet Gynecol. 1988; 158:1044–1049. [PubMed: 3369483]
128. Romero R, Grivel JC, Tarca AL, et al. Evidence of perturbations of the cytokine network in preterm labor. Am J Obstet Gynecol. 2015; 213:836e1–e18. [PubMed: 26232508]
129. Espinoza J, Chaiworapongsa T, Romero R, et al. Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. J Matern Fetal Neonatal Med. 2003; 13:2–21. [PubMed: 12710851]
130. Soto E, Espinoza J, Nien JK, et al. Human beta-defensin-2: a natural antimicrobial peptide present in amniotic fluid participates in the host response to microbial invasion of the amniotic cavity. J Matern Fetal Neonatal Med. 2007; 20:15–22. [PubMed: 17437194]
131. Owen CA, Hu Z, Lopez-Otin C, Shapiro SD. Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. J Immunol. 2004; 172:7791–7803. [PubMed: 15187163]
132. Dejonckheere E, Vandenbroucke RE, Libert C. Matrix metalloproteinase-8 has a central role in inflammatory disorders and cancer progression. Cytokine Growth Factor Rev. 2011; 22:73–81. [PubMed: 21388856]
133. Van Lint P, Libert C. Matrix metalloproteinase-8: cleavage can be decisive. Cytokine Growth Factor Rev. 2006; 17:217–223. [PubMed: 16820317]
134. Herman MP, Sukhova GK, Libby P, et al. Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. Circulation. 2001; 104:1899–1904. [PubMed: 11602491]
135. Prikk K, Maisi P, Pirila E, et al. In vivo collagenase-2 (MMP-8) expression by human bronchial epithelial cells and monocytes/macrophages in bronchiectasis. J Pathol. 2001; 194:232–238. [PubMed: 11400153]
136. Zheng L, Lam WK, Tipoe GL, et al. Overexpression of matrix metalloproteinase-8 and -9 in bronchiectatic airways in vivo. Eur Respir J. 2002; 20:170–176. [PubMed: 12166566]
137. Wahlgren J, Maisi P, Sorsa T, et al. Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. J Pathol. 2001; 194:217–224. [PubMed: 11400151]
138. Toft-Hansen H, Nuttall RK, Edwards DR, Owens T. Key metalloproteinases are expressed by specific cell types in experimental autoimmune encephalomyelitis. J Immunol. 2004; 173:5209–5218. [PubMed: 15470066]

139. Tervahartiala T, Pirila E, Ceponis A, et al. The in vivo expression of the collagenolytic matrix metalloproteinases (MMP-2, -8, -13, and -14) and matrilysin (MMP-7) in adult and localized juvenile periodontitis. J Den Res. 2000; 79:1969–1977.
140. Pirila E, Ramamurthy N, Maisi P, et al. Wound healing in ovariectomized rats: effects of chemically modified tetracycline (CMT-8) and estrogen on matrix metalloproteinases -8, -13 and type I collagen expression. Curr Med Chem. 2001; 8:281–294. [PubMed: 11172683]
141. O'Brien TP, Li QJ, Sauerburger F, et al. The role of matrix metalloproteinases in ulcerative keratolysis associated with perioperative diclofenac use. Ophthalmology. 2001; 108:656–659. [PubMed: 11297478]
142. Pirila E, Ramamurthy NS, Sorsa T, et al. (MMP-2), collagenase-2 (MMP-8), and laminin-5 gamma2-chain expression in murine inflammatory bowel disease (ulcerative colitis). Dig Dis Sci. 2003; 48:93–98. [PubMed: 12645796]
143. Hanemaaijer R, Sorsa T, Konttinen YT, et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. J Biol Chem. 1997; 272:31504–1509. [PubMed: 9395486]
144. Stenman M, Paju A, Hanemaaijer R, et al. Collagenases (MMP-1, -8 and -13) and trypsinogen-2 in fluid from benign and malignant ovarian cysts. Tumour Biol. 2003; 24:9–12. [PubMed: 12743421]
145. Knauper V, Kramer S, Reinke H, Tschesche H. Characterization and activation of procollagenase from human polymorphonuclear leucocytes. N-terminal sequence determination of the proenzyme and various proteolytically activated forms. Eur J Biochem/FEBS. 1990; 189:295–300.
146. Knauper V, Wilhelm SM, Seperack PK, DeClerck YA, Langley KE, Osthues A, et al. Direct activation of human neutrophil procollagenase by recombinant stromelysin. Biochem J. 1993; 295:581–586. [PubMed: 8240261]
147. Balbin M, Fueyo A, Knauper V, et al. Collagenase 2 (MMP-8) expression in murine tissue-remodeling processes. Analysis of its potential role in postpartum involution of the uterus. J Biol Chem. 1998; 273:23959–23968. [PubMed: 9727011]
148. Knauper V, Murphy G, Tschesche H. Activation of human neutrophil procollagenase by stromelysin 2. Eur J Biochem/FEBS. 1996; 235:187–191.
149. Okamoto T, Akaike T, Suga M, et al. Activation of human matrix metalloproteinases by various bacterial proteinases. J Biol Chem. 1997; 272:6059–6066. [PubMed: 9038230]
150. Schmid TM, Mayne R, Jeffrey JJ, Linsenmayer TF. Type X collagen contains two cleavage sites for a vertebrate collagenase. J Biol Chem. 1986; 261:4184–4189. [PubMed: 3005323]
151. Hasty KA, Jeffrey JJ, Hibbs MS, Welgus HG. The collagen substrate specificity of human neutrophil collagenase. J Biol Chem. 1987; 262:10048–10052. [PubMed: 3038863]
152. Pirila E, Sharabi A, Salo T, et al. Matrix metalloproteinases process the laminin-5 gamma 2-chain and regulate epithelial cell migration. Biochem Biophys Res Commun. 2003; 303:1012–1017. [PubMed: 12684035]
153. Shapiro SD. Matrix metalloproteinase degradation of extracellular matrix: biological consequences. Curr Opin Cell Biol. 1998; 10:602–608. [PubMed: 9818170]
154. Van Den Steen PE, Wuyts A, Husson SJ, et al. Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities. Eur J Biochem/FEBS. 2003; 270:3739–3749.
155. Van den Steen PE, Husson SJ, Proost P, et al. Carboxyterminal cleavage of the chemokines MIG and IP-10 by gelatinase B and neutrophil collagenase. Biochem Biophys Res Commun. 2003; 310:889–896. [PubMed: 14550288]
156. McQuibban GA, Gong JH, Wong JP, et al. Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. Blood. 2002; 100:1160–1167. [PubMed: 12149192]
157. Gueders MM, Balbin M, Rocks N, et al. Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. J Immunol. 2005; 175:2589–2597. [PubMed: 16081833]

158. Kuula H, Salo T, Pirila E, et al. Local and systemic responses in matrix metalloproteinase 8-deficient mice during Porphyromonas gingivalis-induced periodontitis. Infect Immun. 2009; 77:850–859. [PubMed: 19029300]

159. Romero, R., Gomez, R., Mazor, M., Ghezzi, F., Yoon, BH. The preterm labor syndrome. In: Elder, MG.Romero, R., Lamont, RF., editors. Preterm labor. New York: Churchill Livingstone; 1997. p. 29-49.

160. Winkler M, Fischer DC, Ruck P, et al. Parturition at term: parallel increases in interleukin-8 and proteinase concentrations and neutrophil count in the lower uterine segment. Hum Reprod. 1999; 14:1096–1100. [PubMed: 10221247]

161. Horwitz AL, Hance AJ, Crystal RG. Granulocyte collagenase: selective digestion of type I relative to type III collagen. Proc Natl Acad Sci USA. 1977; 74:897–901. [PubMed: 191839]

162. Fortunato SJ, Menon R, Lombardi SJ. MMP/TIMP imbalance in amniotic fluid during PROM: an indirect support for endogenous pathway to membrane rupture. J Perinat Med. 1999; 27:362–368. [PubMed: 10642956]

163. Menon R, Fortunato SJ. The role of matrix degrading enzymes and apoptosis in rupture of membranes. J Soc Gynecol Investig. 2004; 11:427–437.

164. Weiss A, Goldman S, Shalev E. The matrix metalloproteinases (MMPS) in the decidua and fetal membranes. Front Biosci. 2007; 12:649–59. [PubMed: 17127325]

165. Sampson JE, Theve RP, Blatman RN, et al. Fetal origin of amniotic fluid polymorphonuclear leukocytes. Am J Obstet Gynecol. 1997; 176:77–81. [PubMed: 9024093]

166. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol. 2015; 16:448–457. [PubMed: 25898198]

167. Gomez-Lopez N, Olson DM, Robertson SA. Interleukin-6 controls uterine Th9 cells and CD8(þ) T regulatory cells to accelerate parturition in mice. Immunol Cell Biol. 2016; 94:79–89. [PubMed: 26073576]

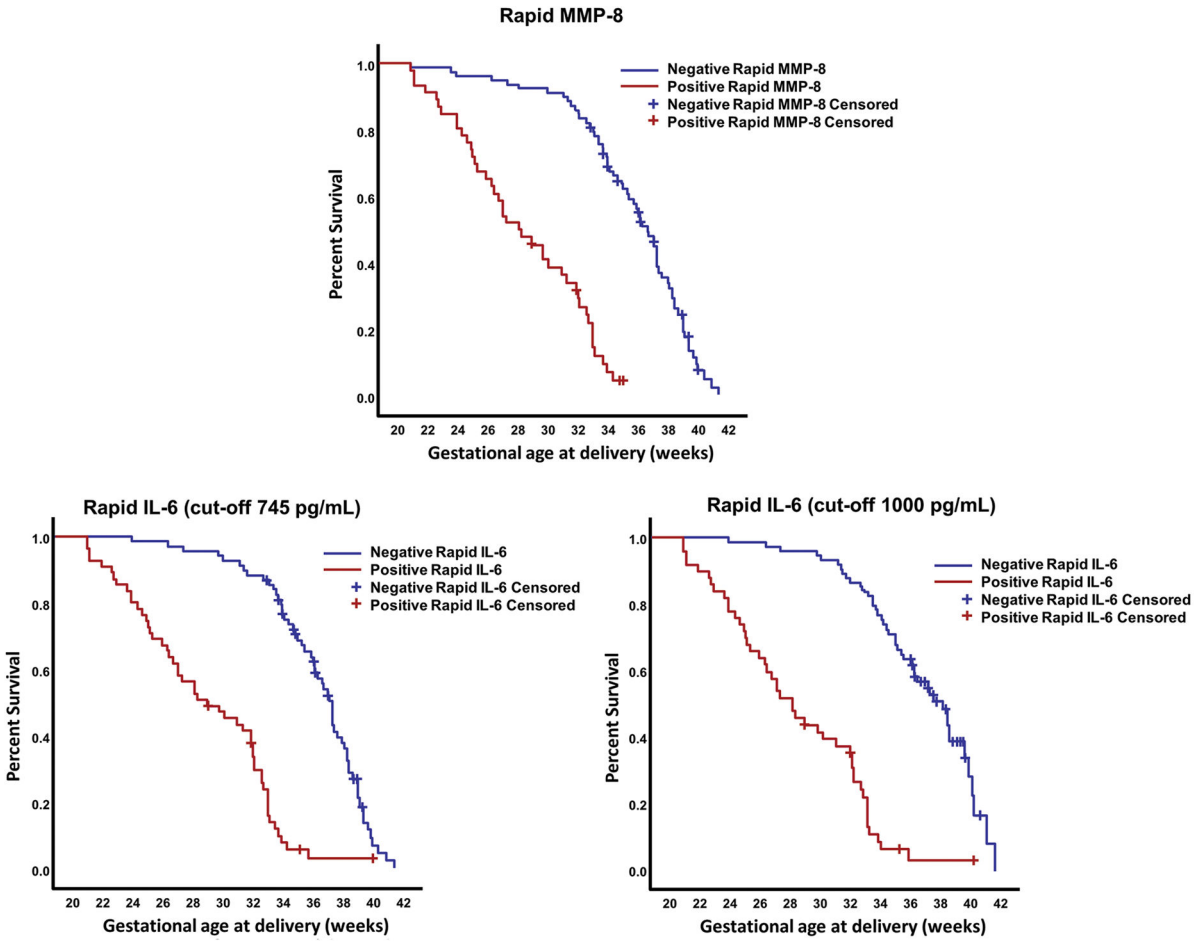

Figure 1.

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.

J Matern Fetal Neonatal Med. Author manuscript; available in PMC 2019 January 01.

Table 3

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

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
Intra-amniotic infection (microbial-associated intra-amniotic inflammation) [9.7% (12/124)]	Sensitivity	100% (12/12)	73.5–100	100% (12/12)	73.5–100
	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.

Table 4

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

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
	Accuracy	79.8% (99/124)	0.72–0.87	79.0% (98/124)	0.71–0.86
Spontaneous delivery within 7 days after amniocentesis [44.4% (55/124)]	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
	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
Spontaneous delivery <28 weeks of gestation [21.8% (27/124)] *	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
	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
Spontaneous delivery <32 weeks of gestation [33.9% (42/124)] **	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).

Table 5

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

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	*Ureaplasma urealyticum*	28^{+6}	13	180	Positive	10,000	9.4	Acute chorioamnionitis	No
2	*Streptococcus agalactiae*	25^{+2}	19	5	Positive	8208	248.9	Acute chorioamnionitis	Umbilical arteritis
3	*Mobiluncus* spp.	32^{+0}	10	570	Positive	8144	76.9	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
4	*Haemophilus influenza*	30^{+6}	10	40	Positive	6467	92.1	Necrotizing chorioamnionitis	Necrotizing funisitis
5	*Fusobacterium* 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	*Bacteroides* spp., *Mobiluncus* spp., *Clostridium sporogenesis*	22^{+4}	10	295	Positive	4748	517.8	Necrotizing chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
9	*Ureaplasma urealyticum*	33^{+0}	10	500	Positive	4628	85.9	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
10	*Mycoplasma hominis*	33^{+0}	1	420	Positive	4613	172.3	N/A	N/A
11	*Candida albicans*	26^{+3}	10	2160	Positive	4448	201.3	Necrotizing chorioamnionitis	No
12	*Staphylococcus capitis*	28^{+6}	20	24	Positive	4374	360.5	Subacute chorioamnionitis	Umbilical arteritis
13	*Candida albicans*	32^{+4}	10	1292	Positive	4252	96.3	Acute chorioamnionitis	Necrotizing funisitis
14	*Streptococcus agalactiae*	25^{+0}	10	4	Positive	3575	93.6	Necrotizing chorioamnionitis	No
15	*Candida albicans, Lactobacillus* spp.	33^{+1}	10	43	Positive	3554	200.6	Acute subchorionitis/chorionitis	No
16	*Prevotella* spp., *Enterococcus faecalis*	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	*Streptococcus* spp., *Gemella morbillorum*	31^{+6}	10	1920	Positive	6796	0.7	Necrotizing chorioamnionitis	Umbilical arteritis
19	*Streptococcus anginosus, Streptococcus mitis*	22^{+6}	10	10	Negative	7246	73.3	Acute chorioamnionitis	Umbilical phlebitis/chorionic vasculitis
20	*Fusobacterium* spp., Gram-negative bacilli	28^{+1}	1	22	Negative	3996	301.4	Acute chorioamnionitis	Umbilical arteritis
21	*Ureaplasma urealyticum*	39^{+2}	19	2	Negative	155	1.3	No	No
22	*Ureaplasma urealyticum*	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.

Table 7

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.

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.