Published in final edited form as:

J Perinat Med. 2017 July 26; 45(5): 539–550. doi:10.1515/jpm-2016-0344.

Clinical chorioamnionitis at term VIII: a rapid MMP-8 Test for the identification of intra-amniotic inflammation

Noppadol Chaiyasit[1,2], **Roberto Romero**[1,3,4,5], **Piya Chaemsaithong**[1,2], **Nikolina Docheva**[1,2], **Gaurav Bhatti**[1,2], **Juan Pedro Kusanovic**[6,7], **Zhong Dong**[1,2], **Lami Yeo**[1,2], **Percy Pacora**[1], **Sonia S. Hassan**[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

[6]Center for Research and Innovation in Maternal-Fetal Medicine (CIMAF), Department of Obstetrics and Gynecology, Sótero del Río Hospital, Santiago, Chile

[7]Division of Obstetrics and Gynecology, Pontificia Universidad Católica de Chile, Santiago, Chile

Abstract

Objective—Clinical chorioamnionitis is the most common infection/inflammatory process diagnosed in labor and delivery units worldwide. The condition is a syndrome that can be caused by: 1) intra-amniotic infection; 2) intra-amniotic inflammation without demonstrable microorganisms (i.e., sterile intra-amniotic inflammation); and 3) maternal systemic inflammation that is not associated with intra-amniotic inflammation. The presence of intraamniotic inflammation is a risk factor for adverse maternal and neonatal outcomes in a broad range of obstetrical syndromes that includes clinical chorioamnionitis at term. Although the diagnosis of intra-amniotic infection has relied on culture results, such information is not immediately available for patient management. Therefore, the diagnosis of intra-amniotic inflammation could be helpful as a proxy for intra-amniotic infection, while results of microbiologic studies are pending. A rapid test is now available for the diagnosis of intraamniotic inflammation, based on the determination of neutrophil collagenase or matrix metalloproteinase-8 (MMP-8). The objectives of this study were to 1) evaluate the diagnostic indices of a rapid MMP-8 test for the identification of intra-amniotic inflammation/infection in patients with the diagnosis of clinical chorioamnionitis at term;

Address correspondence to: 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, romeror@mail.nih.gov.

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

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

and 2) compare the diagnostic performance of a rapid MMP-8 test to that of a conventional enzyme-linked immunosorbent assay (ELISA) interleukin (IL)-6 test for patients with clinical chorioamnionitis at term.

Materials and Methods—A retrospective cohort study was conducted. A transabdominal amniocentesis was performed in patients with clinical chorioamnionitis at term (n=44). Amniotic fluid was analyzed using cultivation techniques (for aerobic and anaerobic bacteria as well as genital Mycoplasmas) and broad-range polymerase chain reaction (PCR) coupled with electrospray ionization mass spectrometry (PCR/ESI-MS). Amniotic fluid IL-6 concentrations were determined by ELISA, and rapid MMP-8 results were determined by Yoon's MMP-8 Check®. Intra-amniotic inflammation was defined as an elevated amniotic fluid IL-6 concentration ≥2.6 ng/mL, and intra-amniotic infection was diagnosed by the presence of microorganisms in the amniotic fluid accompanied by intra-amniotic inflammation. The diagnostic indices of Yoon's MMP-8 Check® for the identification of intra-amniotic inflammation were calculated. In order to objectively compare Yoon's MMP-8 Check® to the ELISA IL-6 test for the identification of intra-amniotic inflammation, we used an amniotic fluid white blood cell (WBC) count ≥50 cells/mm^3 to define intra-amniotic inflammation.

Results—1) A positive rapid MMP-8 test had a sensitivity of 82.4% (28/34), specificity of 90% (9/10), positive predictive value of 96.6% (28/29), negative predictive value of 60% (9/15), positive likelihood ratio 8.2 (95% CI 1.3-53.2), and negative likelihood ratio 0.2 (95% CI 0.1-0.4) for the identification of intra-amniotic inflammation (prevalence 77.3%); 2) a positive rapid MMP-8 test had a sensitivity of 91.7% (22/24), specificity of 65% (13/20), positive predictive value of 75.9% (22/29), negative predictive value of 86.7% (13/15), positive likelihood of ratio 2.6 (95% CI 1.4-4.8), and negative likelihood ratio 0.1 (95% CI 0.03-0.5) for the identification of intra-amniotic infection; 3) the rapid MMP-8 test had a significantly higher specificity than the ELISA IL-6 test in the identification of intra-amniotic inflammation as determined by an amniotic fluid WBC count ≥50 cells/mm^3. The sensitivity and accuracy of the rapid MMP-8 test were comparable to those of the ELISA IL-6 test; and 4) importantly, the rapid MMP-8 test had 100% sensitivity and 100% negative predictive value in the identification of neonates affected with fetal inflammatory response syndrome (FIRS).

Conclusion—The rapid diagnosis of intra-amniotic inflammation is possible by analysis of amniotic fluid using a point-of-care test for MMP-8. Patients with a positive test are at risk for delivering a neonate affected with systemic inflammation, a risk factor for adverse neonatal outcome.

Keywords

amniocentesis; amniotic fluid; biomarkers; interleukin-6 (IL-6); matrix metalloproteinase (MMP-8); microbial invasion of the amniotic cavity (MIAC); point-of-care test; pregnancy

Introduction

Clinical chorioamnionitis is the most common infection/inflammatory process diagnosed in labor and delivery units worldwide (1-9). The condition is a syndrome (10) that can be caused by: 1) intra-amniotic infection (10, 11); 2) intra-amniotic inflammation without demonstrable microorganisms (i.e., sterile intra-amniotic inflammation) (10); and 3)

maternal systemic inflammation that is not associated with intra-amniotic inflammation (12). The presence of intraamniotic inflammation is a risk factor for adverse maternal (5, 13-18) and neonatal (19-36) outcomes in a broad range of obstetrical syndromes (20, 37-44), including clinical chorioamnionitis at term (10, 45). Although the diagnosis of intra-amniotic infection has relied on culture results (46-48), such information is not immediately available for patient management. Therefore, the diagnosis of intra-amniotic inflammation could be helpful as a proxy for intraamniotic infection while results of microbiologic studies are pending. A rapid test is now available for the diagnosis of intra-amniotic inflammation based on the determination of neutrophil collagenase or matrix metalloproteinase-8 (MMP-8).

Amniotic fluid MMP-8 concentration has diagnostic and prognostic value in the identification of intra-amniotic inflammation (49, 50), intra-amniotic infection (50-55), acute inflammatory lesions of the placenta, especially funisitis (56, 57), imminent spontaneous preterm birth (49, 52, 53), and adverse neonatal outcome such as cerebral palsy (49-54, 57-65). However, the determination of MMP-8 using a conventional enzyme-linked immunosorbent assay (ELISA) test is time-consuming, and the results are often not available in time to guide management and clinical decisions for suspected cases of intra-amniotic inflammation.

A rapid MMP-8 test has recently been developed and provides accurate results without the need for costly instruments (49, 50, 57, 64-68). Previous studies reported that the rapid MMP-8 test results strongly correlate with those of conventional MMP-8 ELISA tests (49) and have diagnostic as well as prognostic value in the identification of impending preterm delivery in patients who have preterm labor and intact membranes (49, 66), preterm prelabor rupture of the membranes (preterm PROM) (50, 64), funisitis in preterm delivery (57), microbial invasion of the amniotic cavity (MIAC) (55), and early spontaneous preterm delivery in asymptomatic midtrimester patients (65). Nonetheless, there is no information about the diagnostic value of the amniotic fluid rapid MMP-8 test to identify intra-amniotic inflammation in cases of clinical chorioamnionitis at term.

Therefore, the objectives of this study were to: 1) evaluate the diagnostic indices of a rapid MMP-8 test for the identification of intra-amniotic inflammation/infection in patients with the diagnosis of clinical chorioamnionitis at term; and 2) compare the diagnostic performance of a rapid MMP-8 test to that of a conventional ELISA interleukin (IL)-6 test for patients with clinical chorioamnionitis at term.

Materials and Methods

Study population

This retrospective cohort study was conducted with patients who had clinical chorioamnionitis at term for whom samples of amniotic fluid were available in the Bank of Biological Materials at 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). Inclusion criteria were: 1) singleton gestation; 2) gestational age ≥37 weeks; and 3) transabdominal amniocentesis performed for molecular

microbiologic studies. Exclusion criteria included multiple gestations or fetal malformations. The samples were collected from patients cared for at the Sótero del Río Hospital, a major affiliate of the Catholic University in Santiago, Chile. Patients enrolled in this study comprised a subset of those who had been included in previous studies (8, 10, 12, 36, 45, 69). All amniocenteses were performed for clinical indications at the discretion of the treating physician(s).

Maternal and neonatal data were obtained from clinical chart reviews. Women with the diagnosis of clinical chorioamnionitis were counseled to undergo an amniocentesis. Written informed consent to donate additional amniotic fluid and to collect clinical information for research purposes was obtained from each participant. The Institutional Review Boards of Wayne State University, NICHD, and Sótero del Río Hospital approved the use of samples and biological specimens as well as the use of clinical data for research purposes.

Clinical definitions

Clinical chorioamnionitis was diagnosed using the criteria proposed by Gibbs et al. (1): the presence of maternal fever (temperature >37.8°C) accompanied by two or more of the following: 1) maternal tachycardia (heart rate >100 beats/min); 2) uterine tenderness; 3) foul-smelling amniotic fluid; 4) fetal tachycardia (heart rate >160 beats/min); and 5) maternal leukocytosis (leukocyte count >15,000 cells/mm^3) (1-4, 6, 11, 70-73).

The presence of intra-amniotic inflammation was defined as an elevated amniotic fluid IL-6 concentration ≥2.6 ng/mL (20, 28, 29, 74-79). To compare the diagnostic performance of Yoon's MMP-8 Check® (SK Pharma Co., Ltd., Kyunggi-do, Republic of Korea) and that of a conventional ELISA IL-6 test (Human IL-6 Quantikine ELISA kit, D6050, R&D Systems, Minneapolis, MN, USA), we defined intra-amniotic inflammation as an amniotic fluid white blood cell (WBC) count ≥50 cells/mm^3 (80). Intra-amniotic infection (microbial-associated intraamniotic inflammation) was diagnosed in the presence of both intra-amniotic inflammation and microorganisms detected in the amniotic fluid using cultivation or molecular microbiologic techniques (PCR/ESI-MS; Ibis® Technology, Athogen, Carlsbad, CA, USA) (81-83).

Acute inflammatory lesions of the placenta included acute histologic chorioamnionitis and acute funisitis (84, 85). Acute histologic chorioamnionitis was diagnosed based on the infiltration of inflammatory cells (neutrophils) in the chorionic plate and/or chorioamniotic membranes (20, 84-86). Acute funisitis was diagnosed based on the infiltration of neutrophils in the walls of the umbilical vessels and/or in the Wharton's jelly, also using previously reported criteria (84-87). Fetal inflammatory response syndrome (FIRS) was defined as an elevation of an umbilical cord plasma IL-6 concentration >11 pg/mL (87-92).

Sample collection, amniotic fluid processing, amniotic fluid analysis for microbiologic studies, IL-6 concentrations (10), and the performance and interpretation of Yoon's MMP-8 Check® (49, 50, 57, 65, 93) were previously described.

Study outcomes

The primary outcomes were the diagnostic indices, predictive values, and likelihood ratios of Yoon's MMP-8 Check® for the identification of intra-amniotic inflammation (defined as an amniotic fluid ELISA IL-6 concentration ≥2.6 ng/mL). The secondary outcomes were the diagnostic indices, predictive values, and likelihood ratios of Yoon's MMP-8 Check® for the identification of intra-amniotic infection, the presence of acute inflammatory histologic features of the placenta (acute histologic chorioamnionitis and/or funisitis), and FIRS.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess normality of arithmetic data distributions. Diagnostic indices, predictive values, and likelihood ratios were calculated to identify each outcome. Comparisons of sensitivity, and specificity, between Yoon's MMP-8 Check® and the ELISA IL-6 test were performed using McNemar's test. A p-value of <0.05 indicated statistical significance. All p-values were determined from a two-sided test. Statistical analysis was performed using SPSS Version 19 (IBM Corp, Armonk, NY, USA) and the R statistical language and environment (94).

Results

Characteristics of the study population

A total of 44 patients who had clinical chorioamnionitis at term [39.6 (IQR: 38.8-40.7) weeks of gestation] were included in this study. Table 1 displays the clinical characteristics of the study population. The overall rate of intra-amniotic inflammation was 77.3% (34/44). The rate of intra-amniotic infection was 54.5% (24/44) and that of intra-amniotic inflammation without detectable bacteria was 22.7% (10/44). The most common microorganisms isolated from the amniotic fluid were *Gardnerella vaginalis* (n=10) and *Ureaplasma urealyticum* (n=8), and 66% (16/24) had a polymicrobial infection. Acute histologic chorioamnionitis and acute funisitis were found in 46.5% (20/43) and 30.2% (13/43) of cases, respectively. The prevalence of FIRS was 23.3% (10/43). This information has been extensively covered in prior publications (8, 10, 12, 36, 45, 69).

Performance of the rapid MMP-8 test to identify intra-amniotic inflammation, intra-amniotic infection, acute inflammatory lesions of the placenta, and neonates with FIRS

The diagnostic indices, predictive values, and likelihood ratios of the rapid MMP-8 test for different outcomes of interest are displayed in Table 2. The sensitivity, specificity, and positive and negative likelihood ratios of the rapid MMP-8 test for the identification of intraamniotic inflammation were 82.4% (28/34), 90% (9/10), 8.2, and 0.2, respectively. The positive and negative predictive values were 96.6% (28/29) and 60% (9/15), respectively, in a population with a prevalence of 77.3% (34/44).

This test also demonstrated a high sensitivity [91.7% (22/24)] and negative predictive value [86.7% (13/15)] for the identification of intra-amniotic infection. Importantly, the rapid MMP-8 test had a 100% (10/10) sensitivity and a 100% (14/14) negative predictive value for the identification of mothers who subsequently delivered neonates with FIRS.

Comparison of the diagnostic performances between the rapid MMP-8 test and the ELISA IL-6 test

The prevalence of intra-amniotic inflammation (defined as a WBC count ≥ 50 cells/mm^3) and intra-amniotic infection was 52.3% (23/44) and 54.5% (24/44), respectively (Table 1). The rapid MMP-8 test had a significantly higher specificity than the conventional ELISA IL-6 test in the identification of intra-amniotic inflammation [66.7% (14/21) vs. 42.9% (9/21); p=0.03]. The accuracy of the rapid MMP-8 kit was 81.8% (36/44) and that of the IL-6 ELISA was 70.5% (31/44) (Table 2). In addition, both tests had comparable performances for the identification of intra-amniotic infection, acute inflammatory lesions of the placenta, acute funisitis, and the presence of FIRS in neonates, as the 95% confidence intervals (CI) overlapped (Table 2). Similar results were obtained when intra-amniotic inflammation was defined according to the amniotic fluid WBC counts (> 50 cells/mm^3) (Table 3). Patients with discrepant results between the rapid MMP-8 and the IL-6 ELISA tests are described in the Supplementary Table.

Discussion

Principal findings of this study

In the context of clinical chorioamnionitis at term, 1) the positive rapid MMP-8 test had a sensitivity of 82.4% (28/34), specificity of 90% (9/10), positive predictive value of 96.6% (28/29), negative predictive value of 60% (9/15), positive likelihood ratio of 8.2 (95% CI, 1.3-53.2), and negative likelihood ratio of 0.2 (95% CI, 0.1-0.4) for the identification of intraamniotic inflammation (prevalence, 77.3%); 2) the positive rapid MMP-8 test had a sensitivity of 91.7% (22/24), specificity of 65% (13/20), positive predictive value of 75.9% (22/29), negative predictive value of 86.7% (13/15), positive likelihood ratio of 2.6 (95% CI, 1.4-4.8), and negative likelihood ratio of 0.1 (95% CI, 0.03-0.5) for the identification of intra-amniotic infection; 3) the rapid MMP-8 test had a significantly higher specificity than the ELISA IL-6 test in the identification of intra-amniotic inflammation as determined by an amniotic fluid WBC count ≥50 cells/mm^3. The sensitivity and accuracy of the rapid MMP-8 test were comparable to those of the ELISA IL-6 test; and 4) importantly, the rapid MMP-8 test had a 100% sensitivity and 100% negative predictive value in the identification of neonates affected with FIRS.

Clinical chorioamnionitis at term: the need for a rapid test to identify intra-amniotic inflammation/infection—Clinical chorioamnionitis is the most common infection diagnosed in labor and delivery units (4-7). This condition is associated with substantial maternal and neonatal morbidity, including maternal sepsis (95), dysfunctional labor (16, 96), postpartum endomyometritis (15) and hemorrhage (5, 96), early-onset neonatal sepsis (19, 95, 97-99), and cerebral palsy (100-103). Currently, the diagnosis of clinical chorioamnionitis is based on maternal clinical signs that have only about a 50% accuracy for the identification of proven intra-amniotic infection (8). The management of mothers with suspected clinical chorioamnionitis includes treatment with antimicrobial agents (47, 71, 104-106) and labor augmentation (4, 6, 73, 107). Neonatologists often perform a laboratory work-up for sepsis and initiate antibiotic treatment (108-114).

Interestingly, a fraction of patients with clinical chorioamnionitis at term had proven intra-amniotic infection (10), but others had intra-amniotic inflammation without bacteria or without intra-amniotic inflammation. Only one-fifth (21%) of neonates born to mothers with clinical chorioamnionitis at term had FIRS (36). In our studies, all neonates with FIRS were born to mothers with clinical chorioamnionitis who also had intra-amniotic infection (36).

Patients with intra-amniotic inflammation without microorganisms or without intraamniotic inflammation may not benefit from the administration of antibiotics, which is the standard of practice. In general, the administration of antimicrobial agents in this context has been considered to have little, if any, adverse events. However, recent evidence suggests that antibiotic administration can have adverse maternal and neonatal effects. For example, antibiotic administration is the most common cause of maternal anaphylaxis (115-118); although this condition is rare (prevalence of 2.7 cases/100,000 deliveries) (118), it can lead to maternal death and fetal compromise, including neurological damage (119-121). In addition, excessive antibiotic use increases the development of antibiotic-resistant microorganisms (122-124).

For newborns, consequences from prenatal exposure to antibiotics include: 1) the need for a sepsis work-up and other laboratory testing, which is associated with increased workload and costs (108-114); 2) a prolonged hospital stay that can predispose these neonates to acquire infections caused by multi-drug-resistant bacteria (110, 112); 3) the separation of newborns from parents with the potential for impaired mother-neonate bonding and breastfeeding; and 4) the alteration of gut microbiota (125-128). The change in neonatal gut microbiota due to intrapartum antibiotic exposure is currently a subject of intense investigation. For example, it has been shown that prenatal exposure to antimicrobial agents is associated with a reduction in *Bacteroides* and *Parabacteroides* species and an over-representation of *Enterococcus* and *Clostridium* species within the neonatal gut microbial community (127). Importantly, the disturbance of neonatal gut microbiota can persist until 12 months of age after birth (127) and appears to have short- and long-term effects on the immune response. The latter includes an increased predisposition to the development of allergies (129-131), asthma (132-134), atopic disease (135-137), obesity (138-140), and neonatal sepsis (141-143). The imbalance in the composition of the neonatal gut microbial community in mice due to perinatal antibiotic administration has been shown to prevent granulocytosis and to reduce circulating neutrophils one to three days after birth, thus increasing the susceptibility to blood-borne infections and subsequent development of sepsis (144, 145).

There is mounting evidence indicating that a subset of women with clinical chorioamnionitis at term and their neonates requires intrapartum antibiotics and that a subgroup may not need such treatment. Thus far, clinical signs (8), inflammatory-related proteins in the maternal (12) or cord (36) blood, or placental histopathology (69) have limited value for the identification of intra-amniotic infection/inflammation in patients who have clinical chorioamnionitis at term. The results of several studies in this population suggest that amniotic fluid analysis may help to distinguish between patients with or without intra-amniotic inflammation/infection and to guide clinical decisions. Although amniocentesis is an invasive procedure, transcervical collection of amniotic fluid is now possible with a

simple-to-use device now available for clinical use (146). The optimal method to diagnose intra-amniotic inflammation in fluid retrieved by this device is a subject of current investigation. However, if biomarkers that allow the diagnosis of intra-amniotic inflammation with such biological material are identifiable, then the diagnosis of intra-amniotic inflammation could be made non-invasively.

Rapid MMP-8 test of amniotic fluid—The rapid MMP-8 test of amniotic fluid fulfills the standard requirements for a point-of-care test as results are available in a timely fashion: the test is simple to use, sensitive, inexpensive, and operator- and instrument-independent. Previous studies reported that the rapid MMP-8 test can be used at the bedside to identify intra-amniotic inflammation in patients at risk of imminent preterm delivery (49, 93), preterm PROM (50, 64), funisitis (57), and MIAC (55). Moreover, the diagnostic performance of the rapid MMP-8 test for the identification of intraamniotic inflammation is equivalent to that of a conventional immunoassay for MMP-8 (49). The results of the present study confirm the value of the rapid MMP-8 test for the determination of intra-amniotic inflammation in patients with clinical chorioamnionitis at term.

Given the high positive predictive value and positive likelihood ratio of the rapid MMP-8 test, a positive MMP-8 result can be interpreted as evidence of intra-amniotic inflammation. We propose that the next step in management should be differentiation between sterile and microbial-associated intra-amniotic inflammation; this will determine whether anti-microbial or anti-inflammatory treatment needs to be administered. This can be achieved by sequencing the 16S rRNA gene using novel molecular microbiologic techniques that will allow the rapid detection of microbial signatures (81-83).

The rapid MMP-8 test had a sensitivity of 91.7% (22/24) and a negative predictive value of 86.7% (13/15) for the identification of intra-amniotic infection. Due to the high negative predictive value, a negative result can help to identify women with clinical chorioamnionitis at term who *are not* at risk for intra-amniotic infection, and this information can be used to decrease the index of suspicion of proven intra-amniotic infection, therefore preventing the overuse of anti-microbial agents.

Importantly, the rapid MMP-8 test demonstrated a 100% (10/10) sensitivity and a 100% (14/14) negative predictive value for the identification of FIRS. As neonates with this condition are at an increased risk of neonatal sepsis, antimicrobial treatment should be promptly initiated. Our results indicated that a positive amniotic fluid rapid MMP-8 test can identify most neonates with FIRS, and a negative test result would exclude the presence of FIRS in neonates born to mothers with clinical chorioamnionitis at term. Collectively, the rapid MMP-8 test has optimal characteristics as a point-of-care test.

Strengths and limitations—The strengths of the study include the participation of a homogeneous group of patients with clinical chorioamnionitis at term, the use of both cultivation and molecular microbiologic techniques for the identification of microorganisms, and treatment not influenced by the rapid MMP-8 test. We acknowledge that the major limitation is the sample size; however, this is a unique population in which characterizations of intra-amniotic inflammation and intra-amniotic infection were performed by

amniocentesis, a procedure infrequently used in the identification of patients with clinical chorioamnionitis at term. This procedure was implemented at the Sótero del Río Hospital because of the need to address large-scale use of antibiotics and the performance of a high number of septic work-ups of patients with maternal fever. The samples employed in this study have been used for other studies of amniotic fluid, including the amniotic fluid lipidome (147) and to characterize the nature of the inflammatory response. Therefore, replication of these findings in a separate population is warranted.

Conclusions

A rapid MMP-8 point-of-care test can be used for the diagnosis of intra-amniotic inflammation and to identify the mother at risk for delivering a neonate with systemic inflammation among patients with clinical chorioamnionitis at term.

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, U.S. 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. Gibbs RS. Diagnosis of intra-amniotic infection. Semin Perinatol. 1977; 1(1):71–7. [PubMed: 106471]
2. Hollander D. Diagnosis of chorioamnionitis. Clin Obstet Gynecol. 1986; 29(4):816–25. [PubMed: 3545586]
3. Gilstrap LC 3rd, Cox SM. Acute chorioamnionitis. Obstet Gynecol Clin North Am. 1989; 16(2): 373–9. [PubMed: 2674804]
4. Newton ER. Chorioamnionitis and intraamniotic infection. Clin Obstet Gynecol. 1993; 36(4):795–808. [PubMed: 8293582]
5. Rouse DJ, Landon M, Leveno KJ, Leindecker S, Varner MW, Caritis SN, et al. The Maternal-Fetal Medicine Units cesarean registry: chorioamnionitis at term and its duration-relationship to outcomes. Am J Obstet Gynecol. 2004; 191(1):211–6. [PubMed: 15295368]
6. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. Clin Perinatol. 2010; 37(2):339–54. [PubMed: 20569811]
7. Malloy MH. Chorioamnionitis: epidemiology of newborn management and outcome United States 2008. J Perinatol. 2014; 34(8):611–5. [PubMed: 24786381]
8. Romero R, Chaemsaithong P, Korzeniewski SJ, Kusanovic JP, Docheva N, Martinez-Varea A, et al. Clinical chorioamnionitis at term III: how well do clinical criteria perform in the identification of proven intra-amniotic infection? Journal of perinatal medicine. 2016; 44(1):23–32. [PubMed: 25918914]
9. Mazaki-Tovi S, Vaisbuch E. Clinical chorioamnionitis--an ongoing obstetrical conundrum. J Perinat Med. 2016; 44(1):1–4. [PubMed: 26756087]
10. Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsaithong P, Martinez A, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. J Perinat Med. 2015; 43(1):19–36. [PubMed: 25720095]

11. Gibbs RS, Blanco JD, St Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis. 1982; 145(1):1–8. [PubMed: 7033397]
12. Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Tarca AL, Bhatti G, et al. Clinical chorioamnionitis at term IV: the maternal plasma cytokine profile. J Perinat Med. 2016; 44(1):77–98. [PubMed: 26352068]
13. Koh KS, Chan FH, Monfared AH, Ledger WJ, Paul RH. The changing perinatal and maternal outcome in chorioamnionitis. Obstet Gynecol. 1979; 53(6):730–4. [PubMed: 450343]
14. Duff P, Sanders R, Gibbs RS. The course of labor in term patients with chorioamnionitis. Am J Obstet Gynecol. 1983; 147(4):391–5. [PubMed: 6624808]
15. Hauth JC, Gilstrap LC 3rd, Hankins GD, Connor KD. Term maternal and neonatal complications of acute chorioamnionitis. Obstet Gynecol. 1985; 66(1):59–62. [PubMed: 4011072]
16. Satin AJ, Maberry MC, Leveno KJ, Sherman ML, Kline DM. Chorioamnionitis: a harbinger of dystocia. Obstet Gynecol. 1992; 79(6):913–5. [PubMed: 1579312]
17. Keski-Nisula L, Kirkinen P, Katila ML, Ollikainen M, Suonio S, Saarikoski S. Amniotic fluid U. urealyticum colonization: significance for maternal peripartal infections at term. Am J Perinatol. 1997; 14(3):151–6. [PubMed: 9259918]
18. Edwards RK. Chorioamnionitis and labor. Obstet Gynecol Clin North Am. 2005; 32(2):287–96, x. [PubMed: 15899361]
19. Alexander JM, McIntire DM, Leveno KJ. Chorioamnionitis and the prognosis for term infants. Obstet Gynecol. 1999; 94(2):274–8. [PubMed: 10432142]
20. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001; 185(5):1130–6. [PubMed: 11717646]
21. Jobe AH, Ikegami M. Antenatal infection/inflammation and postnatal lung maturation and injury. Respir Res. 2001; 2(1):27–32. [PubMed: 11686862]
22. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol. 2004; 191(4):1339–45. [PubMed: 15507963]
23. Kallapur SG, Jobe AH. Contribution of inflammation to lung injury and development. Arch Dis Child Fetal Neonatal Ed. 2006; 91(2):F132–5. [PubMed: 16492951]
24. Lee J, Oh KJ, Yang HJ, Park JS, Romero R, Yoon BH. The importance of intra-amniotic inflammation in the subsequent development of atypical chronic lung disease. J Matern Fetal Neonatal Med. 2009; 22(10):917–23. [PubMed: 19718578]
25. Kramer BW, Kallapur S, Newnham J, Jobe AH. Prenatal inflammation and lung development. Semin Fetal Neonatal Med. 2009; 14(1):2–7. [PubMed: 18845493]
26. Bersani I, Thomas W, Speer CP. Chorioamnionitis--the good or the evil for neonatal outcome? J Matern Fetal Neonatal Med. 2012; 25(1):12–6. [PubMed: 22309119]
27. Hofer N, Kothari R, Morris N, Muller W, Resch B. The fetal inflammatory response syndrome is a risk factor for morbidity in preterm neonates. Am J Obstet Gynecol. 2013; 209(6):542 e1–e11. [PubMed: 23994220]
28. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Reprod Immunol. 2014; 72(5):458–74. [PubMed: 25078709]
29. Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol. 2014; 210(2):125 e1–e15. [PubMed: 24274987]
30. Cobo T, Kacerovsky M, Jacobsson B. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol. 2014; 211(6):708.
31. Manuck TA, Varner MW. Neonatal and early childhood outcomes following early vs later preterm premature rupture of membranes. Am J Obstet Gynecol. 2014; 211(3):308 e1–6. [PubMed: 24858202]

32. Bastek JA, Weber AL, McShea MA, Ryan ME, Elovitz MA. Prenatal inflammation is associated with adverse neonatal outcomes. Am J Obstet Gynecol. 2014; 210(5):450 e1–10. [PubMed: 24361788]
33. Pappas A, Kendrick DE, Shankaran S, Stoll BJ, Bell EF, Laptook AR, et al. Chorioamnionitis and early childhood outcomes among extremely low-gestational-age neonates. JAMA Pediatr. 2014; 168(2):137–47. [PubMed: 24378638]
34. 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(1):103 e1–e14. [PubMed: 26772790]
35. Miyazaki K, Furuhashi M, Ishikawa K, Tamakoshi K, Hayashi K, Kai A, et al. Impact of chorioamnionitis on short- and long-term outcomes in very low birth weight preterm infants: the Neonatal Research Network Japan. J Matern Fetal Neonatal Med. 2016; 29(2):331–7. [PubMed: 25567563]
36. Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Tarca AL, Bhatti G, et al. Clinical chorioamnionitis at term V: umbilical cord plasma cytokine profile in the context of a systemic maternal inflammatory response. J Perinat Med. 2016; 44(1):53–76. [PubMed: 26360486]
37. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med. 2006; 11(5):317–26. [PubMed: 16839830]
38. Hassan S, Romero R, Hendler I, Gomez R, Khalek N, Espinoza J, et al. A sonographic short cervix as the only clinical manifestation of intra-amniotic infection. J Perinat Med. 2006; 34(1):13–9. [PubMed: 16489881]
39. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007; 25(1):21–39. [PubMed: 17205421]
40. Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. Nutr Rev. 2007; 65(12 Pt 2):S194–202. [PubMed: 18240548]
41. Menon R, Fortunato SJ. Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Clin Obstet Gynaecol. 2007; 21(3):467–78. [PubMed: 17448730]
42. Lee SE, Romero R, Park CW, Jun JK, Yoon BH. The frequency and significance of intraamniotic inflammation in patients with cervical insufficiency. American journal of obstetrics and gynecology. 2008; 198(6):633 e1–8. Epub 2008/03/18. [PubMed: 18342290]
43. Madan I, Romero R, Kusanovic JP, Mittal P, Chaiworapongsa T, Dong Z, et al. The frequency and clinical significance of intra-amniotic infection and/or inflammation in women with placenta previa and vaginal bleeding: an unexpected observation. Journal of perinatal medicine. 2010; 38(3):275–9. Epub 2010/02/12. [PubMed: 20146660]
44. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014; 345(6198):760–5. [PubMed: 25124429]
45. Romero R, Chaemsaithong P, Korzeniewski SJ, Tarca AL, Bhatti G, Xu Z, et al. Clinical chorioamnionitis at term II: the intra-amniotic inflammatory response. J Perinat Med. 2016; 44(1): 5–22. [PubMed: 25938217]
46. Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, 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(3):817–24. [PubMed: 2675611]
47. Gibbs RS, Duff P. Progress in pathogenesis and management of clinical intraamniotic infection. Am J Obstet Gynecol. 1991; 164(5 Pt 1):1317–26. [PubMed: 2035575]
48. Riggs JW, Blanco JD. Pathophysiology, diagnosis, and management of intraamniotic infection. Semin Perinatol. 1998; 22(4):251–9. [PubMed: 9738989]
49. Nien JK, Yoon BH, Espinoza J, Kusanovic JP, Erez O, Soto E, 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(4):1025–30. [PubMed: 17000236]
50. Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, 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(3):292 e1–5. [PubMed: 17826425]

51. Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol. 2000; 183(1):94–9. [PubMed: 10920315]
52. Maymon E, Romero R, Chaiworapongsa T, Kim JC, Berman S, Gomez R, et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. Am J Obstet Gynecol. 2001; 185(5):1143–8. [PubMed: 11717648]
53. Maymon E, Romero R, Chaiworapongsa T, Berman S, Conoscenti G, Gomez R, et al. Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. Am J Obstet Gynecol. 2001; 185(5):1149–55. [PubMed: 11717649]
54. Angus SR, Segel SY, Hsu CD, Locksmith GJ, Clark P, Sammel MD, et al. Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. Am J Obstet Gynecol. 2001; 185(5):1232–8. [PubMed: 11717662]
55. Lee SJ, Won HS, Kim MN, Lee PR, Shim JY, Kim A. 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(12):1257–60. [PubMed: 18566842]
56. Park JS, Romero R, Yoon BH, Moon JB, Oh SY, Han SY, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. Am J Obstet Gynecol. 2001; 185(5):1156–61. [PubMed: 11717650]
57. Park CW, Lee SM, Park JS, Jun JK, Romero R, Yoon BH. The antenatal identification of funisitis with a rapid MMP-8 bedside test. J Perinat Med. 2008; 36(6):497–502. [PubMed: 19127606]
58. Yoon BH, Oh SY, Romero R, Shim SS, Han SY, Park JS, 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(5):1162–7. [PubMed: 11717651]
59. Moon JB, Kim JC, Yoon BH, Romero R, Kim G, Oh SY, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. J Perinat Med. 2002; 30(4):301–6. [PubMed: 12235718]
60. Biggio JR Jr, Ramsey PS, Cliver SP, Lyon MD, Goldenberg RL, Wenstrom KD. 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(1): 109–13. [PubMed: 15672011]
61. Park CW, Yoon BH, Kim SM, Park JS, Jun JK. The frequency and clinical significance of intraamniotic inflammation defined as an elevated amniotic fluid matrix metalloproteinase-8 in patients with preterm labor and low amniotic fluid white blood cell counts. Obstet Gynecol Sci. 2013; 56(3):167–75. [PubMed: 24327997]
62. Kim A, Lee ES, Shin JC, Kim HY. Identification of biomarkers for preterm delivery in mid-trimester amniotic fluid. Placenta. 2013; 34(10):873–8. [PubMed: 23953866]
63. Kim SM, Romero R, Park JW, Oh KJ, Jun JK, Yoon BH. 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(13):1500–9. [PubMed: 25184305]
64. 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 prematue rupture of membranes. Am J Obstet Gynecol. 2015; 212(1):S174.
65. Kim SM, Romero R, Lee J, Chaemsaithong P, Lee MW, Chaiyasit N, 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(15):2414–22. [PubMed: 26643648]
66. Chaemsaithong, P., Romero, R., Docheva, N., Chaiyasit, N., Kim, YM., Dong, Z., 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. 2015.
67. Chaemsaithong, P., Romero, R., Chaiyasit, N., Docheva, N., Korzeniewski, SJ., Kim, YM., 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. 2015.

68. Chaiyasit, N., Chaemsaithong, P., Romero, R., Docheva, N., Dong, Z., Kim, CJ., 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. 2015.
69. Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Kusanovic JP, Yoon BH, et al. Clinical chorioamnionitis at term VI: acute chorioamnionitis and funisitis according to the presence or absence of microorganisms and inflammation in the amniotic cavity. J Perinat Med. 2016; 44(1): 33–51. [PubMed: 26352071]
70. Gibbs RS, Castillo MS, Rodgers PJ. Management of acute chorioamnionitis. Am J Obstet Gynecol. 1980; 136(6):709–13. [PubMed: 7355955]
71. Gibbs RS, Dinsmoor MJ, Newton ER, Ramamurthy RS. A randomized trial of intrapartum versus immediate postpartum treatment of women with intra-amniotic infection. Obstet Gynecol. 1988; 72(6):823–8. [PubMed: 3186087]
72. Romero R, Chaiworapongsa T, Savasan ZA, Hussein Y, Dong Z, Kusanovic JP, et al. Clinical chorioamnionitis is characterized by changes in the expression of the alarmin HMGB1 and one of its receptors, sRAGE. J Matern Fetal Neonatal Med. 2012; 25(6):558–67. [PubMed: 22578261]
73. Fishman SG, Gelber SE. Evidence for the clinical management of chorioamnionitis. Semin Fetal Neonatal Med. 2012; 17(1):46–50. [PubMed: 21962477]
74. Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, 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(4):329–43. [PubMed: 22752762]
75. Romero R, Kadar N, Miranda J, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, 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(8):757–69. [PubMed: 24028673]
76. Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, 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(4):330–58. [PubMed: 24417618]
77. Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, et al. Sterile intraamniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. J Matern Fetal Neonatal Med. 2014:1–17.
78. Chaemsaithong P, Romero R, Korzeniewski SJ, Dong Z, Yeo L, Hassan SS, 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(13):1510–9. [PubMed: 25182862]
79. Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, 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(3):360–7. [PubMed: 25758620]
80. Romero R, Quintero R, Nores J, Avila C, Mazor M, Hanaoka S, 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(4 Pt 1):821–30. [PubMed: 1951538]
81. Sampath R, Hall TA, Massire C, Li F, Blyn LB, Eshoo MW, et al. Rapid identification of emerging infectious agents using PCR and electrospray ionization mass spectrometry. Ann N Y Acad Sci. 2007; 1102:109–20. [PubMed: 17470915]
82. Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, Eshoo MW, et al. Ibis T5000: a universal biosensor approach for microbiology. Nat Rev Microbiol. 2008; 6(7):553–8. [PubMed: 18521073]
83. Ecker DJ, Sampath R, Li H, Massire C, Matthews HE, Toleno D, et al. New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn. 2010; 10(4):399–415. [PubMed: 20465496]

84. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation--a workshop report. Placenta. 2005; 26(Suppl A):S114–7. [PubMed: 15837060]
85. Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol. 2015; 213(4 Suppl):S29–52. [PubMed: 26428501]
86. Redline RW. Inflammatory responses in the placenta and umbilical cord. Semin Fetal Neonatal Med. 2006; 11(5):296–301. [PubMed: 16621749]
87. Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, et al. Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. J Matern Fetal Neonatal Med. 2002; 11(1):18–25. [PubMed: 12380603]
88. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol. 1998; 179(1):194–202. [PubMed: 9704787]
89. Chaiworapongsa T, Romero R, Kim JC, Kim YM, Blackwell SC, Yoon BH, et al. Evidence for fetal involvement in the pathologic process of clinical chorioamnionitis. Am J Obstet Gynecol. 2002; 186(6):1178–82. [PubMed: 12066094]
90. Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O, et al. The fetal inflammatory response syndrome. Clin Obstet Gynecol. 2007; 50(3):652–83. [PubMed: 17762416]
91. Chaiworapongsa T, Romero R, Berry SM, Hassan SS, Yoon BH, Edwin S, et al. The role of granulocyte colony-stimulating factor in the neutrophilia observed in the fetal inflammatory response syndrome. J Perinat Med. 2011; 39(6):653–66. [PubMed: 21801092]
92. Romero R, Savasan ZA, Chaiworapongsa T, Berry SM, Kusanovic JP, Hassan SS, et al. Hematologic profile of the fetus with systemic inflammatory response syndrome. J Perinat Med. 2012; 40(1):19–32.
93. Chaemsaithong P. A comparison of rapid MMP-8 and rapid interleukin-6 point-of-care tests for the identification of intra-amniotic infection/inflammation and impending preterm delivery. 2016 in preparation.
94. Development Core, R. Team R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2015.
95. Yoder PR, Gibbs RS, Blanco JD, Castaneda YS, St Clair PJ. A prospective, controlled study of maternal and perinatal outcome after intra-amniotic infection at term. Am J Obstet Gynecol. 1983; 145(6):695–701. [PubMed: 6829656]
96. Mark SP, Croughan-Minihane MS, Kilpatrick SJ. Chorioamnionitis and uterine function. Obstet Gynecol. 2000; 95(6 Pt 1):909–12.
97. Yancey MK, Duff P, Kubilis P, Clark P, Frentzen BH. Risk factors for neonatal sepsis. Obstet Gynecol. 1996; 87(2):188–94. [PubMed: 8559521]
98. Soraisham AS, Singhal N, McMillan DD, Sauve RS, Lee SK, Canadian Neonatal N. A multicenter study on the clinical outcome of chorioamnionitis in preterm infants. Am J Obstet Gynecol. 2009; 200(4):372 e1–6. [PubMed: 19217596]
99. Garcia-Munoz Rodrigo F, Galan Henriquez GM, Ospina CG. Morbidity and mortality among very-low-birth-weight infants born to mothers with clinical chorioamnionitis. Pediatr Neonatol. 2014; 55(5):381–6. [PubMed: 24745649]
100. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. JAMA. 1997; 278(3):207–11. [PubMed: 9218666]
101. Wu YW, Colford JM Jr. Chorioamnionitis as a risk factor for cerebral palsy: A meta-analysis. JAMA. 2000; 284(11):1417–24. [PubMed: 10989405]
102. Hagberg H, Wennerholm UB, Savman K. Sequelae of chorioamnionitis. Curr Opin Infect Dis. 2002; 15(3):301–6. [PubMed: 12015466]
103. Wu YW, Escobar GJ, Grether JK, Croen LA, Greene JD, Newman TB. Chorioamnionitis and cerebral palsy in term and near-term infants. JAMA. 2003; 290(20):2677–84. [PubMed: 14645309]
104. Sperling RS, Ramamurthy RS, Gibbs RS. A comparison of intrapartum versus immediate postpartum treatment of intra-amniotic infection. Obstet Gynecol. 1987; 70(6):861–5. [PubMed: 3684121]

105. Gilstrap LC 3rd, Leveno KJ, Cox SM, Burris JS, Mashburn M, Rosenfeld CR. Intrapartum treatment of acute chorioamnionitis: impact on neonatal sepsis. Am J Obstet Gynecol. 1988; 159(3):579–83. [PubMed: 3421256]
106. Hopkins L, Smaill F. Antibiotic regimens for management of intraamniotic infection. Cochrane Database Syst Rev. 2002; (3):CD003254. [PubMed: 12137684]
107. Johnson CT, Farzin A, Burd I. Current management and long-term outcomes following chorioamnionitis. Obstet Gynecol Clin North Am. 2014; 41(4):649–69. [PubMed: 25454996]
108. Polin RA. Committee on F, Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 2012; 129(5):1006–15. [PubMed: 22547779]
109. Polin RA, Watterberg K, Benitz W, Eichenwald E. The conundrum of early-onset sepsis. Pediatrics. 2014; 133(6):1122–3. [PubMed: 24799547]
110. Kiser C, Nawab U, McKenna K, Aghai ZH. Role of guidelines on length of therapy in chorioamnionitis and neonatal sepsis. Pediatrics. 2014; 133(6):992–8. [PubMed: 24799549]
111. Wynn JL, Wong HR, Shanley TP, Bizzarro MJ, Saiman L, Polin RA. Time for a neonatal-specific consensus definition for sepsis. Pediatr Crit Care Med. 2014; 15(6):523–8. [PubMed: 24751791]
112. Mukherjee A, Davidson L, Anguvaa L, Duffy DA, Kennea N. NICE neonatal early onset sepsis guidance: greater consistency, but more investigations, and greater length of stay. Arch Dis Child Fetal Neonatal Ed. 2015; 100(3):F248–9. [PubMed: 25079114]
113. Benitz WE, Wynn JL, Polin RA. Reappraisal of guidelines for management of neonates with suspected early-onset sepsis. J Pediatr. 2015; 166(4):1070–4. [PubMed: 25641240]
114. Higgins RD, Saade G, Polin RA, Grobman WA, Buhimschi IA, Watterberg K, et al. Evaluation and Management of Women and Newborns With a Maternal Diagnosis of Chorioamnionitis: Summary of a Workshop. Obstet Gynecol. 2016; 127(3):426–36. [PubMed: 26855098]
115. Gei AF, Pacheco LD, Vanhook JW, Hankins GD. The use of a continuous infusion of epinephrine for anaphylactic shock during labor. Obstet Gynecol. 2003; 102(6):1332–5. [PubMed: 14662223]
116. Khan R, Anastasakis E, Kadir RA. Anaphylactic reaction to ceftriaxone in labour. An emerging complication. J Obstet Gynaecol. 2008; 28(7):751–3. [PubMed: 19065378]
117. Sengupta A, Kohli JK. Antibiotic prophylaxis in cesarean section causing anaphylaxis and intrauterine fetal death. J Obstet Gynaecol Res. 2008; 34(2):252–4. [PubMed: 18412791]
118. Mulla ZD, Ebrahim MS, Gonzalez JL. Anaphylaxis in the obstetric patient: analysis of a statewide hospital discharge database. Ann Allergy Asthma Immunol. 2010; 104(1):55–9. [PubMed: 20143646]
119. Berardi A, Rossi K, Cavalleri F, Simoni A, Aguzzoli L, Masellis G, et al. Maternal anaphylaxis and fetal brain damage after intrapartum chemoprophylaxis. J Perinat Med. 2004; 32(4):375–7. [PubMed: 15346827]
120. Chaudhuri K, Gonzales J, Jesurun CA, Ambat MT, Mandal-Chaudhuri S. Anaphylactic shock in pregnancy: a case study and review of the literature. Int J Obstet Anesth. 2008; 17(4):350–7. [PubMed: 18691872]
121. Berenguer A, Couto A, Brites V, Fernandes R. Anaphylaxis in pregnancy: a rare cause of neonatal mortality. BMJ Case Rep. 2013; 2013
122. Alarcon A, Pena P, Salas S, Sancha M, Omenaca F. Neonatal early onset Escherichia coli sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. Pediatr Infect Dis J. 2004; 23(4):295–9. [PubMed: 15071281]
123. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG. Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics. 2008; 121(4):689–96. [PubMed: 18381532]
124. Didier C, Streicher MP, Chognot D, Campagni R, Schnebelen A, Messer J, et al. Late-onset neonatal infections: incidences and pathogens in the era of antenatal antibiotics. Eur J Pediatr. 2012; 171(4):681–7. [PubMed: 22134805]
125. Newton DF, Macfarlane S, Macfarlane GT. Effects of antibiotics on bacterial species composition and metabolic activities in chemostats containing defined populations of human gut microorganisms. Antimicrob Agents Chemother. 2013; 57(5):2016–25. [PubMed: 23403424]

126. Arboleya S, Sanchez B, Milani C, Duranti S, Solis G, Fernandez N, et al. Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. J Pediatr. 2015; 166(3):538–44. [PubMed: 25444008]

127. Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG. 2016; 123(6):983–93. [PubMed: 26412384]

128. Aloisio I, Quagliariello A, De Fanti S, Luiselli D, De Filippo C, Albanese D, et al. Evaluation of the effects of intrapartum antibiotic prophylaxis on newborn intestinal microbiota using a sequencing approach targeted to multi hypervariable 16S rDNA regions. Appl Microbiol Biotechnol. 2016; 100(12):5537–46. [PubMed: 26971496]

129. Dom S, Droste JH, Sariachvili MA, Hagendorens MM, Oostveen E, Bridts CH, et al. Pre- and post-natal exposure to antibiotics and the development of eczema, recurrent wheezing and atopic sensitization in children up to the age of 4 years. Clin Exp Allergy. 2010; 40(9):1378–87. [PubMed: 20545699]

130. Romero R, Korzeniewski SJ. Are infants born by elective cesarean delivery without labor at risk for developing immune disorders later in life? Am J Obstet Gynecol. 2013; 208(4):243–6. [PubMed: 23273890]

131. Cho CE, Norman M. Cesarean section and development of the immune system in the offspring. Am J Obstet Gynecol. 2013; 208(4):249–54. [PubMed: 22939691]

132. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. Pediatrics. 2011; 127(6):1125–38. [PubMed: 21606151]

133. Stensballe LG, Simonsen J, Jensen SM, Bonnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J Pediatr. 2013; 162(4):832–8e3. [PubMed: 23140881]

134. Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. Lancet Respir Med. 2014; 2(8):631–7. [PubMed: 25066330]

135. McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: a birth cohort study using the West Midlands General Practice Database. Am J Respir Crit Care Med. 2002; 166(6):827–32. [PubMed: 12231492]

136. Penders J, Stobberingh EE, Thijs C, Adams H, Vink C, van Ree R, et al. Molecular fingerprinting of the intestinal microbiota of infants in whom atopic eczema was or was not developing. Clin Exp Allergy. 2006; 36(12):1602–8. [PubMed: 17177684]

137. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol. 2012; 129(2):434–40. 40 e1–2. [PubMed: 22153774]

138. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014; 158(4):705–21. [PubMed: 25126780]

139. Cox LM, Blaser MJ. Antibiotics in early life and obesity. Nat Rev Endocrinol. 2015; 11(3):182–90. [PubMed: 25488483]

140. Mueller NT, Whyatt R, Hoepner L, Oberfield S, Dominguez-Bello MG, Widen EM, et al. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. Int J Obes (Lond). 2015; 39(4):665–70. [PubMed: 25298276]

141. Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. Arch Dis Child Fetal Neonatal Ed. 2012; 97(6):F456–62. [PubMed: 22562869]

142. Mai V, Torrazza RM, Ukhanova M, Wang X, Sun Y, Li N, et al. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. PLoS One. 2013; 8(1):e52876. [PubMed: 23341915]

143. Drell T, Lutsar I, Stsepetova J, Parm U, Metsvaht T, Ilmoja ML, et al. The development of gut microbiota in critically ill extremely low birth weight infants assessed with 16S rRNA gene based sequencing. Gut Microbes. 2014; 5(3):304–12. [PubMed: 25184833]

144. Deshmukh HS, Liu Y, Menkiti OR, Mei J, Dai N, O'Leary CE, et al. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. Nat Med. 2014; 20(5):524–30. [PubMed: 24747744]

145. Thanabalasuriar A, Kubes P. Neonates, antibiotics and the microbiome. Nat Med. 2014; 20(5): 469–70. [PubMed: 24804751]

146. Lee SM, Romero R, Park JS, Chaemsaithong P, Jun JK, Yoon BH. A transcervical amniotic fluid collector: a new medical device for the assessment of amniotic fluid in patients with ruptured membranes. Journal of perinatal medicine. 2015; 43(4):381–9. [PubMed: 25372723]

147. Maddipati KR, Romero R, Chaiworapongsa T, Chaemsaithong P, Zhou SL, Xu Z, et al. Clinical Chorioamnionitis at Term: The Amniotic Fluid Fatty acyl Lipidome. J Lipid Res. 2016

Table 1
Clinical characteristics of the study population

Characteristics	Median (interquartile range) or percent (n=44)
Maternal age (years)	21 (18-25)
Body mass index (kg/m^2)	23.7 (21.6-24.6)
Nulliparity (%)	65.9% (29/44)
Smoking (%)	11.4% (5/44)
Rupture of the membranes at the time of amniocentesis (%)	65.9% (29/44)
Gestational age at delivery (weeks)	39.6 (38.8-40.7)
Labor (%)	
- Spontaneous	84.1% (37/44)
- Induced	15.9% (7/44)
Birthweight (grams)	3510 (3220-3772)
Amniotic fluid white blood cells (cells/mm^3)	66.5 (5.0-648.8)
Amniotic fluid glucose (mg/dL)	9.0 (9.0-9.0)
Amniotic fluid ELISA IL-6 (ng/mL)	5.7 (2.9-18.3)
Positive amniotic fluid Gram stain (%)	11.4% (5/44)
Intra-amniotic inflammation (ELISA IL-6 ≥2.6 ng/mL) (%)	77.3% (34/44)
Sterile intra-amniotic inflammation (%)	22.7% (10/44)
Intra-amniotic infection (%)	54.5% (24/44)
Amniotic fluid white blood cell count ≥50 cells/mm^3 (%)	52.3% (23/44)
Acute inflammatory lesions of the placenta (%) *	48.8% (21/43)
Acute histologic chorioamnionitis (%) *	46.5% (20/43)
Acute funisitis (%) *	30.2% (13/43)
Fetal inflammatory response syndrome (FIRS) (%) *	23.3% (10/43)

Data presented as median (interquartile range) or % (n).
ELISA, enzyme-linked immunosorbent assay; IL, Interleukin;
Acute inflammatory lesions of the placenta include acute histologic chorioamnionitis and/or acute funisitis.

*Among these patients, a placental histology report was not available for one patient and the fetal plasma IL-6 concentration was not available for one patient.

J Perinat Med. Author manuscript; available in PMC 2018 July 26.

Table 2

Diagnostic performance of point-of-care rapid MMP-8 and ELISA IL-6 tests for the identification of intra-amniotic inflammation/infection, placental lesions consistent with acute inflammation, and fetal inflammatory response syndrome

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation [77.3% (34/44)]	Sensitivity	82.4% (28/34)	65.5-93.2		
	Specificity	90% (9/10)	55.5-99.8		
	Positive Likelihood Ratio	8.2	1.3-53.2		
	Negative Likelihood Ratio	0.2	0.1-0.4		
	Positive predictive value	96.6% (28/29)	82.2-99.9		
	Negative predictive value	60% (9/15)	32.3-83.7		
	Accuracy	84.1% (37/44)	-		
Intra-amniotic infection [54.5% (24/44)]	Sensitivity	91.7% (22/24)	73.0-98.9	100% (24/24)	85.8-100
	Specificity	65% (13/20)	40.8-84.6	50% (10/20)	27.2-72.8
	Positive Likelihood Ratio	2.6	1.4-4.8	2.0	1.3-3.1
	Negative Likelihood Ratio	0.1	0.03-0.5	0	-
	Positive predictive value	75.9% (22/29)	56.5-89.7	70.6% (24/34)	52.5-84.9
	Negative predictive value	86.7% (13/15)	59.5-98.3	100% (10/10)	69.2-100
	Accuracy	79.5% (35/44)	-	77.3% (34/44)	-
Acute inflammatory lesions of the placenta [48.8% (21/43)]	Sensitivity	85.7% (18/21)	63.7-96.9	90.5% (19/21)	69.6-98.8
	Specificity	54.5% (12/22)	32.2-75.6	36.4% (8/22)	17.2-59.3
	Positive Likelihood Ratio	1.9	1.2-3.1	1.4	1.0-2.0
	Negative Likelihood Ratio	0.3	0.1-0.8	0.3	0.1-1.0
	Positive predictive value	64.3% (18/28)	44.1-81.4	57.6% (19/33)	39.2-74.5
	Negative predictive value	80% (12/15)	51.9-95.7	80% (8/10)	44.4-97.5
	Accuracy	69.8% (30/43)	-	62.8% (27/43)	-
Acute funisitis [30.2% (13/43)]	Sensitivity	84.6% (11/13)	54.6-98.1	84.6% (11/13)	54.6-98.1
	Specificity	43.3% (13/30)	25.5-62.6	26.7% (8/30)	12.3-45.9
	Positive Likelihood Ratio	1.5	1.0-2.2	1.2	0.8-1.6
	Negative Likelihood Ratio	0.4	0.1-1.4	0.6	0.1-2.4

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
	Positive predictive value	39.3% (11/28)	21.5-59.4	33.3% (11/33)	17.9-51.8
	Negative predictive value	86.7% (13/15)	59.5-98.3	80% (8/10)	44.4-97.5
	Accuracy	55.8% (24/43)	-	44.2% (19/43)	-
Fetal inflammatory response syndrome [23.3% (10/43)]	Sensitivity	100% (10/10)	69.2-100	100% (10/10)	69.2-100
	Specificity	42.4% (14/33)	25.5-60.8	30.3% (10/33)	15.6-48.7
	Positive Likelihood Ratio	1.7	1.3-2.3	1.4	1.2-1.8
	Negative Likelihood Ratio	0	-	0	-
	Positive predictive value	34.5% (10/29)	17.9-54.3	30.3% (10/33)	15.6-48.7
	Negative predictive value	100% (14/14)	76.8-100	100% (10/10)	69.2-100
	Accuracy	55.8% (24/43)	-	46.5% (20/43)	-

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

Table 3

Diagnostic performances of point-of-care rapid MMP-8 and ELISA IL-6 tests for the identification of intra-amniotic inflammation and infection determined by using an amniotic fluid white blood cell count

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation identified by AF WBC count ≥ 50 cells/mm^3 [52.3% (23/44)]	Sensitivity	95.7% (22/23)	78.1-99.9	95.7% (22/23)	78.1-99.9
	Specificity *	66.7% (14/21)	43.0-85.4	42.9% (9/21)	21.8-66.0
	Positive Likelihood Ratio	2.9	1.6-5.3	1.7	1.1-2.5
	Negative Likelihood Ratio	0.07	0.01-0.5	0.1	0.01-0.7
	Positive predictive value	75.9% (22/29)	56.5-89.7	64.7% (22/34)	46.5-80.3
	Negative predictive value	93.3% (14/15)	68.1-99.8	90.0% (9/10)	55.5-99.8
	Accuracy	81.8% (36/44)	-	70.5% (31/44)	-
Intra-amniotic infection identified by a positive AF culture and an AF WBC count ≥ 50 cells/mm^3 [40.9% (18/44)]	Sensitivity	100% (18/18)	81.5-100	100% (18/18)	81.5-100
	Specificity **	57.7% (15/26)	36.9-76.7	38.5% (10/26)	20.2-59.4
	Positive Likelihood Ratio	2.4	1.5-3.7	1.6	1.2-2.2
	Negative Likelihood Ratio	0	-	0	-
	Positive predictive value	62.1% (18/29)	42.3-79.3	52.9% (18/34)	35.1-70.2
	Negative predictive value	100% (15/15)	78.2-100	100% (10/10)	69.2-100
	Accuracy	75% (33/44)	-	63.6% (28/44)	-

* p-value=0.03,

** p-value=0.058: a comparison between the rapid MMP-8 test and the ELISA IL-6 test using the McNemar's test.