OBSTETRICS

# Antibiotic administration can eradicate intra-amniotic infection or intra-amniotic inflammation in a subset of patients with preterm labor and intact membranes

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**BACKGROUND:** Intra-amniotic infection is present in 10% of patients with an episode of preterm labor, and is a risk factor for impending preterm delivery and neonatal morbidity/mortality. Intra-amniotic inflammation is often associated with intra-amniotic infection, but is sometimes present in the absence of detectable microorganisms. Antibiotic treatment of intra-amniotic infection has traditionally been considered to be ineffective. Intra-amniotic inflammation without microorganisms has a prognosis similar to that of intra-amniotic infection.

**OBJECTIVE:** To determine whether antibiotics can eradicate intra-amniotic infection or intra-amniotic inflammation in a subset of patients with preterm labor and intact membranes.

**MATERIALS AND METHODS:** The study population consisted of women who met the following criteria: 1) singleton gestation between 20 and 34 weeks; 2) preterm labor and intact membranes; 3) transabdominal amniocentesis performed for the evaluation of the microbiologic/inflammatory status of the amniotic cavity; 4) intra-amniotic infection and/or intra-amniotic inflammation; and 5) received antibiotic treatment that consisted of ceftriaxone, clarithromycin, and metronidazole. Follow-up amniocentesis was performed in a subset of patients. Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas, and polymerase chain reaction was performed for *Ureaplasma spp*. Intra-amniotic infection was defined as a positive amniotic fluid culture or positive polymerase chain reaction, and intra-amniotic inflammation was suspected when there was an elevated amniotic fluid white blood cell count or a positive result of a rapid test for matrix metalloproteinase-8. For this study, the final diagnosis of intra-amniotic inflammation was made by measuring the interleukin-6 concentration in stored amniotic fluid (>2.6 ng/mL). These results were not available to managing clinicians. Treatment success was defined as eradication of intra-amniotic infection and/or intra-amniotic inflammation or delivery ≥37 weeks.

**RESULTS:** Of 62 patients with intra-amniotic infection and/or intra-amniotic inflammation, 50 received the antibiotic regimen. Of those patients, 29 were undelivered for ≥7 days and 19 underwent a follow-up amniocentesis. Microorganisms were identified by culture or polymerase chain reaction of amniotic fluid obtained at admission in 21% of patients (4/19) who had a follow-up amniocentesis, and were eradicated in 3 of the 4 patients. Resolution of intra-amniotic infection/inflammation was confirmed in 79% of patients (15/19), and 1 other patient delivered at term, although resolution of intra-amniotic inflammation could not be confirmed after a follow-up amniocentesis. Thus, resolution of intra-amniotic inflammation/infection or term delivery (treatment success) occurred in 84% of patients (16/19) who had a follow-up amniocentesis. Treatment success occurred in 32% of patients (16/50) with intra-amniotic infection/inflammation who received antibiotics. The median amniocentesis-to-delivery interval was significantly longer among women who received the combination of antibiotics than among those who did not (11.4 days vs 3.1 days: $P = .04$).

**CONCLUSION:** Eradication of intra-amniotic infection/inflammation after treatment with antibiotics was confirmed in 79% of patients with preterm labor, intact membranes, and intra-amniotic infection/inflammation who had a follow-up amniocentesis. Treatment success occurred in 84% of patients who underwent a follow-up amniocentesis and in 32% of women who received the antibiotic regimen.

**Key words:** amniotic fluid, ceftriaxone, chorioamnionitis, clarithromycin, interleukin-6, intra-amniotic inflammation, metronidazole, MMP-8, pregnancy, prematurity, white blood cell

Preterm labor is a syndrome caused by multiple pathologic processes.[1] The following mechanisms of disease have been implicated: intra-amniotic infection,[2–25] "sterile" intra-amniotic inflammation,[26–39] uterine overdistention,[40,41] maternal anti-fetal rejection,[42–46] decidual senescence,[47–50] and possibly other mechanisms that are yet to be identified.

EDITORS' CHOICE

One of every 10 patients with preterm labor and intact membranes will have intra-amniotic infection[2–7,12,16,17,22–25,33,35,51] that is largely subclinical,[2,6,22,26,33,38,39,52,53] and these patients are at increased risk for early preterm delivery,[3,6–8,22,33,53] neonatal complications,[6,8,21,26,33,54–66] and maternal morbidity (such as acute pulmonary edema, when treated with tocolytics and steroids[67–69]) or maternal sepsis.[70] Similar risks occur in patients with preterm premature rupture of membranes (PROM) and intra-amniotic infection.[4,13,17,71,72]

Given the frequency and importance of intra-amniotic infection in the pathogenesis of preterm labor with intact membranes, several randomized clinical trials have tested the efficacy and safety of antibiotic administration.[73–76] Despite initial enthusiasm,[10,73,75,77] subsequent trials have not shown beneficial effects,[74,78–83] and currently, antibiotic administration is restricted to

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## AJOG at a Glance

### Why was this study conducted?

This study was conducted to determine whether intra-amniotic infection or intra-amniotic inflammation can be treated with antibiotics in patients with preterm labor and intact membranes.

### Key findings

Resolution of intra-amniotic inflammation or intra-amniotic infection was objectively demonstrated by analysis of amniotic fluid after treatment with antibiotics in 79% of patients.

### What does this add to what is known?

Contrary to what is widely believed, antimicrobial treatment of intra-amniotic infection or intra-amniotic inflammation can be successful in a subset of patients with preterm labor and intact membranes. These observations encourage new therapeutic alternatives and call for personalized assessment of patients with preterm labor and intact membranes to identify those who can benefit from this intervention.

patients with an episode of premature labor who are carriers of group B streptococcus (GBS)[84,85] or have unknown GBS status[86] to prevent vertical transmission and neonatal sepsis.[87,88]

Intra-amniotic inflammation, defined as an elevated concentration of interleukin-6 or matrix metalloproteinase-8 (MMP-8) in amniotic fluid in the absence of demonstrable microorganisms detected with culture or molecular methods ("sterile" intra-amniotic inflammation), has also been associated with adverse pregnancy outcomes, including acute histologic chorioamnionitis and funisitis.[25–28,33,71,89] Activation of the inflammasome has been implicated in the mechanisms responsible for preterm labor induced by "sterile" intra-amniotic inflammation.[31,32,90–92]

Important advances have been made in the identification of patients at risk for spontaneous preterm delivery by assessing cervical length in the midtrimester,[93–103] as well as in the treatment of patients with a sonographic short cervix with vaginal progesterone.[104–115] However, the optimal treatment of patients with an episode of preterm labor, intact membranes, and intra-amniotic infection or intra-amniotic inflammation has not been determined. Previous reports demonstrated the eradication of microorganisms in the amniotic cavity of patients with a short cervix[116,117] and preterm PROM.[27,118] A recent report suggests that a subset of patients with preterm labor and intra-amniotic infection may benefit from antibiotic administration.[119]

We have recently reported that antibiotic treatment in patients with preterm PROM can reduce the rate of intra-amniotic infection and intra-amniotic inflammation, as well as funisitis and the fetal systemic inflammatory response, using a combination of antibiotics (ceftriaxone, clarithromycin, and metronidazole) that target microorganisms frequently isolated from the amniotic cavity in these cases.[118,120]

The purpose of this study was to determine whether antibiotics could eradicate intra-amniotic infection or intra-amniotic inflammation without demonstrable microorganisms in patients with preterm labor and intact membranes.

## Materials and Methods

### Study design

This is a retrospective case series study of pregnant women admitted to Seoul National University Hospital between January 2004 and March 2014 who met the following criteria: 1) singleton gestation between 20 and 34 weeks; 2) preterm labor and intact amniotic membranes determined by sterile speculum examination; 3) transabdominal amniocentesis performed for the evaluation of the microbiologic and inflammatory status of the amniotic cavity; 4) positive amniotic fluid culture or intra-amniotic inflammation; and 5) antibiotic treatment (regimen consisted of ceftriaxone, clarithromycin, and metronidazole). Follow-up amniocentesis was performed in a subset of patients at the discretion of the managing physician.

At the Seoul National University Hospital, a transabdominal amniocentesis is routinely offered to all patients admitted with the diagnosis of preterm labor to assess the microbiologic status of the amniotic cavity and fetal lung maturity. Retrieval of amniotic fluid was performed after written informed consent was obtained. Preterm labor was diagnosed as the presence of regular uterine contractions (4 or more contractions in 20 minutes or 8 or more in 60 minutes). The Institutional Review Board of the Seoul National University Hospital approved the collection and use of these samples and clinical information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protection (OHRP) of the Department of Health and Human Services (DHHS) of the United States.

### Amniotic fluid analysis

Amniotic fluid was cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas. Beginning in 2007, samples were also assayed for *Ureaplasma spp.* by means of polymerase chain reaction (PCR) with specific primers using methods previously described.[8] An aliquot of amniotic fluid was examined in a hemocytometer chamber to determine the white blood cell count.[33,121] In a subset of patients, MMP-8 concentration in amniotic fluid was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Amersham Pharmcia Biotech, Inc, Bucks, UK) and the results were available to clinicians. Intra-amniotic inflammation was suspected when the concentration of MMP-8 in the amniotic fluid was higher than 23 ng/mL, as previously reported.[71,89,122–125]

Between March 2005 and December 2010, a rapid MMP-8 bedside test

(MMP-8 PTD Check test, SK Pharma Co, Ltd, Kyunggi-do, Korea) was performed and used in patient management. Details of the MMP-8 rapid test have been previously described.[53,60,126,127] Amniotic fluid not used for diagnostic tests was centrifuged at 800 *g* and stored at −80°C.

Intra-amniotic infection was defined as a positive amniotic fluid culture or positive PCR for *Ureaplasma spp.* For the purposes of this study, a definitive diagnosis of intra-amniotic inflammation was made when the interleukin-6 concentration of stored amniotic fluid was higher than 2.6 ng/mL.[33] The amniotic fluid interleukin-6 concentration was measured with a commercially available ELISA kit (R&D Systems, Minneapolis, MN) in 2017 and 2018. The sensitivity of the assay was 0.7 pg/mL. The intra- and interassay coefficients of variation were <10%. These results were not available to managing clinicians.

### Clinical management

Intra-amniotic inflammation was suspected when there was an elevated amniotic fluid white blood cell count (defined as ≥19 cells/mm$^3$),[122] a positive MMP-8 rapid test result,[53,126,127] or an elevated concentration of amniotic fluid MMP-8 (>23 ng/mL) measured by ELISA.[71,89,122] Suspicion of intra-amniotic inflammation, isolation of microorganisms by amniotic fluid culture, or the detection of *Ureaplasma* nucleic acids was an indication for the administration of antibiotics. We used a combination of antimicrobial agents previously prescribed in the management of patients with preterm PROM,[118,120] including ceftriaxone 1 g (intravenous) every 24 hours, clarithromycin 500 mg (oral) every 12 hours, and metronidazole 500 mg (intravenous) every 8 hours. Metronidazole was administered for a maximum of 4 weeks. A follow-up amniocentesis was offered to monitor the microbiologic and inflammatory status of amniotic cavity and fetal lung maturity. The use, discontinuation, or change of antibiotic regimen or tocolytics, or the interval to follow-up amniocentesis, was left to the discretion of the treating clinicians because there was no uniformity among attending physicians about these issues. Tocolytics used were ritodrine, magnesium sulphate, or atosiban. Nonsteroidal anti-inflammatory agents, such as indomethacin, were not used as tocolytic agents. GBS screening and intrapartum treatment are not routinely performed in our institution because neonatal GBS sepsis is extremely rare in our patient population.[128,129]

### Definition of treatment success in this study

Treatment success was defined as 1) eradication of intra-amniotic infection or intra-amniotic inflammation; or 2) delivery at or after 37 weeks of gestation.

### Diagnosis of acute histologic chorioamnionitis and clinical chorioamnionitis

Acute histologic chorioamnionitis was diagnosed in the presence of acute inflammatory changes in tissue samples collected from the amnion and chorion-decidua.[130] Funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly.[71,89,122,131–134]

Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature >37.8°C) accompanied by 2 or more of the following criteria: 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$^3$).[135] The limitations of these criteria in the identification of intra-amniotic infection have been recently described.[118,125,136–138] The criteria for the diagnosis of neonatal morbidity can be found in Supplementary Material S1.

## Statistical analysis

Continuous variables were compared between 2 groups with the Mann–Whitney *U* test. Proportions were compared with a Fisher's exact test. The amniocentesis-to-delivery interval was compared by using the generalized Wilcoxon test for survival analysis. A *P* value <.05 was considered statistically significant. Statistical analyses were performed using SPSS software (Version 22; SPSS Inc, Chicago, IL).

## Results

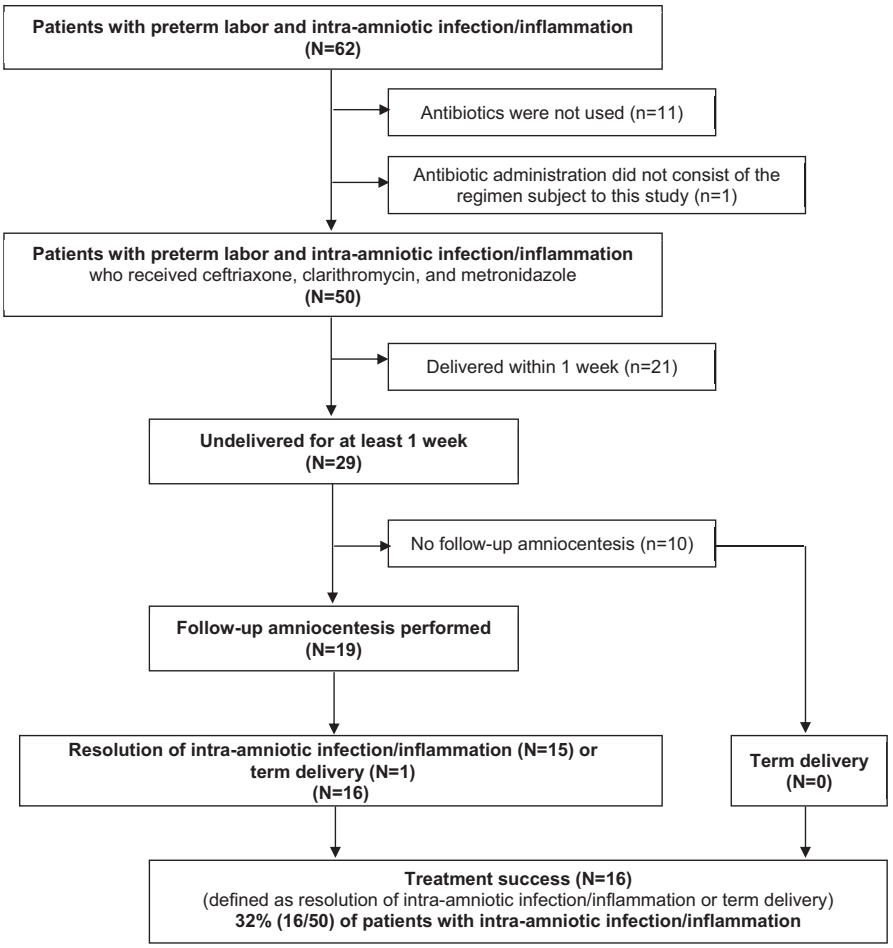
### Characteristics of study population

Figure 1 shows a flow diagram of patients included in this study. A total of 62 patients with intra-amniotic infection and/or intra-amniotic inflammation were identified. A positive amniotic fluid culture was present in 11 patients; *Ureaplasma spp.* was detected by the PCR method in 8 patients; and intra-amniotic inflammation was identified in 51 patients with an amniotic fluid culture negative for microorganisms. Bacteria identified by culture included *Ureaplasma urealyticum* (n = 9), *Mycoplasma hominis* (n = 2), and 1 isolate each of *Streptococcus anginosus* and *Gardnerella vaginalis.*

Of 62 patients with intra-amniotic infection and/or intra-amniotic inflammation, 50 received the combination of ceftriaxone, clarithromycin, and metronidazole. The remaining 12 patients did not receive this antibiotic regimen (11 patients did not receive any antibiotics; 1 patient received an alternative regimen, consisting of ceftriaxone, azithromycin, and metronidazole). Of the 11 patients who did not receive any antibiotics, 1 patient had an amniotic fluid culture positive for *Ureaplasma urealyticum,* and antibiotics were not administered because of rapid progression of preterm labor to delivery.

The lack of antibiotic administration in the other 10 patients occurred for the following reasons: 1) intra-amniotic infection/inflammation was not suspected because the patients had a low amniotic fluid white blood cell count when the MMP-8 rapid test was not available (n = 4); however, intra-amniotic inflammation was diagnosed by elevated concentrations of interleukin-6 retrospectively; 2) the managing clinician preferred to rely on the results of the amniotic fluid white blood cell count rather than on those of the rapid MMP-8 test (n = 2); 3) rapid progression of labor (n = 2); 4) declined antibiotic treatment (n = 1); and 5)

**FIGURE 1**
**Flow diagram of the study population**

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transfer to another hospital because of unavailability of a neonatal intensive care bed (n = 1).

Table 1 compares the characteristics and outcomes of patients who received the antibiotic regimen to those of patients who did not. There were no significant differences between the study groups in maternal age, cerclage use, gestational age at amniocentesis, interleukin-6 concentration, frequency of a positive amniotic fluid culture, use of tocolytics and antenatal corticosteroids, delivery within 7 and 14 days of amniocentesis, delivery at <30, <34 and ≥37 weeks, clinical and acute histologic chorioamnionitis, and funisitis ($P > 0.1$ for each). Patients who received the antibiotic regimen had a significantly higher median amniotic fluid white blood cell count (79 cells/mm$^3$ vs 3 cells/mm$^3$), longer median amniocentesis-to-delivery interval (11.4 days vs 3.1 days), and lower rate of delivery within 4 weeks of amniocentesis (58% vs 91.7%) than those in whom the antibiotic regimen was not used ($P < .05$ for each).

Of 50 patients treated with the antibiotic regimen, 29 remained undelivered for at least 1 week (Figure 1). Microorganisms identified in the amniotic fluid of 29 patients undelivered for at least 1 week included *Ureaplasma urealyticum* (n = 4) and *Mycoplasma hominis* (n = 1). One patient had a mixed infection of *Ureaplasma urealyticum* and *Mycoplasma hominis*. Microorganisms identified in the amniotic fluid of 21 patients who delivered within 7 days of amniocentesis included *Ureaplasma urealyticum* (n = 4), and 1 isolate each of *Mycoplasma hominis*, *Streptococcus anginosus,* and *Gardnerella vaginalis*. One patient had a mixed infection of *Ureaplasma urealyticum* and *Mycoplasma hominis*.

Table 2 compares the characteristics and outcomes of patients who delivered within 7 days of amniocentesis and those who were undelivered for at least 7 days. There were no significant differences in the median gestational age at amniocentesis and the frequency of a positive amniotic fluid culture for microorganisms between the 2 groups ($P > .1$ for each). Patients who remained undelivered for at least 1 week had a significantly lower median concentration of amniotic fluid interleukin-6 and white blood cell count than those who delivered before 1 week ($P < .005$ for both).

Of 29 patients undelivered for ≥7 days, 10 did not have a follow-up amniocentesis (5 declined the procedure, 2 had severe oligohydramnios due to rupture of membranes, 2 were transferred to another hospital, and for 1 patient, the treating physician did not recommend the procedure). The remaining 19 patients had a follow-up amniocentesis to determine whether (1) intra-amniotic infection had been eradicated, (2) intra-amniotic inflammation was being treated, and (3) antibiotic treatment should be continued or stopped. Generally, antibiotics were discontinued if patients had a negative MMP-8 test result or if the amniotic fluid white blood cell count became normal. However, the final decision was made by the attending obstetrician.

There were no significant differences in the median gestational age at amniocentesis, amniotic fluid interleukin-6 concentration and white blood cell count, and the frequency of a positive amniotic fluid culture between patients who were undelivered for at least 1 week and had a follow-up amniocentesis and those who had not ($P > .1$ for each). Patients who did not have a follow-up amniocentesis delivered significantly earlier than those who had a follow-up amniocentesis (27.3 weeks [interquartile range, 25.0–33.9

**TABLE 1**
**Clinical characteristics and outcomes of patients who did vs did not use the regimen of antibiotics consisting of ceftriaxone, clarithromycin, and metronidazole**

| | Use of ceftriaxone, clarithromycin, and metronidazole (n = 50) | No antibiotics or use of other antibiotics (n = 12) | *P* value |
|---|---|---|---|
| Maternal age (y) | 31 (29–34) | 34 (31–36) | .12 |
| Nulliparity (%) | 62.0% (31/50) | 25.0% (3/12) | .027 |
| Cerclage before onset of preterm labor | 12.0% (6/50) | 8.3% (1/12) | .99 |
| Cerclage after onset of preterm labor and preterm labor stopped | 4.0% (2/50) | 8.3% (1/12) | .48 |
| Initial amniocentesis | | | |
| Gestational age at amniocentesis (wk) | 25.4 (22.1–27.5) | 25.7 (22.6–28.6) | .63 |
| Positive amniotic fluid culture (%) | 20.0% (10/50) | 9.1% (1/12) | .68 |
| Positive amniotic fluid PCR for *Ureaplasma spp.* | 21.2% (7/33) | 11.1% (1/9) | .66 |
| Amniotic fluid WBC count (cells/mm$^3$) | 79 (2–860) | 3 (0–65) | .048 |
| Amniotic fluid WBC count (≥19 cells/mm$^3$) | 58.3% (28/48) | 25.0% (3/12) | .054 |
| Amniotic fluid interleukin-6 (ng/mL) | 18.2 (4.1–43.0) | 7.8 (3.2–16.9) | .12 |
| Amniotic fluid interleukin-6 (>2.6 ng/mL) | 100% (49/49) | 100% (12/12) | >.99 |
| Cervical dilatation >3 cm (%) | 10.0% (5/50) | 16.7% (2/12) | .61 |
| Use of tocolytics (%) | 98.0% (49/50) | 91.7% (11/12) | .35 |
| Antenatal corticosteroids administration (%) | 62.0% (31/50) | 58.3% (7/12) | >.99 |
| Gestational age at delivery (wk) | 28.9 (25.5–33.9) | 27.3 (23.4–31.7) | .29 |
| Interval between amniocentesis to delivery (days) | 11.4 (2.8–57.0) | 3.1 (0.3–17.8) | .04[b] |
| Delivery within 7 days of amniocentesis | 42.0% (21/50) | 58.3% (7/12) | .35 |
| Delivery within 14 days of amniocentesis | 52.0% (26/50) | 67% (8/12) | .52 |
| Delivery within 4 wk of amniocentesis | 58.0% (29/50) | 91.7% (11/12) | .042 |
| Delivery before 30 wk[a] | 57.4% (27/47) | 81.8% (9/11) | .18 |
| Delivery before 34 wk | 76.0% (38/50) | 91.7% (11/12) | .43 |
| Delivery at term (≥37 wk) | 8.0% (4/50) | 8.3% (1/12) | >.99 |
| Clinical chorioamnionitis | 12.0% (6/50) | 0% (0/12) | .59 |
| Acute histologic chorioamnionitis | 69.2% (27/39) | 88.9% (8/9) | .41 |
| Funisitis | 30.8% (12/39) | 22.2% (2/9) | >.99 |

Data are median (interquartile range) or percentage (n/N).

*PCR*, polymerase chain reaction; *WBC*, white blood cell.

[a] Patients who underwent amniocentesis at or beyond 30 wk were excluded from the analysis; [b] The amniocentesis-to-delivery interval was compared by using the generalized Wilcoxon test for survival analysis.

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weeks] vs 34.1 weeks [interquartile range, 31.7–35.6 weeks]; $P < .05$).

## Treatment success with antibiotics in this study

Of the 19 patients who had a follow-up amniocentesis, intra-amniotic inflammation was successfully resolved in 15, and intra-amniotic infection was eradicated in 3 (1 patient with a positive culture and a positive PCR result *for Ureaplasma spp.*, and 2 with a negative culture but a positive PCR result for *Ureaplasma spp.*). All patients with intra-amniotic infection also had intra-amniotic inflammation.

Microbiologic or biochemical evidence of successful treatment was demonstrated in 79% (15/19). One patient who did not have confirmation of eradication of intra-amniotic infection/inflammation at the follow-up amniocentesis delivered at term. None of the 10 patients who did not have a follow-up amniocentesis delivered at term. Thus, treatment success of antibiotics (defined as eradication of intra-amniotic infection/inflammation or delivery ≥37 weeks of gestation) occurred in 84% of patients (16/19)

TABLE 2
**Characteristics and outcomes of patients delivered within 7 days of amniocentesis and those who were undelivered for at least 7 days**

| | Delivery before 1 week (n = 21) | Undelivered for ≥ 1 week (n = 29) | *P* value |
|---|---|---|---|
| Maternal age (y) | 33 (30–36) | 30 (28–33) | .07 |
| Nulliparity (%) | 47.6% (10/21) | 72.4% (21/29) | .09 |
| Initial amniocentesis | | | |
| Gestational age at amniocentesis (wk) | 26.4 (22.6–28.4) | 24.3 (21.9–26.9) | .13 |
| Positive amniotic fluid culture (%) | 28.6% (6/21) | 13.8% (4/29) | .29 |
| Positive amniotic fluid PCR for *Ureaplasma spp.* | 14.3% (2/14) | 26.3% (5/19) | .67 |
| Amniotic fluid WBC count (cells/mm$^3$) | 725 (94 to >1000) | 5 (1–100) | .002 |
| Amniotic fluid WBC count (≥ 19 cells/mm$^3$) | 81.0% (17/21) | 40.7% (11/27) | .008 |
| Amniotic fluid interleukin-6 (ng/mL) | 28.2 (14.0–46.5) | 10.3 (3.4–21.8) | .001 |
| Amniotic fluid interleukin-6 (>2.6 ng/mL) | 100% (21/21) | 100% (28/28) | >.99 |
| Cervical dilatation > 3 cm (%) | 14.3% (3/21) | 6.9% (2/29) | .64 |
| Use of tocolytics (%) | 95.2% (20/21) | 100% (29/29) | .42 |
| Antenatal corticosteroids administration (%) | 52.4% (11/21) | 69.0% (20/29) | .26 |
| Gestational age at delivery (wk) | 26.6 (22.9–28.7) | 33.1 (27.3–34.9) | <.001 |
| Delivery within 14 days of amniocentesis | 100% (21/21) | 17.2% (5/29) | <.001 |
| Delivery within 4 wk of amniocentesis | 100% (21/21) | 27.6% (8/29) | <.001 |
| Delivery before 30 wk[a] | 94.7% (18/19) | 32.1% (9/28) | <.001 |
| Delivery before 34 wk | 100% (21/21) | 58.6% (17/29) | .001 |
| Delivery at term (≥37 wk) | 0% (0/21) | 13.8% (4/29) | .13 |
| Clinical chorioamnionitis | 23.8% (5/21) | 3.4% (1/29) | .07 |
| Acute histologic chorioamnionitis | 81.3% (13/16) | 60.9% (14/12) | .29 |
| Funisitis | 37.5% (6/16) | 26.1% (6/23) | .50 |

Data are median (interquartile range) or percentage (n/N).

*PCR*, polymerase chain reaction; *WBC*, white blood cell.

[a] Patients who underwent amniocentesis at or beyond 30 weeks were excluded from the analysis.

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who had a follow-up amniocentesis and was possible in at least 32% of patients (16/50) with intra-amniotic infection/inflammation who received the antimicrobial agents.

### Clinical outcome of patients treated with antimicrobial agents who had a follow-up amniocentesis

Table 3 shows the details and Table 4 summarizes the characteristics and outcomes of 19 patients who were treated with the antimicrobial agents and had a follow-up amniocentesis. A detailed description of each patient can be found in Supplementary Material S2.

## Comment

### Principal findings of the study

The principal findings of the study were as follows: 1) antibiotics were effective in treating intra-amniotic infection/inflammation in women with preterm labor and intact membranes, as demonstrated by analysis of amniotic fluid obtained before and after antibiotics were administered; 2) resolution of intra-amniotic infection/inflammation was objectively demonstrated in 79% of patients (15/19) who received the antimicrobial agents and had a follow-up amniocentesis; 3) the overall treatment success (defined as resolution of intra-amniotic inflammation or infection, or delivery ≥37 weeks) rate among patients who underwent follow-up amniocentesis was 84% (16/19). The overall success rate among all women with intra-amniotic infection/inflammation who received the antimicrobial agents was 32% (16/50).

### Prevalence and clinical importance of intra-amniotic infection/inflammation in patients with preterm labor and intact membranes

The frequency of an amniotic fluid culture positive for microorganisms in patients presenting with an episode of

**TABLE 3**
**Details of presentations of initial amniocentesis, and outcomes of patients who were treated with antibiotics (ceftriaxone, clarithromycin, and metronidazole) and had follow-up amniocentesis**

| Case no. | Gestational age, wk | | Initial amniocentesis | | | | | Interval between initial amniocentesis and resolution (days)[a] | Birth weight (gm) | Steroid for fetal lung maturity (wk) | Acute histologic chorioamnionitis/ funisitis | Neonatal outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amnio-centesis | Delivery | Culture | PCR for *Ureaplasma spp.* | Interleukin-6 (ng/mL) | WBC count (cells/mm$^3$) | MMP-8 rapid test | | | | | |
| Group A: | | | Resolution confirmed and delivered after 34 weeks of gestation | | | | | | | | | |
| 1 | 25.1 | 35.6 | Neg | Pos | 5.0 | 11 | Pos | 28 | Unknown | 25.3 | N/A | Survival without morbidity |
| 2 | 22.7 | 38.3 | Neg | N/A | 33.4 | 100 | Pos | 14 | 2620 | 22.9 | —/— | Survival without morbidity |
| 3 | 26.0 | 40.1 | Neg | N/A | 2.7 | 5 | Pos | 7 | 3860 | 26.0 | —/— | Survival without morbidity |
| 4 | 29.6 | 34.1 | Neg | N/A | 2.7 | 0 | Pos | 7 | 2200 | 33.3 | —/— | Survival without morbidity |
| 5 | 22.9 | 38.0 | Neg | Neg | 2.8 | 5 | Pos | 38 | 2850 | | —/— | Survival without morbidity |
| 6 | 26.7 | 34.7 | Neg | N/A | 4.1 | 0 | Pos | 7 | 2610 | 26.3 | +/— | Survival without morbidity |
| 7 | 27.6 | 35.4 | Neg | Neg | 4.0 | 1 | Pos | 10 | unknown | | N/A | Survival without morbidity |
| 8 | 20.9 | 34.7 | Neg | Neg | 3.3 | 1 | Pos | 67 | 2040 | | +/— | Survival without morbidity |
| 9 | 25.6 | 35.0 | Neg | Neg | 3.6 | 25 | N/A | 48 | 2290 | | —/— | Survival without morbidity |
| Group B: | | | Resolution confirmed but delivered before 34 weeks of gestation | | | | | | | | | |
| 10 | 28.4 | 31.7 | Neg | Pos | 42.6 | 720 | N/A | 13 | 1840 | 28.4 | +/— | Survival without morbidity |
| 11 | 24.0 | 32.6 | Neg | Neg | 2.9 | 2 | N/A | 30 | 1990 | 29.7 | +/— | Survival without morbidity |
| 12 | 25.4 | 30.4 | Neg | N/A | 2.6 | 0 | Pos | 15 | 1400 | 24.0 | +/+ | Survival without morbidity |
| 13 | 21.0 | 33.3 | Neg | Neg | 51.5 | 105 | Pos | 22 | 1800 | 27.9 | —/— | Survived and diagnosed as BPD |
| 14 | 21.0 | 29.6 | Pos | Pos | 4.8 | 0 | N/A | 30 | 1640 | 29.4 | +/— | Survival without morbidity |
| 15 | 20.1 | 25.4 | Neg | Neg | 11.1 | 16 | Pos | 15 | 700 | 25.4 | +/+ | Shortly died (5 hours from birth) |
| Group C: | | | Resolution not confirmed but delivered after 37 weeks of gestation | | | | | | | | | |
| 16 | 21.6 | 38.0 | Neg | Neg | 19.4 | 2 | N/A | — | 2760 | | —/— | Survival without morbidity |
| Group D: | | | Resolution not confirmed and delivered before 34 weeks of gestation | | | | | | | | | |
| 17 | 31.4 | 32.9 | Neg | Neg | 22.0 | 100 | Pos | — | 1710 | 30.9 | +/+ | Survival without morbidity |

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(continued)

**TABLE 3**
**Details of presentations of initial amniocentesis, and outcomes of patients who were treated with antibiotics (ceftriaxone, clarithromycin, and metronidazole) and had follow-up amniocentesis** *(continued)*

| Case no. | Gestational age, wk | | Initial amniocentesis | | | | | Interval between initial amniocentesis and resolution (days)[a] | Birth weight (gm) | Steroid for fetal lung maturity (wk) | Acute histologic chorioamnionitis/ funisitis | Neonatal outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amnio-centesis | Delivery | Culture | PCR for *Ureaplasma spp.* | Interleukin-6 (ng/mL) | WBC count (cells/mm$^3$) | MMP-8 rapid test | | | | | |
| 18 | 26.4 | 32.6 | Pos | N/A | 18.2 | 50 | Pos | — | 2060 | | +/— | Survival without morbidity |
| 19 | 22.1 | 23.6 | Neg | N/A | 23.8 | 54 | Pos | — | 620 | | +/— | Survived and diagnosed as RDS, BPD, IVH, PVL |

*BPD*, bronchopulmonary dysplasia; *IVH*, intraventricular hemorrhage; MMP-8, matrix metalloproteinase-8; *N/A*, not assessed; *Neg*, negative result; *PCR*, polymerase chain reaction; *Pos*, positive result; *RDS*, respiratory distress syndrome; *PVL*, periventricular leukomalacia.

[a] The first amniocentesis without evidence of intra-amniotic infection/inflammation.

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preterm labor and intact membranes is approximately 10%,[3,6,12,14,33,139] and these patients are more likely to develop maternal complications such as clinical chorioamnionitis[3] and pulmonary edema while receiving tocolytics,[68,69] and to deliver a preterm neonate shortly after admission.[3,14,16,33,140,141] In addition, patients with intra-amniotic infection are more likely to show evidence of histologic chorioamnionitis (a maternal host response) or funisitis/ chorionic vasculitis (pathologic hallmarks of the fetal inflammatory response syndrome [FIRS]).[59,124,131–133] One of every 4 preterm neonates is born to a mother with microorganisms in the amniotic cavity.[3,11,12,22,142–146]

When microorganisms invade the human fetus, a systemic inflammatory response can be elicited, and this condition is referred to as FIRS (diagnosed by an elevated umbilical cord blood plasma interleukin-6 concentration). This condition is associated with a higher rate of neonatal complications[147,148] because, before birth, these fetuses have multisystemic involvement or dysfunction.[149] Examples include leukocyte activation,[150] leukocytosis,[151] adrenal gland hyperactivity (elevated concentrations of cortisol in peripheral blood),[152] cardiac dysfunction,[153,154] and increased concentrations of matrix-degrading enzymes in the amniotic fluid and fetal blood.[155–160] FIRS is a risk factor for neonatal morbidity as well as long-term complications such as cerebral palsy[54,161] and chronic lung disease.[162–165]

In summary, a strong body of evidence indicates that fetal exposure to microorganisms or intra-amniotic inflammation is associated with adverse outcome.[1,5,6,8,9,39,55,64,71,131,161,166–170] Despite this overwhelming evidence, obstetricians in practice do not routinely ascertain whether patients with preterm labor have intra-amniotic infection/ inflammation. The reason is 2-fold: first, the best method to determine the presence of intra-amniotic infection/inflammation is analysis of amniotic fluid, which requires an invasive procedure (amniocentesis); second, the evidence that treatment with antimicrobial agents can eradicate intra-amniotic infection has been based on case reports.

TABLE 4
**Characteristics and outcomes of 19 patients who were treated with antibiotics (ceftriaxone, clarithromycin, and metronidazole) and had follow-up amniocentesis**

| | Resolution of intra-amniotic inflammation | | | |
|---|---|---|---|---|
| | Confirmed (n = 15) | | Not confirmed (n = 4) | |
| | Group A: Delivery at or after 34 wk (n = 9) | Group B: Delivery before 34 wk (n = 6) | Group C: Delivery at or after 34 wk (n = 1) | Group D: Delivery before 34 wk (n = 3) |
| Nulliparity | 88.9% (8/9) | 66.7% (4/6) | 0% (0/1) | 66.6% (2/3) |
| History of preterm delivery | 0% (0/9) | 16.7% (1/6) | 0% (0/1) | 0% (0/3) |
| Progesterone treatment | 0 | 0 | 0 | 0 |
| Cerclage before onset of preterm labor | 11.1% (1/9) | 16.7% (1/6) | 0% (0/1) | 33.3% (1/3) |
| Cerclage after onset of preterm labor and labor stopped | 0% (0/9) | 16.7% (1/6) | 0% (0/1) | 0% (0/1) |
| Initial amniocentesis | | | | |
| Gestational age at amniocentesis | 25.6 (22.9–26.7) | 22.5 (21.0–25.4) | 21.6 | 26.4 (22.1–31.4) |
| Positive amniotic fluid culture (%) | 0% (0/9) | 16.7% (1/6) | 0% (0/1) | 33.3% (1/3) |
| Positive amniotic fluid PCR for *Ureaplasma spp.* | 20% (1/5) | 40% (2/5) | 0% (0/1) | 0% (0/1) |
| Amniotic fluid WBC count (cells/mm$^3$) | 5 (1–11) | 16 (2–105) | 2 | 54 (50–100)[a] |
| Positive MMP-8 rapid test | 87.5% (7/8) | 100% (3/3) | 0% (0/0) | 100% (3/3) |
| Amniotic fluid interleukin-6 (ng/mL) | 3.56 (2.79–4.09) | 7.02 (2.6–42.6) | 19.40 | 21.97 (18.22–23.84) |
| Days from initial amniocentesis to resolution | 14 (7–38) | 18 (13–22) | N/A | N/A |
| Duration of new antibiotic regimen use (days)[b] | 21 (14–25) | 25.5 (21–31) | 14 | 10 (10–33) |
| Number of amniocenteses | 4 (4–4) | 3.5 (3–4) | 2 | 2 (2–3) |
| Gestational age at delivery (wk) | 35.4 (34.7–38.0) | 31.1 (29.6–32.6)[a] | 38.0 | 32.6 (23.6–32.9)[a] |
| Days from initial amniocentesis to delivery | 73 (56–103) | 48 (32–66) | 115 | 10 (10–43)[a] |
| Delivery within 14 days of amniocentesis | 0% (0/9) | 0% (0/6) | 0% (0/1) | 66.7% (2/3)[a] |
| Delivery within 4 wk of amniocentesis | 0% (0/9) | 16.7% (1/6) | 0% (0/1) | 66.7% (2/3)[a] |
| Delivery before 30 wk | 0% (0/9) | 33.3% (2/6) | 0% (0/1) | 50.0% (1/2)[c] |
| Delivery before 34 wk | 0% (0/9) | 100% (6/6)[a] | 0% (0/1) | 100% (3/3)[a] |
| Delivery at term (≥37 wk) | 66.7% (6/9) | 0% (0/6) | 100% (1/1) | 0% (0/3) |
| Birth weight (g) | 2610 (2200–2850) | 1720 (1225–1878)[a] | 2760 | 1710 (620–2060)[a] |
| Clinical chorioamnionitis | 0% (0/9) | 0% (0/6) | 0% (0/1) | 0% (0/3) |
| Acute histologic chorioamnionitis | 28.6% (2/7) | 83.3% (5/6) | 0% (0/1) | 100% (3/3) |
| Funisitis | 0% (0/7) | 50% (3/6) | 0% (0/1) | 33.3% (1/3) |
| Significant neonatal morbidity | 0% (0/9) | 33.3% (2/6) | 0% (0/1) | 33.3% (1/3) |

Data are median (interquartile ranges for group A and B, range for group D) or percentage (n/N).

*MMP-8,* matrix metalloproteinase–8; *PCR,* polymerase chain reaction; *WBC,* white blood cell.

[a] $P < .05$ compared to Group A; [b] Some patients restarted antibiotic administration because they developed preterm rupture of membranes or preterm labor and intra-amniotic infection/inflammation after the discontinuation of antibiotics as intra-amniotic infection/inflammation resolved and preterm labor stopped. This duration was not included in this analysis; [c] Patient case who underwent amniocentesis at or beyond 30 weeks was excluded from the analysis.

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Therefore, in practice, clinicians rely on signs and symptoms of clinical chorioamnionitis (eg, fever, maternal tachycardia, etc) to exclude intra-amniotic infection. However, it is now well established that these clinical signs are both insensitive and nonspecific for the identification of intra-amniotic infection in both preterm[6,33,121,125,171] and term[136,137,172–176] gestations. This is also the case for maternal circulating white blood cell count and other biomarkers of the acute phase response (such as serum C-reactive protein).[18,121,167,177,178] One argument against the analysis of amniotic

fluid has been that results were not immediately available to affect patient management, as culture for microorganisms may take several days. However, rapid tests are now available for the diagnosis of intra-amniotic inflammation (such as amniotic fluid white blood cell count, glucose, amniotic fluid MMP-8, or interleukin-6, among others),[4,19,38,53,60,126,127,167,176,177,179–184] and for the diagnosis of infection using PCR.[8,23,123,140,185–188]

## Antibiotic administration to patients in preterm labor with intact membranes

The evidence that intra-amniotic infection is causally linked to spontaneous preterm labor and delivery coalesced in the 1980s;[2,3,13,14,144,189,190] this led to the conduct of several randomized clinical trials in which patients with an episode of preterm labor were allocated to antimicrobial agents vs placebo or no treatment.[73,74,77–80,82,191–194] Although the initial trials reported pregnancy prolongation[75,77,191] and, in some cases, a lower frequency in the rate of preterm delivery,[73,77] these findings were not supported by subsequent clinical trials[78,81] or systematic reviews and meta-analyses.[195-197] This led professional organizations, including the American College of Obstetricians and Gynecologists[198] and the National Institute for Clinical Excellence,[199] to recommend that antibiotics not be administered to patients with preterm labor and intact membranes, with the objective of prolonging pregnancy or reducing the rate of preterm birth. Antibiotics have been recommended in the context of preterm labor with intact membranes when delivery is impending and the patient is a carrier of GBS or *Streptococcus agalactiae*.[85,86,199,200]

## Why are antibiotics considered ineffective in prolonging pregnancy and preventing preterm delivery in patients with preterm labor and intact membranes?

Preterm labor is a syndrome defined by the presence of increased uterine contractility, cervical dilatation, and decidual membrane activation, each caused by multiple pathologic processes.[15,201,202] Intra-amniotic infection is only 1 of the potential mechanisms of disease responsible for this syndrome. If the frequency of intra-amniotic infection is only 10%,[22] then antimicrobial agents can be effective only in that small fraction of patients.[26]

The ORACLE II trial randomized 6295 women with an episode of preterm labor with intact membranes to placebo or antibiotics; these patients did not have clinical evidence of infection, and amniocenteses were not performed to diagnose intra-amniotic infection.[78] Therefore, 90% of patients enrolled in the ORACLE II trial could not have benefitted from antibiotic administration, and the negative results are not surprising. The same applies to all other randomized clinical trials of antibiotics in patients with preterm labor and intact membranes.[74,79–82] However, these results should not be interpreted as indicating that antibiotics are ineffective when administered to the "right" patients, namely, those who have proven intra-amniotic infection.

## Experimental evidence that anti-microbial agents can eradicate intrauterine infection and prolong pregnancy

McDuffie et al reported that, in pregnant rabbits, antibiotic administration (ampicillin and sulbactam) at or before inoculation with *Escherichia coli* led to fewer preterm deliveries and more live pups than in rabbits whose treatment was delayed for more than 4 hours.[203] Subsequently, Fidel et al, using the same experimental model, showed that antibiotic administration within 12 hours of inoculation—but not after 18 hours—increased the duration of pregnancy and reduced perinatal mortality.[204] Collectively, these results suggest that antibiotics can be beneficial in cases of intrauterine infection.

These observations were subsequently confirmed in non-human primates. Investigators at the Oregon Primate Center administered GBS to pregnant rhesus monkeys (*Macaca mulatta*) on day 130 of gestation (term, 167 days), and observed an increase in uterine contractility at a median of 28 hours (range, 14–40 hours) after inoculation.[5] This model of intra-amniotic infection has many features in common with intra-amniotic infection in humans. Importantly, the onset of contractions was preceded by an increase in amniotic fluid concentrations of proinflammatory cytokines (interleukin-1$\beta$, tumor necrosis factor-$\alpha$, interleukin-6) and prostaglandins (E2 and F2a). None of the animals became febrile or had leukocytosis; yet, all delivered preterm. Subsequent studies demonstrated that dexamethasone,[205] indomethacin,[206] and interleukin-10 blocked interleukin-1-induced uterine contractility (a model of intra-amniotic inflammation), suggesting a role for anti-inflammatory agents in the treatment of inflammation-induced preterm labor.[207]

## Antibiotics used in this study to treat intra-amniotic infection/inflammation

An important principle in the treatment of infectious diseases is that antibiotic selection should be tailored to the microorganisms causing the infection. The rationale for the antibiotic regimen used in our study was described in previous studies of patients with preterm PROM.[118,120] Briefly, 2 macrolides, erythromycin and azithromycin, have each been used to treat intra-amniotic infection in women, and there is experimental evidence in non-human primates that azithromycin can eradicate *Ureaplasma* spp. from the amniotic cavity and can reduce fetal lung injury.[208,209] Clarithromycin was chosen at our institution because it has a much higher rate of transplacental passage than erythromycin or azithromycin, and this agent is effective against *Ureaplasma* spp. the most common microorganism identified in the amniotic fluid of patients at risk for preterm delivery.[210] Ceftriaxone was included because of its enhanced coverage of aerobic bacteria and high rate of transplacental passage.[211,212] Metronidazole was selected because anaerobic organisms are frequently present in the amniotic cavity, and this drug provides optimal coverage for these microorganisms. We reported

that, in patients with preterm PROM, this antibiotic combination eradicated intra-amniotic infection and/or inflammation in at least 33% of patients, as demonstrated by repeat analysis of amniotic fluid.[118] Whether other antimicrobial combinations can achieve the same result would need to be determined.

## Evidence that intra-amniotic infection can be treated

Intra-amniotic infection has been successfully treated in patients with a sonographic short cervix without clinical manifestations of infection (fever, uterine tenderness, etc).[117] Eradication of intra-amniotic infection has also been reported in cases of preterm PROM[118,213] and preterm labor.[214,215] Whether this approach is effective in patients with preterm labor with intact membranes had not been studied until recently.[119] In patients with preterm labor and proven intra-amniotic infection, there was a shorter diagnosis-to-delivery interval.[3,6–8,22,33,53] Indeed, it was generally believed that once patients presented with preterm labor, an intra-amniotic "cytokine storm" would inevitably lead to preterm delivery.

The results reported herein represent the first objective confirmation, in a case series, that antibiotic treatment can eradicate intra-amniotic infection in preterm labor with intact membranes. This was demonstrated in 3 patients: the first patient had microbial invasion of the amniotic cavity with *Ureaplasma* spp. detected by culture; the other 2 patients had microbial nucleic acids detected by PCR.

In all 3 cases, repeat amniocentesis yielded a negative amniotic fluid culture and negative PCR for microorganisms. Details of each specific case are illustrated in Table 3 (see cases 1, 10, and 14). It is interesting that in case 14, the first amniocentesis at 21 weeks was positive for *Ureaplasma* spp. and showed elevated interleukin-6 (4.8 ng/mL). Antibiotic treatment eradicated both the microorganisms and evidence of the intra-amniotic inflammatory process (interleukin-6: 1.93 ng/mL). The treating physician elected to continue oral clarithromycin. Four weeks after successful treatment, the patient was suspected to have rupture of membranes, and the amniotic fluid became positive for *Morganella morganii,* a Gram-negative bacilli frequently implicated in nosocomial infections.[216] Intra-amniotic inflammation (interleukin-6: 6.89 ng/mL) recurred, labor progressed, and the patient delivered at 29.4 weeks a 1640-g neonate who had no major complications. This case indicates that patients with an intra-amniotic infection may be susceptible to recurring infection with other microorganisms. Whether this indicates a deficit in host defense or an opportunistic infection during the course of antimicrobial therapy is unclear. Chorioamnionitis caused by *Morganella morganii* has been reported in an immunocompetent patient.[217] Recent evidence derived from whole exome sequencing indicates that some patients may have deleterious mutations in genes encoding for proteins implicated in host defense against microbial invasion.[218–221] There is evidence that acute chorioamnionitis may be recurrent in successive pregnancies;[222] therefore, the predisposition to intra-amniotic infection may have a genetic basis.[29,223–227]

Recently, a group of investigators reported that antimicrobial agents in patients with intra-amniotic infection may result in prolongation of pregnancy and a decreased rate of admission to the neonatal intensive care unit without a change in perinatal morbidity.[119] No follow-up amniocenteses were performed in that study; therefore, there was no objective evidence to determine whether antimicrobial therapy was effective in treating intra-amniotic infection/inflammation. Nonetheless, the reports of such studies represent indirect evidence consistent with our findings.

## Successful treatment of intra-amniotic inflammation in preterm labor and intact membranes with antibiotics

Intra-amniotic inflammation in the absence of demonstrable microorganisms is more frequent than intra-amniotic infection in patients with preterm labor and intact membranes,[24,26,33,36,39] a sonographic short cervix,[28,117] and even preterm PROM.[27,71] This type of intra-amniotic inflammation may be caused either by microorganisms that escaped detection[27,28] or by danger signals, or alarmins,[30–32,91,228,229] that are released by cells under stress or during the course of cell death, such as necrosis.[230–233] Examples of danger signals include high mobility group box 1 (HMGB1), S100 calcium-binding protein B (S100B), and interleukin-1$\alpha$, which can induce preterm labor by the activation of the inflammasome.[31,90,92,234–238]

The treatment of sterile inflammation is a major challenge in medicine. The conventional approach is to use anti-inflammatory agents, such as glucocorticoids[239,240] or nonsteroidal anti-inflammatory drugs.[241,242] In some cases, treatment is possible with a specific agent that decreases the concentration of the danger signal, such as allopurinol, to decrease the concentration of uric acid in gout. However, in the case of intra-amniotic inflammation without demonstrable microorganisms, the optimal treatment is uncertain. Preliminary evidence from our laboratory suggests that inhibitors of the inflammasome may have therapeutic benefits in preventing preterm labor induced by specific danger signals such as S100B.[236]

How can antibiotics be effective in cases of intra-amniotic inflammation without demonstrable microorganisms? The antibiotic combination used at the Seoul National University Hospital included clarithromycin, which has been shown to have immunomodulatory properties and specifically inhibits activator protein 1 (AP-1) and nuclear factor-kappa B (NF-$\kappa$B), 2 transcription factors that induce production of proinflammatory cytokines and effectively act as anti-inflammatory agents.[243,244] We have previously shown that NF$\kappa$B is upregulated by interleukin-1B.[245–249]

Our study shows that intra-amniotic infection/inflammation was successfully treated in 84% of cases (16/19) in which follow-up amniocentesis was performed. It is unlikely that this therapeutic success can be attributed to

glucocorticoids, because these agents were not used in 31% of patients (5/16) in whom intra-amniotic inflammation improved.

The results of the current study are consistent with our previous observations in the context of preterm PROM. The antimicrobial agents used in this study were able to treat and prevent intra-amniotic infection/inflammation, prolong the latency period, reduce acute histologic chorioamnionitis and funisitis, and improve neonatal outcomes in patients with preterm PROM.[120]

## Strengths and limitations

Strengths of the study include the following: first, this is the first case series in which women with intra-amniotic infection/inflammation were treated with antibiotics and monitored with serial amniocentesis to determine whether there was therapeutic success in patients with preterm labor and intact membranes. Second, assessment of intra-amniotic infection/inflammation was performed by analysis of amniotic fluid using the amniotic fluid white blood cell count or a rapid test for MMP-8. Third, the retrospective diagnosis of intra-amniotic inflammation was performed using amniotic fluid concentrations of interleukin-6, which has been demonstrated to be a reliable marker, widely used in many reports to diagnose this condition. Finally, this study used serial evaluation of amniotic fluid. This is the only objective method to ascertain whether there is therapeutic efficacy.

Limitations of this study include its observational nature. This is not a randomized clinical trial in which there was a placebo arm. However, clinicians in our institution are unwilling to randomize patients with intra-amniotic infection/inflammation to placebo because such patients are at increased risk for clinical chorioamnionitis, maternal sepsis, and neonatal complications, such as early neonatal sepsis, among others. In a previous observational study, we reported that 91% of patients with intra-amniotic inflammation delivered within 1 week of amniocentesis.[33] In contrast, in the current study, only 42% (21/50) delivered within 1 week: this is also indirect evidence of efficacy.

We have grouped together patients with intra-amniotic infection and intra-amniotic inflammation without demonstrable microorganisms. A limitation of our study is that there were only 3 patients with intra-amniotic infection who were successfully treated and that most of the patients had intra-amniotic inflammation without microorganisms detectable with the methods used in our institution. It is possible that more organisms could have been detected by using assays for the conserved region of the microbial genome or sequencing of microbial cell-free DNA.[250–252] Further studies using molecular microbiologic methods are required to address this issue.

Another potential limitation to our interpretation of the results of this case series is that we used a definition of "success" in delivery $\geq$37 weeks of gestation rather than $\geq$34 weeks. This may be a very stringent criterion to assess the prognosis of a patient with preterm labor and intra-amniotic infection/inflammation; however, use of this definition strengthens the evidence of the effectiveness of antibiotics, as patients may benefit from antimicrobial agents without delivering at term (eg, delivery at 36 weeks). Indeed, we performed a sensitivity analysis, and if treatment success was defined as eradication of intra-amniotic infection/inflammation or delivery $\geq$32 weeks of gestation, the overall efficacy would be 44% (22/50).

## Conclusion

The administration of antibiotics to patients with preterm labor and intact membranes with proven intra-amniotic infection/inflammation is associated with eradication of infection and inflammation in a subset of patients. ■


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## Supplementary Material S1

### Diagnosis of neonatal morbidity

Respiratory distress syndrome (RDS) was diagnosed as the presence of respiratory distress, an increased oxygen requirement ($FiO_2$ >0.4), and diagnostic radiological and laboratory findings in the absence of evidence of any other causes of respiratory distress.[1] Bronchopulmonary dysplasia (BPD) was diagnosed using the criteria of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Workshop definition, namely, treatment with oxygen >21% for at least 28 days, and also diagnosed in the presence of typical findings at autopsy.[2] Congenital neonatal sepsis was diagnosed in the presence of a positive blood culture result within 72 hours of delivery.[3] Necrotizing enterocolitis (NEC) was diagnosed in the presence of abdominal distension and feeding intolerance (vomiting or increased gastric residual) for at least 24 hours with clear evidence of intramural air, perforation, and meconium plug syndrome by radiological examination, or definite surgical or autopsy findings of NEC.[4] Intraventricular hemorrhage (IVH) was diagnosed by ultrasonographic examination or magnetic resonance imaging (MRI) of the neonatal head (≥ Grade II).[5] Periventricular leukomalacia (PVL) was diagnosed by the presence of cystic lesions within the periventricular white matter by ultrasonographic examination or MRI. Significant neonatal morbidity was defined when 1 or more neonatal outcomes including RDS, BPD, congenital neonatal sepsis, NEC, and IVH were diagnosed.

## Supplementary Material S2

### Description of patients who received antimicrobial agents

The reasons for the administration of antibiotics include the following: 1) the MMP-8 rapid test was positive (n = 9); 2) the MMP-8 rapid test was positive and amniotic fluid white blood cell count was elevated (n = 5); 3) the amniotic fluid white blood cell count was elevated, and the MMP-8 rapid test was not available (n = 2); 4) rupture of membranes was suspected (positive result of a nitrazine test but without gross leakage or pooling of amniotic fluid by speculum examination) (n = 2) (cases 11 and 14 in Table 3); rupture of the membranes was confirmed afterward in these patients (case 11, 7 weeks, and case 14, 8 weeks, after the initial amniocentesis, respectively); and 5) MMP-8 concentration was elevated (80.1 ng/mL) (n = 1, case 16 in Table 3).

### Outcome of patients with intra-amniotic infection

Two patients (2/19) had a positive culture for microorganisms; details are listed in Table 3 (case 14 had a positive culture for *Ureaplasma* spp. and case 18 had a mixed infection with *Ureaplasma* spp. and *Mycoplasma hominis*). One patient responded favorably to antimicrobial treatment and had a negative amniotic fluid culture 1 week after the initial amniocentesis (case 14 in Table 3). The other patient (case 18 in Table 3) had persistent intra-amniotic infection with the same microorganisms at serial amniocenteses and delivered 6 weeks later at 32.6 weeks. The placenta revealed acute histologic chorioamnionitis and funisitis; the newborn weighed 2060 g and did not develop complications.

Although antibiotics did not eradicate the infection, this case demonstrates that the magnitude of the inflammatory response decreased as the amniotic fluid interleukin-6 concentration dropped from 18 ng/mL to 12 ng/mL and the amniotic fluid white blood cell count dropped from 50 to 10 cells/$mm^3$ (from 26 to 31 weeks of gestation).

Two patients had a positive amniotic fluid polymerase chain reaction (PCR) result for *Ureaplasma* spp. and a negative culture. In 1 patient (case 1 in Table 3), amniotic fluid PCR for *Ureaplasma* spp. became negative in the follow-up amniocentesis (26.4 weeks) performed 9 days after the initial amniocentesis (25.1 weeks). Resolution of intra-amniotic inflammation was confirmed retrospectively by a decrease in amniotic fluid interleukin-6 concentration at the time of the third amniocentesis (29.1 weeks, interleukin-6 concentration: from 5.01 ng/mL in initial amniocentesis to 0.56 ng/mL in the third amniocentesis). The patient delivered spontaneously at 35.6 weeks. The newborn did not develop any major complications. In the other patient with a positive amniotic fluid PCR result for *Ureaplasma* spp. (case 10 in Table 3), eradication of intra-amniotic infection and resolution of intra-amniotic inflammation was confirmed in the follow-up amniocentesis performed 13 days after the initial amniocentesis (interleukin-6 concentration from 42.6 ng/mL to 2.2 ng/mL).

### Patients with intra-amniotic inflammation without demonstrable microorganisms

Fifteen patients had intra-amniotic inflammation without intra-amniotic infection (positive culture or positive PCR result for *Ureaplasma* spp.) at the time of initial amniocentesis and were treated with antimicrobial agents. Antibiotics were continued despite culture and PCR results, because it was not possible to exclude the presence of microorganisms that had escaped detection with conventional culture techniques used in the clinical setting.[6-8] These patients continued to receive antibiotics and were subsequently offered amniocentesis to monitor fetal lung maturity (depending on gestational age) and the intra-amniotic inflammatory response.

### Resolution of intra-amniotic infection/inflammation and delivery after 34 weeks (Group A in Tables 3 and 4)

A description of the patients who presented objective evidence of the resolution of intra-amniotic inflammation at the follow-up amniocentesis, classified according to whether delivery occurred after (Group A) or before (Group B) 34 weeks, is listed in Table 3.

Nine patients (cases 1-9 in Table 3, Group A in Tables 3 and 4) delivered after 34 weeks, and resolution of intra-amniotic inflammation was confirmed by interleukin-6 concentrations measured retrospectively in amniotic fluid obtained during follow-up amniocenteses. Neonates born to these 9

mothers did not have major complications. The median gestational age at initial amniocentesis and delivery were 25.6 weeks and 35.4 weeks, respectively. The median interval from the first amniocentesis to diagnosis of the resolution of intra-amniotic inflammation was 14 days (range, 7−67) and to delivery, 73 days (range, 32−109). There were no significant differences in the median gestational age at amniocentesis, interleukin-6 concentration, and the rate of a positive amniotic fluid culture among the 4 groups of patients (A, B, C, and D described in Table 4; $P > .1$ for each).

## Resolution of intra-amniotic infection/inflammation and delivery before 34 weeks (Group B in Tables 3 and 4)

Resolution of intra-amniotic inflammation was confirmed retrospectively by a decreased concentration of interleukin-6 in amniotic fluid determined at the follow-up amniocentesis; however, 6 patients delivered before 34 weeks (cases 10−15 in Table 3, Group B in Tables 3 and 4). The median gestational ages at initial amniocentesis and delivery were 22.5 weeks and 31.1 weeks, respectively. The median interval from the first amniocentesis to the diagnosis of resolution of amniotic fluid inflammation was 18 days (range, 10−30), and to delivery, 48 days (range, 23−86).

Case 10 had a positive PCR result for *Ureaplasma* spp. in amniotic fluid from the initial amniocentesis. Amniotic fluid PCR for *Ureaplasma* spp. became negative in the second amniocentesis (30.3 weeks) performed 13 days after the initial procedure (28.4 weeks). The amniotic fluid white blood cell count was persistently high (720, 342, and 486 cells/$mm^3$) in this patient, despite the use of antibiotics for 3.5 weeks. The patient underwent a cesarean delivery because of documented fetal lung maturity at 31.7 weeks and breech presentation. Amniotic fluid interleukin-6 concentration measured retrospectively revealed resolution of intra-amniotic inflammation (from 42.6 ng/mL to 1.22 ng/mL). The rapid MMP-8 test was not available at that time (October 2011). The newborn weighed 1840 g and had no complications.

Resolution of intra-amniotic inflammation was observed in 4 patients (Cases 11−14) in whom rupture of membranes occurred several weeks after the procedure (67% [4/6]) (7 weeks, 3 weeks, 10 weeks, and 8 weeks, respectively). If patients presented ruptured membranes before term, they were offered admission, amniocentesis, and antibiotic administration, and management occurred as previously described.[9] The newborns of cases 11 and 12 delivered at 32.6 weeks and 30.4 weeks, respectively, and had no significant complications. The newborn of case 13 delivered at 33.3 weeks, weighed 1800 g, and developed atypical BPD.[2] However, placental pathology showed no evidence of acute histologic chorioamnionitis or funisitis.

Case 14 had a positive amniotic fluid culture for *Ureaplasma* spp. at 21 weeks of gestation and was given antibiotics. The amniotic fluid culture was negative for microorganisms at the follow-up amniocentesis (performed at 21.9 weeks); the interleukin-6 concentration, measured from the stored amniotic fluid obtained during the third procedure (23.9 weeks), decreased (from 4.84 ng/mL at initial to 1.93 ng/mL). However, at the time of a subsequent amniocentesis, performed given the suspicion of rupture of membranes at 29 weeks, there was a positive amniotic fluid culture for *Morganella morganii.* The patient delivered shortly thereafter (29.6 weeks), and the placenta showed acute histologic chorioamnionitis and funisitis; however, the newborn had no significant complications. This case demonstrates that intra-amniotic infection can recur after successful treatment.

In case 15, the first amniocentesis at 20.1 weeks of gestation showed a positive MMP-8 rapid test, and antibiotics were administered. Repeat amniocentesis revealed another positive MMP-8 rapid test, and the patient went into spontaneous labor and delivered a neonate weighing 700 g at 25.4 weeks of gestation. Retrospective amniotic fluid analysis showed that there was an improvement in intra-amniotic inflammatory processes, as amniotic fluid interleukin-6 concentrations at the follow-up amniocenteses performed at 21.6 weeks and at 23.1 weeks had decreased substantially (from 11.1 ng/mL to 0.9 ng/mL, and 0.49 ng/mL). There was no evidence of funisitis; however, the newborn died 5 hours after delivery. The parents declined an autopsy.

## Patients without evidence of intra-amniotic infection/inflammation resolution who delivered after 34 weeks (Group C in Tables 3 and 4)

One patient (case 16 in Table 3, Group C in Tables 3 and 4) delivered at term after antibiotic treatment. The patient had received antibiotics for 2 weeks because the MMP-8 concentration of amniotic fluid obtained at initial amniocentesis (21.6 weeks of gestation) was elevated (80.1 ng/mL): antibiotics were discontinued after the follow-up amniocentesis at 23.7 weeks, which showed an absence of white blood cells in the amniotic fluid, and preterm labor had stopped. The maternal C-reactive protein concentration decreased over time (from 1.99 mg/mL to 0.12 mg/mL). When this patient was admitted (January 2004), the rapid MMP-8 test was not yet available. However, the interleukin-6 concentration of the amniotic fluid obtained at the follow-up amniocentesis was 6.6 ng/mL (down from 19.4 ng/ml at the first amniocentesis). Resolution of intra-amniotic inflammation could not be confirmed. The patient delivered at 38 weeks, the newborn had no complications, and the placenta was not examined.

## Patients without evidence of resolution of intra-amniotic infection/inflammation who delivered before 34 weeks (Group D in Tables 3 and 4)

In 3 patients, the resolution of intra-amniotic fluid inflammation was not confirmed; these patients delivered before 34 weeks (cases 17, 18, and 19 in Table 3; Group D in Tables 3 and 4).

Case 17 received antibiotics for 10 days and delivered because of placental abruption at 32.9 weeks of gestation. The

effect of antibiotics could not be assessed because there was no remaining amniotic fluid for interleukin-6 determination obtained at the follow-up amniocentesis. However, the amniotic fluid white blood cell count dropped from 100 to 14 cells/mm$^3$. The newborn had no significant complications. The placenta showed acute histologic chorioamnionitis but not funisitis.

Cases 18 and 19 did not have a demonstrable response to antibiotic treatment. Both had intra-amniotic inflammation at the time of admission. However, after antibiotic treatment, follow-up amniocenteses showed persistent inflammation. Although the absolute amniotic fluid interleukin-6 concentrations decreased, both patients went into spontaneous labor. Acute histologic chorioamnionitis was present in both cases, but funisitis was absent. Case 18 delivered at 32.6 weeks and did not have significant neonatal complications. However, the neonate in case 19 was born at 23.6 weeks, weighed 620 g, had RDS , IVH grade II, and PVL, and was discharged from the hospital on day 105.

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