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A New Antibiotic Regimen Treats and Prevents Intra-Amniotic Infection/Inflammation in Patients with Preterm PROM

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Abstract

Objective—To determine if a new antibiotic regimen could reduce the frequency of intra-amniotic inflammation/infection in patients with preterm PROM.

Study design—This retrospective cohort study was conducted to evaluate the effect of antibiotics on the frequency of intra-amniotic inflammation/infection based on the results of follow-up transabdominal amniocenteses from 89 patients diagnosed with preterm PROM who underwent serial amniocenteses. From 1993–2003, ampicillin and/or cephalosporins or a combination was used ("regimen 1"). A new regimen (ceftriaxone, clarithromycin, and metronidazole) was used from 2003–2012 ("regimen 2"). Amniotic fluid was cultured and matrix metalloproteinase-8 (MMP-8) concentrations were measured.

Results—1) The rates of intra-amniotic inflammation and intra-amniotic inflammation/infection in patients who received regimen 2 decreased during treatment from 68.8% to 52.1% and from

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75% to 54.2%, respectively. In contrast, in patients who received regimen 1, the frequency of intra-amniotic inflammation and infection/inflammation increased during treatment (31.7% to 55% and 34.1% to 58.5%, respectively); and 2) intra-amniotic inflammation/infection was eradicated in 33.3% of patients who received regimen 2, but in none who received regimen 1.

Conclusion—The administration of ceftriaxone, clarithromycin, and metronidazole was associated with a more successful eradication of intra-amniotic inflammation/infection and prevented secondary intra-amniotic inflammation/infection more frequently than an antibiotic regimen which included ampicillin and/or cephalosporins in patients with preterm PROM.

Keywords

ceftriaxone; chorioamnionitis; clarithromycin; erythromycin; funisitis; metronidazole; neonatal morbidity; neonatal sepsis; pregnancy; prematurity; preterm birth; preterm labor; placental pathology

Introduction

Premature rupture of the membranes (PROM) occurs in 10% of pregnancies [1–5] and is a risk factor for adverse pregnancy and neonatal outcomes [6–24]. Preterm PROM is a major complication of pregnancy [25–39], and accounts for 30% of spontaneous preterm births [40–42]. Microorganisms in the amniotic fluid are present in approximately 30% of patients, based on the results of culture techniques [43–66], and in 50% of patients using the combination of culture and molecular microbiologic techniques [67–72]. The frequency of infection increases over time (latency period), so that when a patient with preterm PROM eventually goes into labor, microorganisms are detected in 75% of cases [48]. Intra-amniotic inflammation is frequently present in patients with microorganisms in the amniotic fluid, even though, in some cases, sterile inflammation is present [69,73–77].

Antibiotic administration has become the standard of care for patients with preterm PROM [1,35,36,78–88]. Antimicrobial agents are prescribed to eradicate existing subclinical intra-amniotic infection, or to prevent secondary ascending infection into the amniotic cavity [1,35,36,78–89]. Randomized clinical trials [90–104] and systematic reviews [2,105–108] indicate that antibiotic administration has short-term benefits for both pregnant women and their neonates, including prolonged pregnancy [2,91–97,99–102,105,109], reduction of neonatal respiratory distress syndrome [2,92,100,102], infection-related morbidity [2,90–92,94,98–100,102,105,106,109], and necrotizing enterocolitis [2,100,102]. Whether the short-term benefits translate into long-term health outcomes is not clear. Indeed, a follow-up study of infants exposed to antibiotics due to preterm PROM indicated that there was not a demonstrable benefit at 7 years of age [110].

Several antibiotic regimens have been used in patients with preterm PROM, including ampicillin [90,93,97,99,100,108,111–113], amoxicillin-clavulanate [93,102,108], penicillin [94], erythromycin [92,95,97,100,102,108,114], mezlocillin [97], pipericillin [96], cefexin [115], cefizox [116], gentamicin [93], clindamycin [93], azithromycin [113,117] and combinations of different agents [89,109]. The currently recommended practice varies in the United States and Europe. These choices appear to be influenced by randomized clinical

trials conducted in each country. For example, the current recommendation in the United States includes intravenous ampicillin and erythromycin, followed by oral amoxicillin and erythromycin [100]. In the United Kingdom, the recommendation is to administer oral erythromycin [102].

Genital mycoplasmas are the most frequent organisms invading the amniotic cavity in preterm PROM [57,69,118–122,123]. The antibiotics used in randomized clinical trials of preterm PROM have limited effectiveness against these organisms. Penicillin and cephalosporins are not effective, and more than 80% of *Ureaplasma* spp. are resistant to erythromycin [124–129]. Therefore, we designed a combination of antibiotic therapies for patients with preterm PROM, which was implemented in clinical practice in 2003 with the goal of providing antimicrobial activities to most organisms found in the amniotic cavity in preterm PROM. This combination included ceftriaxone, clarithromycin, and metronidazole. We recently reported that the use of this regimen was associated with a prolonged latency period and the reduced frequency of funisitis [109].

We performed follow-up amniocenteses as a part of our standard practice to monitor the response to therapy. The information derived from follow-up amniocenteses and microbiologic studies provides a unique opportunity to gain insight into the efficacy of antibiotic administration in preterm PROM. The purpose of this study was to examine whether the antibiotic regimen with expanded coverage (regimen 2) would decrease the frequency of intra-amniotic inflammation/infection.

Materials and Methods

Study design

This is a retrospective cohort study. The effect of antibiotics on the microbial state of the amniotic cavity and intra-amniotic inflammation was analyzed in the samples of amniotic fluid obtained at the time of the initial and follow-up amniocenteses. The study population comprised patients admitted to Seoul National University Hospital, Seoul, Republic of Korea, between January 1993 and June 2012, with the diagnosis of preterm PROM, who met the following criteria: 1) singleton pregnancy, 2) gestational age < 34 weeks, 3) follow-up amniocentesis performed within 15 days of the initial amniocentesis. The last criterion was used to evaluate changes in the microbial state of the amniotic cavity and intra-amniotic inflammation after the initial amniocentesis.

At Seoul National University Hospital, a transabdominal amniocentesis is routinely offered to all patients admitted with the diagnosis of preterm PROM to assess the microbiologic status of the amniotic cavity and fetal lung maturation. A follow-up amniocentesis could then be performed at the discretion of the managing clinician to further assess the microbiologic state of amniotic cavity and fetal lung maturity after the completion of corticosteroid administration. This could have been performed with either a transabdominal amniocentesis or the aspiration of fluid at the time of cesarean delivery. Although all patients admitted with the diagnosis of preterm PROM were offered an initial amniocentesis, there was no uniform agreement on the performance and timing of a follow-up amniocentesis in our unit. Some physicians elected not to offer a second amniocentesis; therefore, many

patients did not undergo the procedure. The diagnosis of rupture of the membranes was based on the patient having a previous history of watery vaginal discharge and a combination of the following tests: confirming leakage of amniotic fluid from the cervical os, vaginal pooling of amniotic fluid, and a positive nitrazine test through a sterile speculum examination.

Antibiotic administration was initiated when rupture of the membranes was diagnosed and continued until delivery. Corticosteroids were administered to patients at 24 weeks of gestation or more for the induction of fetal lung maturity. Between January 1993 and August 2003, patients received ampicillin and/or cephalosporin. Fifteen patients received intravenous ampicillin alone and seven patients received intravenous cephalosporins. Three patients were treated with intravenous ampicillin combined with cephalosporins. The remaining 16 patients received erythromycin, azithromycin, gentamicin, or metronidazole combined with ampicillin or cephalosporins [regimen 1 (N=41)]. The precise selection of antimicrobial agents was also at the discretion of the managing clinicians.

Between September 2003 and June 2012, the antibiotic regimen administered was 1g of intravenous ceftriaxone every 24 hours, 500 mg of oral clarithromycin every 12 hours, and 500 mg of intravenous metronidazole every 8 hours until delivery [regimen 2 (N=48)] with the exception of metronidazole, which was administered for a maximum of 4 weeks. The study population was divided into two groups according to the antibiotic regimen 1 or 2.

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

Amniotic fluid

Amniotic fluid was retrieved by transabdominal amniocentesis and cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas using methods previously described [52, 57, 130–133]. Amniotic fluid not used for diagnostic studies was centrifuged and stored at -70°C until assay. Matrix metalloproteinase-8 (MMP-8) concentrations in the amniotic fluid were used to diagnose intra-amniotic inflammation based on the criteria of previous studies [55,131,134–141]. Stored amniotic fluid as well as a commercially available enzyme-linked immunosorbent assay (ELISA) (Amersham Pharmacia Biotech, Inc., Bucks, UK) was used, according to the manufacturer's instructions. The sensitivity of the test was 0.3 ng/mL, and the intra- and inter-assay coefficients of variation were less than 10%.

Criteria for the diagnosis of intra-amniotic inflammation, intra-amniotic infection/ inflammation, acute histologic chorioamnionitis, and funisitis

Intra-amniotic inflammation was defined as an elevated amniotic fluid MMP-8 concentration (>23 ng/mL), as reported previously [55,131,134–141]. Intra-amniotic inflammation/ infection was diagnosed when either a positive amniotic fluid culture or the presence of intra-amniotic inflammation was detected. Using criteria previously described, the diagnosis of acute histologic chorioamnionitis was made upon examination of the extraplacental

chorioamniotic membrane roll and/or the chorionic plate of the placenta when a patient presented with acute inflammatory changes [142–145]. Funisitis was diagnosed when neutrophil infiltration was detected in the umbilical vessel walls or Wharton's jelly using criteria previously reported [135,143–148].

Statistical analysis

To determine continuous variables, the Mann-Whitney U test was used. Paired non-parametric analyses were used to compare the changes in the amniotic fluid MMP-8 concentration between the initial and follow-up amniocenteses. For categorical variables, the χ^2 test or Fisher's exact test was used. McNemar's test was used to compare the frequency of positive amniotic fluid culture, intra-amniotic inflammation, and intra-amniotic infection/inflammation between the initial and follow-up amniocenteses. Medians and ranges were reported for continuous variables, whereas frequencies and percentages were calculated for categorical variables. Statistical analyses were conducted using SPSS Version 19.0 (SPSS Inc., Chicago, IL, USA). All p-values were two-sided and a p-value of <0.05 was considered statistically significant.

Results

Eighty-nine patients with preterm PROM and a singleton gestation (<34 weeks) met the inclusion criteria of this study; 41 patients received regimen 1 and 48 patients received regimen 2. After the initial amniocentesis, the antibiotic regimen was changed in 12 patients and discontinued in one patient among those who received regimen 1, and also changed in 4 patients and discontinued in one patient among those who received regimen 2. After the follow-up amniocentesis, the antibiotic treatment regimen was changed in 6 patients and discontinued in another 6 patients among those who received regimen 1, and also changed in 6 patients and discontinued in 4 patients who received regimen 2. Table 1 shows the clinical and demographical characteristics of the study population. Patients who received regimen 2 (ceftriaxone, clarithromycin, and metronidazole) had a significantly lower median gestational age at the initial amniocentesis, follow-up amniocentesis, and delivery as well as a significantly lower rate of funisitis, a marker of fetal systemic inflammatory response syndrome, than those who received regimen 1 ($p<0.05$ for each).

Table 2 compares the rates of positive amniotic fluid culture, intra-amniotic inflammation, intra-amniotic inflammation/infection, and the median MMP-8 concentration according to the antibiotic regimen and timing of amniocentesis. Microorganisms isolated at the initial amniocentesis in patients who received regimen 1 included *Ureaplasma urealyticum* (n=7), coagulase (-) *Staphylococcus* (n=2), *Streptococcus anginosus* (n=1), *peptostreptococcus* (n=1), and in those who received regimen 2 were *Ureaplasma urealyticum* (n=7), *Mycoplasma hominis* (n=4), *Streptococcus anginosus* (n=1), Gram (+) cocci (n=2), *Lactobacillus* (n=1), and *Enterococcus faecium* (n=1). Microorganisms identified at follow-up amniocentesis in patients who received regimen 1 included *Ureaplasma urealyticum* (N=8), *Mycoplasma hominis* (N=1), *Klebsiella pneumoniae* (N=2), *Escherichia coli* (N=1), coagulase (-) *Staphylococcus* (N=1), *Staphylococcus epidermidis* (N=2), *Staphylococcus aureus* (N=1), and *Streptococcus agalactiae* (N=1), and in those who received regimen 2, we

found *Ureaplasma urealyticum* (N=5), *Mycoplasma hominis* (N=7), and *Candida albicans* (N=3).

The frequency of intra-amniotic inflammation in patients who received regimen 1 increased over time [initial amniocentesis: 31.7% (13/41) vs. the follow-up amniocentesis 55% (22/40); p=0.006, McNemar's test]. Similarly, the frequency of intra-amniotic inflammation/infection also increased over time in patients who received regimen 1 [initial amniocentesis: 34.1% (14/41) vs. the follow-up amniocentesis: 58.5% (24/41), p=0.002, McNemar's test] (Table 2).

In contrast, the rates of intra-amniotic inflammation and intra-amniotic inflammation/infection in patients who received regimen 2 decreased over time from 68.8% (33/48) to 52.1% (25/48) and from 75% (36/48) to 54.2% (26/48), respectively ($p<0.05$ for each, McNemar's test) (Table 2). In other words, the frequency of intra-amniotic inflammation/infection in patients who received regimen 2 decreased while it increased over time in those who received regimen 1.

There was no difference in the frequency of positive amniotic fluid cultures between the initial and follow-up amniocenteses in patients who received either regimen 1 or regimen 2 ($p>0.2$ for each, McNemar's test). However, the magnitude of the intra-amniotic inflammatory response significantly increased over time in patients who received regimen 1 (median amniotic fluid MMP-8 concentration 1.8 ng/mL vs. 29.0 ng/mL, p=0.003, Wilcoxon signed rank test), while there was no difference in the magnitude of the intra-amniotic inflammatory response between the initial and follow-up amniocenteses in patients who received regimen 2 (Table 2).

In terms of treatment of an existing intra-amniotic inflammation/infection, regimen 2 was superior to regimen 1 (Figure 1A). Specifically, 33.3% (12/36) of patients with intra-amniotic inflammation/infection who received regimen 2 at the initial amniocentesis had a negative amniotic fluid culture and no evidence of intra-amniotic inflammation at the follow-up amniocentesis. In contrast, none (0/14) of the patients who had intra-amniotic inflammation/infection at the initial amniocentesis who received antibiotic regimen 1 had a negative amniotic fluid culture and intra-amniotic inflammation at the follow-up amniocentesis [33.3% (12/36) versus 0% (0/14); $p<0.05$] (Figure 1A). Among the patients without intra-amniotic inflammation/infection at the time of the initial amniocentesis, 83.3% (10/12) of patients who received regimen 2 and 63% (17/27) of those who received regimen 1 remained without evidence of intra-amniotic inflammation/infection (Figure 1B).

Twenty-two percent (2/9) of patients with a positive amniotic fluid culture at the initial amniocentesis who received regimen 1 had a negative amniotic fluid culture at the follow-up amniocentesis. In contrast, in 69.2% (9/13) of patients who received regimen 2, the amniotic cavity became sterile; however, this trend did not reach statistical significance (p=0.08) (Figure 2A). There was no difference in the rate of development of *de novo* intra-amniotic infection according to the antibiotic regimen (Figure 2B).

Discussion

Principal findings of this study

The administration of ceftriaxone, clarithromycin, and metronidazole was associated with a more successful eradication of intra-amniotic inflammation/infection and also prevented secondary intra-amniotic inflammation/infection more frequently than that observed with a conventional antimicrobial regimen used in preterm PROM. Moreover, this new combination was associated with a lower rate of funisitis, the histologic hallmark of fetal inflammatory response syndrome.

Antibiotic administration to patients with preterm PROM based on randomized clinical trials

The practice of administering antibiotics to patients with preterm PROM is grounded in the results of multiple randomized clinical trials [90–104] and a large systematic review and meta-analysis [2,105,106]. Antimicrobial agents have been shown to prolong the latency period [2,91–97,99–102,105], decrease neonatal infection [2,90–92,94,98–100,102,105,106], and reduce respiratory morbidity, including the need for oxygen and surfactant [2,92,100,102]. Moreover, antibiotic administration also reduces the frequency of clinical chorioamnionitis [2,91,94,97,98,100,102,105]. Long-term follow-up of infants to the age of 7 exposed to antimicrobial agents *in utero* because of preterm PROM has not shown convincing evidence that the short-term gain translates into long-term benefit [110].

Are antibiotics effective in treating and eradicating intra-amniotic infection in preterm PROM?

A challenge in assessing the efficacy of antibiotic administration is the clinical context in which they are prescribed. Pregnant women with sub-clinical intra-amniotic infection are difficult to monitor non-invasively. Serial amniocenteses were performed in the current study, and this provided a unique opportunity to assess which antibiotic regimen eradicated intra-amniotic inflammation/infection present at the time of admission or prevented secondary intra-amniotic inflammation/infection which frequently occurs over time prior to the onset of spontaneous labor or induction of labor.

Although eradication of intra-amniotic infection has been demonstrated in patients with preterm PROM by using serial amniocenteses, one study has raised important questions about efficacy [149]. Gomez et al. followed 46 patients with preterm PROM for whom amniocenteses were performed between 18–32 weeks (median 27 weeks). The prevalence of intra-amniotic inflammation for the initial amniocentesis was 39% (18/46); seven patients had a positive amniotic fluid culture for bacteria [149]. At the time of the follow-up amniocentesis, six of these seven patients still had a positive amniotic fluid culture despite having received antibiotics (ampicillin and erythromycin) for 7 days; the intra-amniotic inflammatory process had been successfully treated in only three of 18 patients. Importantly, among patients with no evidence of intra-amniotic inflammation, 32% (9/28) developed intra-amniotic inflammation despite antibiotic therapy, and five of the nine had a positive amniotic fluid culture [149]. These observations suggest that antimicrobial agents are not

effective in eradicating or preventing intra-amniotic inflammation/infection in preterm PROM.

Potential risks associated with inadequate therapy or prophylaxis of intra-amniotic infection

Experimental studies designed to generate an animal model of brain injury after intrauterine infection have shown that inoculation of bacteria is followed by the onset of labor [150]. If antibiotics are administered around the time of bacterial inoculation, this prevents the onset of labor, but intrauterine infection can be observed at the time of euthanasia [151]. Moreover, the fetuses whose gestation was prolonged by the administration of antibiotics were subsequently found to have lesions of periventricular leukomalacia, a major risk factor for cerebral palsy [151]. Studies in humans have suggested an association between acute chorioamnionitis and neurodevelopmental disorders [136,151–159]. The observation that the administration of ampicillin and erythromycin in the randomized clinical trial conducted by Mercer et al. [100] did not result in a lower rate of funisitis (compared to the placebo group) [160] indicates that short-term antimicrobial therapy did not reduce the likelihood of a fetal inflammatory response [79]. This observation is consistent with a subsequent report indicating that there was no change in the concentrations of inflammatory cytokines in umbilical cord blood after the administration of antibiotics [161]. Therefore, in women with preterm PROM, antibiotic administration prolongs pregnancy but does not eradicate infection or inflammation.

Efficacy of erythromycin and azithromycin in eradicating intra-amniotic infection in animal models

Recent studies in sheep and non-human primates have reported that the administration of erythromycin does not eradicate intrauterine infection [162,163]. This has been attributed to the fact that maternal administration of macrolide antibiotics achieved only sub-therapeutic doses in the amniotic fluid and fetal plasma. In contrast, studies in rhesus monkeys [164,165] and sheep [166] demonstrated that the administration of azithromycin can eradicate *Ureaplasma parvum* from the amniotic fluid and fetal organs. Yet, residual acute histologic chorioamnionitis has been observed, indicating that such therapy is not completely successful [164,166].

A new antibiotic regimen for patients with preterm PROM

Our study has examined the changes in microbiology and intra-amniotic inflammation in patients with preterm PROM treated with two different antimicrobial regimens. The first regimen used antibiotics used by clinicians concerned with polymicrobial infections. The second regimen was developed and implemented based upon studies of the microbiology of amniotic fluid in patients with preterm PROM, which showed that *Ureaplasmas* were a predominant species not treated successfully with erythromycin [124–129]. The rationale for using a combination of clarithromycin [167,168], ceftriaxone [169–171], and metronidazole [172–175] was based on previous studies about the pharmacokinetics of antibiotics during pregnancy and the need for expanded coverage and duration of treatment [129,167,168,176,177]. The key finding of the current study is that antimicrobial therapy is more effective in eradicating intra-amniotic inflammation/infection than the antimicrobial

therapy used in the past. The improved efficacy can be attributed to the improved bioavailability of antibiotics and expanded coverage. It is unlikely that the improved result can be attributed to the duration of therapy, as patients in both groups were treated from admission to delivery. The clinical benefit of this therapy is the subject of a separate communication. However, there is evidence that this new antimicrobial therapy is associated with prolonged pregnancy and a lower frequency of neonatal complications, acute histologic chorioamnionitis, and funisitis [109]. When broad-spectrum antibiotics are used, the emergence of antibiotic-resistant bacteria is a possibility.

Strengths and limitations

The major strength of this study is that it is the first to examine the effect of the new antibiotic regimen for the treatment of intra-amniotic inflammation/infection. In our practice, we used serial amniocenteses to evaluate the microbial state and inflammatory response in the amniotic fluid. Moreover, we have also used well-established assays to detect inflammation in the amniotic cavity.

A limitation of this study is that it was not a randomized clinical trial but rather a retrospective cohort study conducted over a period of 20 years. However, none of the parameters serving as an endpoint (amniotic fluid culture, MMP-8 determination, and histologic examination of the placenta) changed fundamentally during this time, so it is unlikely that the historical nature of the control group could have affected the result reported herein. To conduct this study required the performance of serial amniocenteses, a procedure demanding considerable skill; this could introduce bias because not all patients are able to undergo serial amniocenteses. We have recently developed a transcervical amniotic fluid collector to obtain this material for the assessment of intra-amniotic inflammation [178]. Preliminary evidence indicates that fluid retrieved with this device can be used to reliably identify intra-amniotic inflammation, and this could enable serial evaluation of a large number of patients and reduce potential biases introduced by the requirement of amniocentesis [178].

Conclusion

The combination of ceftriaxone, clarithromycin, and metronidazole can treat and prevent intra-amniotic inflammation/infection in patients with preterm PROM.

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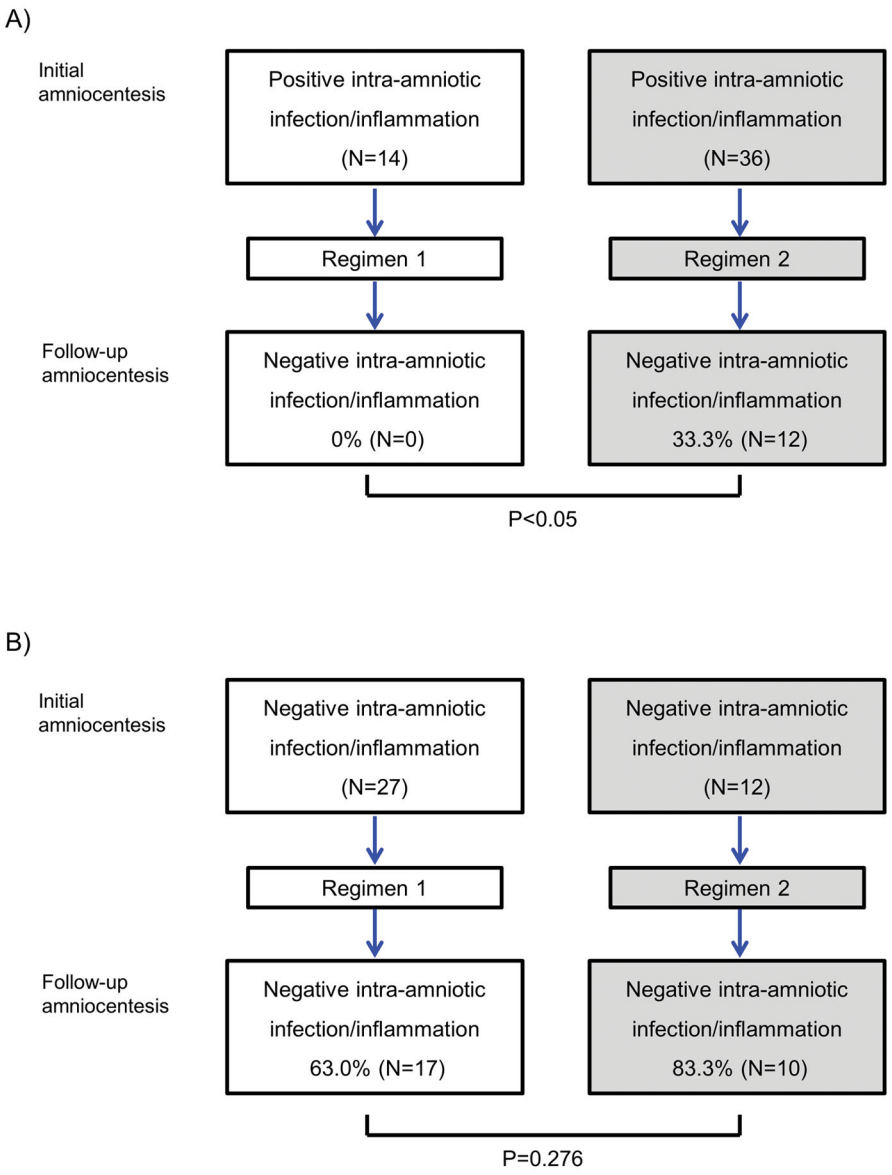

Figure 1. Conversion of intra-amniotic infection/inflammation according to the antibiotic regimen

(a) Intra-amniotic infection/inflammation was eradicated in 33.3% of patients who had intra-amniotic infection/inflammation at the time of the initial amniocentesis and received regimen 2, while none of patients who received regimen 1 showed negative conversion of intra-amniotic infection/inflammation ($p<0.05$). (b) Among the patients without intra-amniotic inflammation/infection at the time of the initial amniocentesis, 83.3% of patients who received regimen 2 and 63% of those who received regimen 1 remained without evidence of intra-amniotic infection/inflammation, which did not reach statistical significance ($p=0.276$).

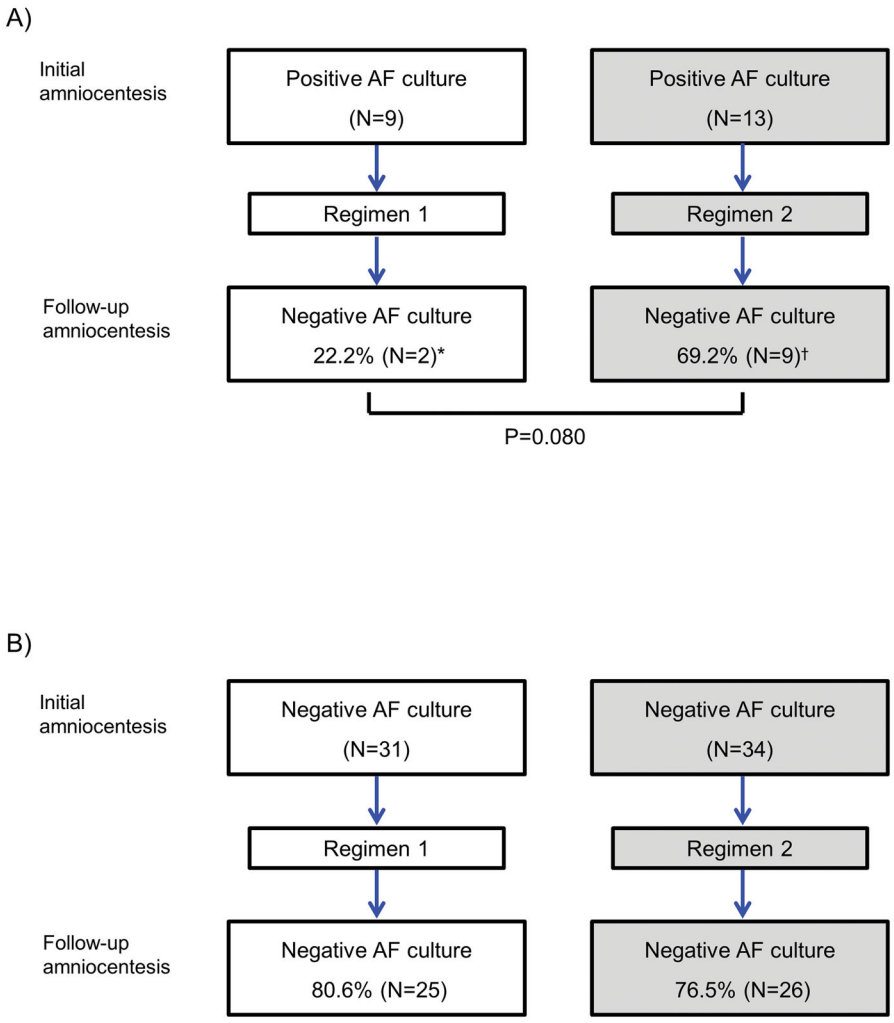

Figure 2. Conversion of intra-amniotic infection according to the antibiotic regimen

(a) 22.2% of patients with a positive amniotic fluid culture at the time of initial amniocentesis and who received regimen 1 had a conversion to a negative amniotic fluid culture at the follow-up amniocentesis, while 69.2% of patients who received regimen 2, the amniotic cavity became sterile (p=0.080). (b) There was no difference in the rate of development of *de novo* intra-amniotic infection according to the antibiotic regimen.

*, All cases (2/2) with a negative conversion of intra-amniotic infection had intra-amniotic inflammation.

†, Four (44.4%) of 9 cases with a negative conversion of intra-amniotic infection had intra-amniotic inflammation.

Table 1

Demographics and clinical characteristics of the study population

	Regimen 1 (N=41)	Regimen 2 (N=48)	p value
Maternal age (years) *	30 (20–36)	32 (25–40)	0.003
Nulliparity (%)	41.5 (17/41)	45.8 (22/48)	0.679
Prior preterm delivery (%)	17.1 (7/41)	10.4 (5/48)	0.359
Cesarean delivery (%)	36.6 (15/41)	35.4 (17/48)	0.909
Gestational age at delivery (weeks) *	34.0 (23.1–41.6)	30.9 (20.7–41.0)	0.027
Birth-weight (g) *	2160 (530–4100)	1500 (260–3100)	0.077
Baby gender, male (%)	51.2 (21/41)	54.2 (26/48)	0.781
Gestational age at initial amniocentesis (weeks) *	29.7 (20.4–33.9)	27.2 (17.7–32.0)	0.001
Gestational age at follow-up amniocentesis (weeks) *	30.9 (21.3–35.4)	28.4 (19.7–34.7)	0.003
Interval between initial and follow-up amniocentesis (days) *	7 (2–15)	9 (2–15)	0.077
Initial amniocentesis-to-delivery interval (days) *	14 (3–148)	20 (3–124)	0.087
Follow-up amniocentesis-to-delivery interval (days) *	3 (0–141)	9 (0–109)	0.065
Acute histologic chorioamnionitis (%)	54.5 (18/33)	58.5 (24/41)	0.730
Funisitis (%)	36.4 (12/33)	14.0 (6/43)	0.023

* median (range)

Table 2

Amniotic fluid culture, intra-amniotic inflammation, and intra-amniotic infection/inflammation according to the antibiotics regimen and timing of amniocentesis

	Regimen 1 (N=41)		P value	Regimen 2 (N=48)		P-value
	Initial	F/U		Initial	F/U	
Positive amniotic fluid culture (%)	22.0 (9/41)	32.5 (13/40)	0.289≠	27.1 (13/48)	25.5 (12/47)	>0.999≠
Amniotic fluid MMP-8 concentration (ng/mL)*	1.8 (0.3–1460.9)	29.0 (0.3–2608.0)	0.003§	83.5 (0.3–6874.0)	43.4 (0.3–5271.5)	0.678§
Intra-amniotic inflammation (%)†	31.7 (13/41)	55.0 (22/40)	0.006≠	68.8 (33/48)	52.1 (25/48)	0.021≠
Intra-amniotic infection/inflammation (%)	34.1 (14/41)	58.5 (24/41)	0.002≠	75.0 (36/48)	54.2 (26/48)	0.013≠

*median (range), MMP-matrix metalloproteinase

†amniotic fluid MMP-8 concentration >23ng/mL

≠P-value by McNemar's test

§P-value by Wilcoxon signed rank test