Published in final edited form as:

Am J Obstet Gynecol. 2017 June ; 216(6): 604.e1–604.e11. doi:10.1016/j.ajog.2017.02.035.

Twenty-four percent of patients with clinical chorioamnionitis in preterm gestations have no evidence of either culture-proven intra-amniotic infection or intra-amniotic inflammation

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Abstract

Background—Recent studies on clinical chorioamnionitis at term suggest that some patients with this diagnosis have neither intra-amniotic infection nor intra-amniotic inflammation. A false-positive diagnosis of clinical chorioamnionitis in preterm gestation may lead to unwarranted preterm delivery.

Objective—To determine the frequency of intra-amniotic inflammation and microbiologically proven amniotic fluid infection in patients with preterm clinical chorioamnionitis.

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

Study design—Amniocentesis was performed in singleton pregnant women with preterm clinical chorioamnionitis (<36 weeks of gestation). Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas and assayed for matrix metalloproteinase-8 concentration. Microbial invasion of the amniotic cavity was defined as a positive amniotic fluid culture; intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration of >23 ng/mL. Non-parametric and survival techniques were used for analysis.

Results—Among patients with preterm clinical chorioamnionitis, 24% (12/50) had microbiologic evidence of neither intra-amniotic infection nor intra-amniotic inflammation. Microbial invasion of the amniotic cavity was present in 34% (18/53) and intra-amniotic inflammation in 76% (38/50) of patients. The most common microorganisms isolated from the amniotic cavity were the *Ureaplasma* species. Finally, patients without microbial invasion of the amniotic cavity or intra-amniotic inflammation had significantly lower rates of adverse outcomes (including lower gestational age at delivery, a shorter amniocentesis-to-delivery interval, acute histologic chorioamnionitis, acute funisitis, and significant neonatal morbidity) than those with microbial invasion of the amniotic cavity and/or intra-amniotic inflammation.

Conclusion—Among patients with preterm clinical chorioamnionitis, 24% had no evidence of either intra-amniotic infection or intra-amniotic inflammation, and 66% had negative amniotic fluid cultures, using standard microbiologic techniques. These observations call for a re-examination of the criteria used to diagnose preterm clinical chorioamnionitis.

Keywords

amniocentesis; matrix metalloproteinase-8 (MMP-8); microbial invasion of the amniotic cavity (MIAC); pregnancy; preterm delivery

Introduction

Clinical chorioamnionitis is the most frequent pregnancy-related infection diagnosed in labor and delivery units worldwide [1]. The prevalence of this condition in term gestations ranges from 5% to 12% [2], while in preterm gestations, it is diagnosed in 20% of patients with preterm premature rupture of the membranes (PROM) [3–5] and in about 10% of patients with preterm labor and intact membranes [6–11]. Clinical chorioamnionitis is a well-known risk factor for adverse maternal and perinatal outcomes [12–14]. Affected mothers have an increased rate of endometritis [2, 15], post-operative wound infections [2, 16], sepsis [17, 18], disseminated intravascular coagulation [19], septic pelvic thrombophlebitis [20, 21], postpartum hemorrhage [22], pelvic abscess [2], and other complications [2, 4, 23–26]. Neonatal complications include congenital sepsis [11, 25, 27–34] as well as localized infections such as pneumonia [27, 28], dermatitis [35], and otitis media [36]. In addition, clinical chorioamnionitis has been associated with an increased risk of long-term complications such as cerebral palsy [30, 37–44].

Clinical chorioamnionitis at term is frequently diagnosed during labor, and treatment consists of antibiotic administration and acceleration of the labor process to reduce the risk of exposure to infection [2, 4, 5, 24, 28, 44]. In the context of preterm gestation, the

diagnosis of clinical chorioamnionitis is an indication for delivery to spare an immature neonate the serious consequences of congenital neonatal sepsis, multiple organ involvement, and the long-term adverse effects of exposure to infection [5, 24]. Thus, clinical chorioamnionitis in preterm gestation is frequently associated with either the spontaneous onset of preterm labor or the induction of preterm labor.

Most studies of clinical chorioamnionitis have focused on term gestation. However, there is a paucity of studies done in the context of preterm gestation. Recent evidence derived from studies on clinical chorioamnionitis at term, as well as some early studies in preterm labor with intact membranes, suggests that a fraction of patients with this diagnosis have neither intra-amniotic infection nor intra-amniotic inflammation [45, 46]. In other words, some patients with preterm clinical chorioamnionitis appear to have a false-positive diagnosis. The implications of a false-positive diagnosis in term gestations are less severe than those associated with the delivery of a preterm neonate. The objective of this study was to determine the prevalence of intra-amniotic infection and intra-amniotic inflammation in patients with the diagnosis of clinical chorioamnionitis in preterm gestation.

Material and methods

Study design

This was a retrospective cohort study conducted at the Seoul National University Hospital, Seoul, South Korea, from 1993 through 2012. Patients with the diagnosis of preterm clinical chorioamnionitis (gestational age <36 weeks) were identified. Clinical chorioamnionitis was diagnosed in the presence of a maternal temperature of ≥ 37.8°C and two or more of the following criteria: (1) uterine tenderness; (2) malodorous vaginal discharge; (3) maternal leukocytosis (white blood cell count of >15,000 cells/mm^3); (4) maternal tachycardia (>100 beats/minute); and (5) fetal tachycardia (>160 beats/minute), following the recommendations of Gibbs et al. [47–50].

The criteria for inclusion in this study were as follows: (1) transabdominal amniocentesis performed <36 weeks of gestation to obtain amniotic fluid (AF) for microbiologic studies; (2) singleton gestation; (3) clinical chorioamnionitis diagnosed within 48 hours of amniocentesis (this criterion was used to preserve a meaningful relationship between the results of AF studies and clinical chorioamnionitis); and (4) no evidence of other causes of fever at the time of amniocentesis. Patients had no evidence of being immunocompromised (i.e., evidence of HIV or similar diseases). Corticosteroids (dexamethasone) and the selection of antimicrobial agents were at the discretion of the managing physician. Intra-amniotic inflammation was defined as an elevated concentration of AF matrix metalloproteinase-8 (MMP-8) (>23 ng/ml), according to our previous publications that described in detail the methodology for this test, performance characteristics, diagnostic indices, predictive values, and likelihood ratios in preterm and term gestations [51–53].

Microbial invasion of the amniotic cavity (MIAC) was diagnosed in the presence of a positive AF culture for microorganisms. The methodology for culture included both aerobic and anaerobic bacteria as well as genital mycoplasmas. We have reported extensively on

these results and characterized the microbial burden of the amniotic cavity in preterm gestation with these techniques.

Written informed consent was obtained from all subjects to donate AF for research purposes. The Institutional Review Board of the Seoul National University Hospital approved the collection and use of these samples and the information for research purposes. The Seoul National University Hospital has received a Federalwide Assurance with the Office for Human Research Protection of the U. S. Department of Health and Human Services.

Amniotic fluid analysis

Amniotic fluid was retrieved by transabdominal amniocentesis. The fluid was then immediately transported to the Department of Laboratory Medicine of our hospital, using specific transport media, and cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas (*Ureaplasma* species and *Mycoplasma hominis*). The culture techniques are similar to those previously described [54]. An aliquot of AF was transported to the laboratory and examined in the hemocytometer chamber to determine the AF white blood cell count. Amniotic fluid not used for diagnostic studies was centrifuged for 10 minutes at 4°C and stored at −70°C until assayed. MMP-8 concentrations were measured by a commercially available enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN, USA). Inter-assay and intra-assay coefficients of variation were <10% for each.

Placental pathology, diagnosis of acute histologic chorioamnionitis, acute funisitis, and neonatal morbidity

Acute histologic chorioamnionitis was defined as the presence of acute inflammatory changes characterized by the infiltration of neutrophils in the choriodecidua and amnion [55, 56], respectively; acute funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly, using previously published criteria [55–60]. Significant neonatal morbidity was defined as the presence of any of the following conditions: respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage (grade ≥II), periventricular leukomalacia, proven congenital neonatal sepsis, or necrotizing enterocolitis. The definitions of each of these complications have been previously described [61].

Statistical analysis

Mann-Whitney U tests were used to compare continuous non-parametric variables, and Fisher's exact tests were used for comparison of proportions. Survival analysis was performed with the Kaplan-Meier method to compare the amniocentesis-to-delivery interval for patients with and without MIAC and/or intra-amniotic inflammation. Patients delivered for maternal or fetal indications were treated as censored observations with a censoring time equal to the amniocentesis-to-delivery interval. A Cox proportional hazards model was used to examine the relationship between the interval from amniocentesis to delivery and the presence or absence of MIAC and/or intra-amniotic inflammation after adjusting for gestational age. Logistic regression analysis was used to examine the relationship between

the presence or absence of MIAC and/or intra-amniotic inflammation and the pregnancy outcome of interest after adjustment for gestational age; the Firth method was used if the outcome variable was perfectly separated by the presence or absence of MIAC and/or intra-amniotic inflammation [62]. A probability value <0.05 was considered as significant. SPSS Version 22.0 for Windows (IBM, Armonk, NY, USA) and SAS 9.3 (SAS Institute, Cary, NC, USA) were used for statistical analyses.

Results

The frequency of microbial invasion of the amniotic cavity and intra-amniotic inflammation in preterm clinical chorioamnionitis

A total of 53 patients with clinical chorioamnionitis met the entry criteria. The prevalence of a positive AF culture for microorganisms was 34% (18/53). Thus, 66% (35/53) of patients with the diagnosis of clinical chorioamnionitis had a negative AF culture for microorganisms. *Ureaplasma* species were the most frequent microorganisms isolated from the amniotic cavity (n = 8). Other isolates included *Streptococcus anginosus* (n = 3), *Escherichia coli* (n = 2), *Candida* species (n = 2), *Mycoplasma hominis* (n = 2), *Klebsiella pneumoniae* (n = 1), *Listeria monocytogenes* (n = 1), and *Gardnerella vaginalis* (n = 1).

Intra-amniotic inflammation (defined as an MMP-8 >23 ng/mL) was present in 76% (38/50) of patients. Amniotic fluid was not available from three women for MMP-8 determinations (two women with a positive AF culture and 1 with a negative AF culture); therefore, results about the frequency of inflammation are based on 50 patients. Importantly, 24% (12/50) of patients with the clinical diagnosis of chorioamnionitis did not have either bacteria in the AF or an elevated MMP-8 concentration. These patients could be considered to have a false-positive diagnosis of preterm clinical chorioamnionitis.

All patients with bacteria in the AF (n = 16) had an elevated AF MMP-8 concentration, and 44% (22/50) of patients had an elevated MMP-8 concentration with a negative AF culture, indicating that intra-amniotic inflammation was more frequent than proven intra-amniotic infection. Importantly, there were no patients who had bacteria in the AF without intra-amniotic inflammation.

Characteristics of the study population according to the presence or absence of microbial invasion of the amniotic cavity and/or intra-amniotic inflammation

Table 1 compares the characteristics of the study population according to the presence or absence of MIAC and/or intra-amniotic inflammation. Patients with MIAC and/or intra-amniotic inflammation had a significantly lower median gestational age at amniocentesis than those without MIAC or intra-amniotic inflammation (25.8 weeks [interquartile range (IQR), 23.7–30.0 weeks] versus 31.4 weeks [IQR, 28.3–33.3 weeks], P = .006). There were no significant differences in the median maternal body temperature and maternal white blood cell count between the two study groups (P > 0.1).

Outcomes according to the presence or absence of microbial invasion of the amniotic cavity and/or intra-amniotic inflammation

Table 2 compares the pregnancy outcomes according to the presence or absence of MIAC and/or intra-amniotic inflammation. Women with MIAC and/or intra-amniotic inflammation had a significantly higher rate of amniocentesis-to-delivery intervals of <7 days and <14 days and preterm delivery (including spontaneous preterm delivery) <34 weeks and <37 weeks of gestation, even after adjusting for gestational age at amniocentesis.

Figure 1 illustrates the interval from amniocentesis to delivery according to the presence or absence of MIAC and/or intra-amniotic inflammation. Of the 23 cases in which delivery resulted from induction of labor or cesarean delivery before the onset of labor, this interval was censored. Multivariate survival analysis demonstrated that women with MIAC and/or intra-amniotic inflammation had a significantly shorter median amniocentesis-to-delivery interval than those without MIAC or intra-amniotic inflammation, after adjusting for the gestational age at amniocentesis (hazards ratio, 7.55; 95% confidence interval, 1.74–32.8; $P < 0.01$ by the Cox proportional hazards model analysis).

Neonatal outcomes

Table 3 compares neonatal outcomes according to the presence or absence of MIAC and/or intra-amniotic inflammation. All fetal deaths (n = 3) were observed in mothers with MIAC and/or intra-amniotic inflammation. Neonates born to mothers with MIAC and/or intra-amniotic inflammation had a significantly higher rate of serious neonatal morbidity than those born to mothers without MIAC or intra-amniotic inflammation. Among the 40 women with MIAC and/or intra-amniotic inflammation, 78% (31/40) delivered within two days of amniocentesis. Among the remaining nine women, two women remained undelivered for more than seven days. However, one patient had a fetal death and the other underwent induction of labor at a non-viable gestational age due to maternal sepsis.

Amniotic fluid analysis, placental pathology, and pregnancy outcome

Table 4 displays the clinical characteristics, AF analyses, histopathologic characteristics of the placenta, and pregnancy and neonatal outcomes of 12 patients without MIAC and/or intra-amniotic inflammation. One-third (4/12) of women without MIAC or intra-amniotic inflammation delivered their neonates at term, and two-thirds (8/12) of neonates did not have any significant neonatal morbidity. The rate of histologic chorioamnionitis was 25% (2/8); however, acute funisitis was not found in any case.

Comment

Principal findings of the study

The principal findings of the study are as follows: 1) among patients with preterm clinical chorioamnionitis, 24% had neither culture-proven microbiologic evidence of intra-amniotic infection nor intra-amniotic inflammation; 2) demonstrable intra-amniotic infection was present in 34% of cases (18/53); 3) the most common microorganisms isolated from the amniotic cavity were the *Ureaplasma* species; 4) patients without MIAC or intra-amniotic inflammation had significantly better outcomes than those with MIAC and/or intra-amniotic

inflammation. The main conclusion of this study is that the current clinical criteria used for the diagnosis of clinical chorioamnionitis in preterm gestation may result in unnecessary preterm deliveries and, therefore, neonatal morbid events. There is an urgent need to improve the diagnosis of this condition in preterm gestation.

Clinical diagnosis of chorioamnionitis

The most widely used definition of clinical chorioamnionitis was proposed by Gibbs et al. [47–50]. This definition uses a combination of clinical and laboratory parameters derived from the mother or the fetus. Maternal fever, tachycardia, and an elevation of white blood cells in the peripheral blood count reflect signs of maternal systemic inflammation. In contrast, uterine tenderness and foul-smelling amniotic fluid/discharge are indicative of a localized response to microbial invasion or uterine inflammation. Fetal tachycardia could reflect a response to infection/inflammation or, alternatively, a physiologic response to a change in maternal temperature, as the fetus is less able to dissipate heat than the mother [24, 63].

The sensitivity and specificity of each clinical and laboratory sign of clinical chorioamnionitis in preterm gestation has not been examined in the context of AF cultures and intra-amniotic inflammation. The original diagnostic indices were based on the results of AF microbiologic studies obtained using a transcervical catheter in term gestations [49]. Retrieval of AF via such a catheter is susceptible to contamination of the specimen with vaginal/cervical bacteria. In support of this concept is the study of Gibbs et al. [49]; there were 52 patients with clinical chorioamnionitis, and 52 others matched for gestational age without this diagnosis. Notably, approximately 75% of samples from patients without clinical chorioamnionitis had $>10^2$ colony-forming units/mL in AF cultures. These findings sharply contrast with a systematic study of the amniotic cavity in women in spontaneous labor at term with intact membranes, in which only 17% had positive AF cultures for microorganisms in the absence of clinical signs of infection. Collectively, this evidence suggests that samples obtained by transvaginal/transcervical catheterization are prone to bacterial contamination and, therefore, cannot be relied on to accurately represent the microbial state of the amniotic cavity.

A recent study of the accuracy of the clinical signs proposed by Gibbs et al. [47–50] in clinical chorioamnionitis at term using AF obtained by transabdominal amniocentesis indicated that all clinical signs have an accuracy of around 50% for the identification of intra-amniotic infection (presence of bacteria and intra-amniotic inflammation). The implications of a high inaccuracy rate in the diagnosis of preterm clinical chorioamnionitis could be serious because the standard intervention is the induction of labor [64].

False-positive diagnosis of clinical chorioamnionitis

While 76% (38/50) of patients with the clinical diagnosis of chorioamnionitis had MIAC and/or intra-amniotic inflammation, it is noteworthy that 24% had neither; therefore, the diagnoses should be considered as false-positive. This observation was unexpected because there is a widespread belief among clinicians that the diagnosis of clinical chorioamnionitis may not be sensitive to the detection of intra-amniotic infection in preterm gestations, but is

quite specific for the diagnosis of intra-amniotic infection. Indeed, in a study in which serial amniocenteses were performed in patients with preterm PROM, the frequency of MIAC increased as a function of time, and, in particular, when patients with preterm PROM began to contract (i.e. evidence of preterm labor, even in the absence of clinical signs of infection).

Our results call for a re-examination of the reliability of the criteria because a clinical diagnosis is frequently used as an indication for delivery. The consequences of preterm delivery because of a false-positive diagnosis could be serious. Future studies are required to determine the mechanisms of disease that mimic the clinical syndrome of chorioamnionitis. Also, examination of AF retrieved by a transvaginal collector [65] in patients with preterm PROM and the determination of inflammatory cytokines may dramatically improve the accuracy of the diagnosis of "true" intra-amniotic inflammation without resorting to an invasive procedure.

Microbiologically proven clinical chorioamnionitis

Of the cases with clinical chorioamnionitis, 34% (18/53) had microbiologic evidence of infection. The most common microorganisms isolated were the *Ureaplasma* species—this is consistent with previous reports [4, 24, 45, 53, 54, 66–77]. We reported that the intensity of the systemic fetal inflammatory response in cases with a positive AF culture is greater than that observed in cases of intra-amniotic inflammation with a negative culture [78].

False-negative diagnosis of intra-amniotic infection

A consistent observation across many studies, including the present one, is that the frequency of intra-amniotic inflammation is greater than that of AF cultures positive for microorganisms [7, 52, 53, 79–81]. The mere presence of microbial footprints, using molecular microbiologic techniques in a fraction of these patients, is a poor prognostic factor [76, 81–87]. Therefore, failure to grow microorganisms in the laboratory should not be interpreted as the absence of bacteria *in vivo*. Cultivation techniques are helpful when the results are positive, but negative culture results when inflammation is present should be interpreted with caution [81, 85, 86]. The possibility that some intra-amniotic infections may be caused by viruses or other organisms requiring special isolation techniques should not be overlooked, and future studies are required to identify novel pathogens—thus far, our studies have not identified viruses as major organisms causative of intra-amniotic infection. Another potential limitation is that antibiotics were used to treat one-half of the patients prior to amniocentesis. It is possible that this could have reduced the rate of positive AF cultures. Further studies using molecular microbiologic techniques, which are able to identify bacterial footprints even after treatment with antibiotics, would be required to address this question. However, our study spans 20 years, and during much of this time, molecular microbiologic techniques were not available, and they are still not part of routine practice in obstetrics. Finally, inflammation is a non-specific mechanism of host defense and can be caused by non-infection-related insults, which we have termed "sterile" inflammation [81, 88, 89]. The precise nature of these "danger signals" has not been identified, but there is evidence that they may engage alarmins and the inflammasome system in the onset of term or preterm labor [90–94].

Strengths and limitations

The current study represents the most comprehensive examination of AF analysis for microorganisms using cultivation techniques, assessment of intra-amniotic inflammation, neonatal outcome, and placental pathology in preterm gestation. The focus of this study—preterm clinical chorioamnionitis—is one of the major indications for the induction of labor in preterm gestations. The major finding of the study—that a substantial number of patients diagnosed with preterm clinical chorioamnionitis had no evidence of either intra-amniotic infection or intra-amniotic inflammation—is unexpected and calls for very careful documentation of the criteria used for diagnosis and intervention in daily clinical practice. A limitation of this study is that it was conducted over a long period of time, given the low prevalence of proven clinical chorioamnionitis in preterm gestations. However, we used a uniform protocol for the diagnosis of intra-amniotic infection, as the cultivation techniques used in our unit have been in place for more than 20 years. Moreover, we have used objective criteria for the diagnosis of intra-amniotic inflammation, and samples have been stored under optimal conditions (−70° C). The criteria for the diagnosis of placental pathologic lesions have been used by our placental pathologists for more than 25 years as well as being the subject of extensive publication. The main message of this article is to alert clinicians to the potential diagnosis of preterm clinical chorioamnionitis, and such a conclusion is solidly derived from the findings reported herein.

Clinical implications

The clinical diagnosis of chorioamnionitis is thought to be a reliable indicator of intra-amniotic infection. While most such infections are known to be sub-clinical, false-positive diagnoses of chorioamnionitis are believed to be rare. Our observations call for a re-examination of this view. This is particularly the case in preterm gestation when an erroneous diagnosis may lead to unwarranted induction of labor or cesarean delivery. Amniotic fluid analysis, including rapid tests for the detection of inflammation [95–98] and molecular microbiologic methods [34, 45, 72, 81–87, 99–107], are likely to become important in the management of preterm clinical chorioamnionitis. Recent observations suggest that assessment of AF retrieved using an AF collector [65] and the measurement of inflammatory mediators could be of value in the assessment of patients with preterm PROM [108].

Research implications

The findings of this study have implications for both clinical and translational research. Clinical research is necessary to estimate the impact of interventions based on false-positive diagnoses of clinical chorioamnionitis. Outcomes could include neonatal morbidity and the cost of care for an unindicated preterm delivery. The causes of the clinical syndrome that resembles chorioamnionitis in these patients need to be determined. Although, in the context of PROM, the onset of preterm contractions in a patient who has been quiescent in the antepartum period has been associated with MIAC [3], it is now clear that the syndrome of clinical chorioamnionitis can occur without microbial invasion or even intra-amniotic inflammation. Although negative cultures could be explained by treatment with antibiotics,

this is unlikely to be the explanation for the absence of intra-amniotic inflammation in our study.

The gold standard used for the diagnosis of intra-amniotic infection/inflammation in our study required examination of AF obtained by amniocentesis. Recently, a device was developed for the retrieval of AF in patients with preterm PROM [65]. The assay of such fluid for inflammatory markers may help in the identification of patients with true intra-amniotic inflammation [109–111]. In patients with preterm labor with intact membranes or acute cervical insufficiency, examination of AF retrieved by amniocentesis is probably the best method to assess the true state of the amniotic cavity. Emphasis needs to be placed on the development of bedside tests that allow a rapid answer [95–98, 112, 113]. Research in non-invasive methods to assess the state of the amniotic cavity is desirable. For example, it would be ideal if assessment of cervical fluid could provide information without requiring amniocentesis.

Acknowledgments

We are grateful to the patients who volunteered for the study, attending physicians, residents, faculty, nursing staff, and administrative personnel at the Seoul National University for the contributions they have made over the years to the conduct of these and many other studies related to clinical chorioamnionitis.

Source of Funding: This work was supported in part, by the SNUH research fund (Grant No. 04-2016-0920), and 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.

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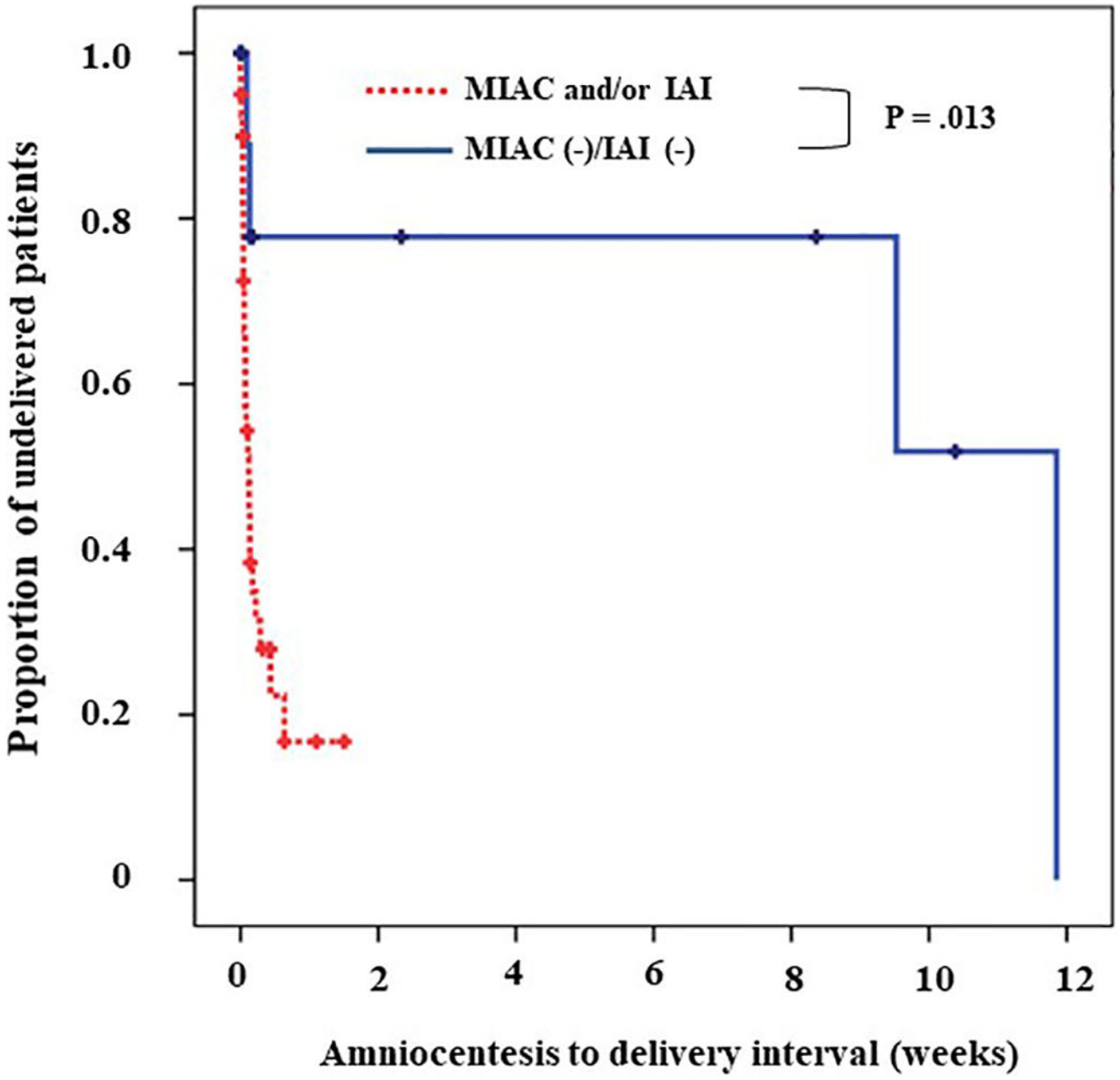

Figure 1.

Survival analysis of the amniocentesis-to-delivery intervals, according to the presence or absence of microbial invasion of the amniotic cavity (MIAC) and/or intra-amniotic inflammation (IAI) (median 0.1 [range, 0–1.5 vs. median 4.8 range 0–11.8 weeks, respectively; $P = .013$).

Table 1

Clinical characteristics of the study population according to the presence or absence of microbial invasion of the amniotic cavity and/or intra-amniotic inflammation

	MIAC and/or IAI (n=40)	MIAC (−)/IAI (−) (n=12)	*P*
Maternal age, years	31.5 (28.5–36)	29.5 (27–31)	.112
Nulliparity	40 (16/40)	66.7 (8/12)	.186
Gestational age at amniocentesis, weeks	25.8 (23.7–30.0)	31.4 (28.3–33.3)	.006
Diagnosis			
Preterm PROM	32.5 (13/40)	8.3 (1/12)	.144
Preterm labor	47.5 (19/40)	75.0 (9/12)	.133
Acute cervical insufficiency	10.0 (4/40)	0 (0/12)	.562
Clinical chorioamnionitis alone	10.0 (4/40)	16.7 (2/12)	.612
Maternal body temperature, °C	38.2 (38.0–38.3)	38.3 (38.0–38.6)	.758
Maternal blood WBC count, $\times 10^3$ cells/mm^3	15.2 (12.3–17.7)	16.3 (8.5–18.0)	.862
Antibiotic use	95.0 (38/40)	69.2 (8/12)	.021
Before amniocentesis	55.0 (22/40)	25.0 (3/12)	.101
Corticosteroid use	42.5 (17/40)	41.7 (5/12)	1.000
Before amniocentesis	32.5 (13/40)	25.0 (3/12)	.733

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

IAI: intra-amniotic inflammation, defined as an amniotic fluid matrix metalloproteinase-8 concentration >23 ng/mL.

MIAC: microbial invasion of amniotic cavity, defined as a positive amniotic fluid culture.

PROM: preterm rupture of the membranes.

WBC: white blood cell.

Table 2

Pregnancy outcomes of the study population according to the presence or absence of microbial invasion of the amniotic cavity and/or intra-amniotic inflammation

	MIAC and/or IAI (n=40)	MIAC (−)/IAI (−) (n=12)	*P*	Adjusted *P*[a]
Gestational age at delivery, weeks	26.0 (23.8–30.2)	34.8 (30.2–37.8)	<.001	
Amniocentesis-to-delivery interval				
<48 hours	77.5 (31/40)	58.3 (7/12)	.267	-
<7 days	95.0 (38/40)	58.3 (7/12)	.005	.004
<14 days	100 (40/40)	58.3 (7/12)	<.001	.011[b]
Amniocentesis-to-spontaneous-delivery interval				
<48 hours	71.9 (23/32)	28.6 (2/7)	.075	-
<7 days	92.9 (26/28)	28.6 (2/7)	.001	.005
<14 days	100 (26/26)	28.6 (2/7)	<.001	.004[b]
Preterm delivery				
<30 weeks	96.7 (29/30)	60 (3/5)	.047	.106
<34 weeks	97.4 (37/38)	41.7 (5/12)	<.001	.008
<37 weeks	100 (40/40)	66.7 (8/12)	.002	.015[b]
Spontaneous preterm delivery				
<30 weeks	94.4 (17/18)	33.3 (1/3)	.041	.991
<34 weeks	95.8 (23/24)	22.2 (2/9)	<.001	.005
<37 weeks	100 (26/26)	33.3 (2/6)	<.001	.006[b]
Acute histologic chorioamnionitis	91.2 (31/34)	25 (2/8)	<.001	.003
Acute funisitis	57.6 (19/33)	0 (0/8)	.004	.024[b]

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

IAI, intra-amniotic inflammation, defined as an amniotic fluid matrix metalloproteinase-8 concentration >23 ng/mL.

MIAC, microbial invasion of amniotic cavity, defined as a positive amniotic fluid culture.

[a] Logistic regression analysis was used to adjust gestational age at amniocentesis.

[b] Firth's method was used in the analysis.

Table 3

Neonatal outcomes of the study population according to the presence or absence of microbial invasion of the amniotic cavity and/or intra-amniotic inflammation

	MIAC and/or IAI (n=38)[a]	MIAC (−)/IAI (−) (n=12)	*P*	Adjusted *P*[b]
1-min Apgar score <7[c]	89.2 (33/37)	41.7 (5/12)	.002	.035
5-min Apgar score <7[c]	70.3 (26/37)	25.0 (3/12)	.008	.185
Fetal death *in utero*	7.9 (3/38)	0 (0/12)	1.000	-
Neonatal death and/or stillbirth[c]	40.5 (15/37)	16.7 (2/12)	.175	-
Significant morbidity[c,d]	84.6 (22/26)	33.3 (4/12)	.003	.015
Respiratory distress syndrome	34.6 (9/26)	33.3 (4/12)	1.000	-
Bronchopulmonary dysplasia	52.2 (12/23)	0 (0/10)	.005	.120[e]
Intraventricular hemorrhage	26.9 (7/26)	0 (0/12)	.074	-
Periventricular leukomalacia	19.2 (5/26)	0 (0/12)	.158	-
Necrotizing enterocolitis	7.7 (2/26)	0 (0/12)	1.000	-
Proven early neonatal sepsis	7.7 (2/26)	0 (0/12)	1.000	-

Data are presented as n/N (%).

MIAC: microbial invasion of amniotic cavity, defined as a positive amniotic fluid culture.

IAI: intra-amniotic inflammation, defined as an amniotic fluid matrix metalloproteinase-8 concentration >23 ng/mL.

[a] Two cases with congenital anomaly were excluded from the analysis.

[b] Logistic regression analysis was used to adjust gestational age at amniocentesis.

[c] One case with unavailable data was excluded from the analysis.

[d] Eleven neonates who died *in utero* (n=3) or immediately after birth because of extreme prematurity (n=8) and thus could not be evaluated with respect to the presence or absence of complications were excluded from the analysis.

[e] Firth's method was used in the analysis.

Table 4

Cases without microbial invasion of the amniotic cavity or intra-amniotic inflammation

No	Gestational age at amniocentesis (weeks)	Gestational age at birth (weeks)	Interval from amniocentesis(days)	Diagnosis	Birth-weight (gram)	Maternal white blood cell count (cells/mm^3)	Amniotic fluid MMP-8 concentration (ng/mL)	Acute histologic chorioamnionitis	Acute funisitis	Remarks
1	28.9	29	1	PROM	950	18400	5.8	Present	Absent	Maternal preeclampsia, RDS, neonatal death due to pulmonary hemorrhage
2	25.1	25.3	2	PTL	911	15500	1.7	Present	Absent	Progression of spontaneous labor, RDS, neonatal death due respiratory failure
3	35.4	35.4	0	PTL	2390	18200	0.5	Absent	Absent	Delivery due to sustained fever, survival without significant morbidity
4	32.4	42.4	70	CCA	4040	18100	1.4	N/A	N/A	Survival without significant morbidity
5	33.7	36	16	PTL	2970	8000	0.3	Absent	Absent	Cesarean delivery due to placenta previa
6	27.6	38.1	74	PTL	2450	6000	0.5	N/A	N/A	Survival without significant morbidity
7	34.1	34.1	0	PTL	2010	8900	0.7	Absent	Absent	Cesarean delivery due to breech presentation and spontaneous labor progression
8	31.3	31.3	0	PTL	1700	17090	0.3	Absent	Absent	Cesarean delivery due to maternal bleeding, RDS
9	25.7	37.4	83	PTL	2850	17860	0.5	Absent	Absent	Survival without significant morbidity
10	31.4	41	67	CCA	3320	7000	0.3	N/A	N/A	Survival without significant morbidity
11	29.1	29.1	0	PTL	1140	15030	0.3	Absent	Absent	Cesarean delivery due to non-reassuring fetal status, RDS
12	32.9	33	1	PTL	1980	17460	2.1	N/A	N/A	Spontaneous labor progression

CCA: clinical chorioamnionitis without preterm rupture of the membranes or preterm labor; MMP-8: matrix metalloproteinase-8; N/A: not assessed; PTL: preterm labor; PROM: premature rupture of the membranes; RDS: respiratory distress syndrome;