

Antibiotic administration to patients with preterm labor and intact membranes: Is there a beneficial effect in patients with endocervical inflammation?

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Abstract

Objective. To determine whether broad-spectrum antibiotic administration to patients with preterm labor and intact membranes is associated with an improvement in neonatal and maternal outcomes, particularly in patients with microbial invasion of the amniotic cavity (MIAC) or endocervical inflammation (ECI).

Methods. A prospective clinical trial was conducted in which women in premature labor were alternately allocated to receive either antibiotics or placebo, and information about MIAC and ECI collected. Eighty-four pregnant women between 24 and 34 weeks of gestation with spontaneous preterm labor were enrolled. Exclusion criteria were cervical dilatation greater than 3 cm, clinical chorioamnionitis, abruption, rupture of membranes, vaginal bleeding, and several additional fetal and maternal conditions that may influence perinatal outcome. Amniocentesis was offered to all patients and the cervix and vagina were sampled for microbiological and cytological studies. Eligible patients were allocated to receive either clindamycin–gentamycin or placebo for 7 days. Corticosteroids and tocolysis with beta-adrenergic agents were used according to the standard management of our institution. MIAC was defined as the presence of a positive amniotic fluid culture obtained by trans-abdominal amniocentesis. ECI was diagnosed when a significant increase in the white blood cell count of the endocervical secretions was found. A composite neonatal morbidity/mortality outcome was created, including severe neonatal morbidity (respiratory distress syndrome, asphyxia, sepsis, pneumonia, intraventricular hemorrhage) and mortality.

Results. Thirty-nine women received antibiotics and 40 received placebo. The prevalence of ECI and MIAC in both groups was comparable (antibiotic group ECI 61.5% (24/39) and MIAC 20.5% (8/39); placebo group ECI 62.5% (25/40) and MIAC 20% (8/40); $p > 0.05$). Overall, there were no significant differences in maternal infections and composite neonatal outcomes between antibiotic and placebo groups. Women who received antibiotics had a lower rate of subsequent rupture of membranes compared to patients who received placebo (2.6% (1/39) vs. 25% (10/40), respectively; $p = 0.007$). A sub-analysis showed that among patients with ECI, antibiotic administration was associated with a lower rate of composite neonatal morbidity/mortality outcome compared to those who received placebo (4.2% (1/24) vs. 28% (7/25), respectively; $p < 0.05$). This association was also present in patients with ECI without MIAC (0% (0/16) vs. 27.8% (5/18); $p < 0.05$), but not in patients with ECI and MIAC (antibiotic group 12.5% (1/8) vs. placebo group 28.6% (2/7); $p > 0.05$).

Conclusions. The combination of antibiotics used in this study did not improve maternal or perinatal outcome in patients with preterm labor and intact membranes. Further studies are required to determine if women with endocervicitis presenting with preterm labor and intact membranes may benefit from antibiotic administration.

Keywords: Preterm labor, antibiotic therapy, chorioamnionitis, neonatal complications, randomized clinical trials

Introduction

Preterm labor and delivery is the leading cause of perinatal morbidity and mortality worldwide [1]. One third of women with preterm birth deliver after presenting with spontaneous preterm labor and intact

membranes [2,3]. Infection has been causally linked to spontaneous preterm parturition [4–6]. Indeed, 20% of all women with intact membranes who deliver a preterm neonate have a documented microbial invasion of the amniotic cavity (MIAC) using conventional microbiologic culture techniques [5,6]. Moreover, the

frequency of intra-amniotic inflammation is approximately double that of MIAC [7], and the clinical implications of these two conditions are similar.

Previous randomized clinical trials of antibiotic administration to women with premature labor and intact membranes have not shown a prolongation of pregnancy, or a reduction in the rate of preterm delivery, perinatal morbidity or mortality [8]. Moreover, randomized clinical trials of antibiotic administration to patients with bacterial vaginosis yielded largely negative results with a few exceptions [9]. It has recently been proposed that it is not the presence of bacterial vaginosis but vaginal inflammation that confers risk for spontaneous preterm delivery [10].

A clinical trial was conducted at our institution to test the effect of antibiotic administration in women with preterm labor and intact membranes. Our study included the performance of amniocentesis to evaluate the microbiologic state of the amniotic cavity and the determination of the presence of endocervical inflammation. Therefore, our study offers a unique opportunity to determine whether a subset of patients with preterm labor and intact membranes may benefit from antibiotic administration (such as those with intra-amniotic infection and/or endocervical inflammation).

Patients and methods

Study population

Pregnant women with preterm labor and intact membranes between 24 and 34 weeks of gestation were asked to participate in a prospective clinical trial to determine the effects of antibiotics (combination of clindamycin and gentamycin) versus placebo on adverse pregnancy outcome.

Preterm labor was defined as the presence of four or more uterine contractions in 30 minutes for at least 1 hour, a sonographic cervical length measurement < 5 mm and/or cervical dilatation of 1 to 3 cm. Exclusion criteria were: clinical chorioamnionitis, preterm PROM at admission, vaginal bleeding that required delivery, abruptio placentae, previous cervical cerclage, uterine anomalies, major fetal anomalies or death, multiple gestation, fetal distress, a small for gestational age neonate (estimated fetal weight $< 10^{\text{th}}$ percentile), preeclampsia, eclampsia, chronic hypertension, intrahepatic cholestasis, use of antibiotics within 30 days of admission, diabetes, hyperthyroidism, heart disease with cardiac functional capacity II to IV, arrhythmias, leukemia, HIV, alcoholism and drug abuse, extrauterine infection including acute pyelonephritis, and documented allergy to clindamycin or gentamycin.

Gestational age was determined using the last menstrual period and/or an ultrasound performed

before 24 weeks in cases without a reliable last menstrual period. Trans-abdominal amniocentesis was offered to all patients as part of the study protocol. Written informed consent was obtained from all women. This study was approved by the Institutional Review Board of Hospital San Borja Arriarán.

Microbiologic studies

Endocervical mucus, vaginal fluid, and amniotic fluid samples were obtained for microbiological studies. Gram stains were performed on endocervical mucus, vaginal fluid, and amniotic fluid. *Chlamydia trachomatis* was identified using direct immunofluorescence in samples obtained from the endocervical canal [11]. An additional sample of endocervical mucus was placed in sucrose phosphate (SP) media for the isolation of genital mycoplasmas [12]. Samples of vaginal fluid were placed on Stuart media for the identification of *Streptococcus agalactiae* and other aerobic and facultative anaerobic bacteria. The presence and number of white blood cells in endocervical secretions was determined using direct microscopic examination of freshly collected samples.

Amniotic fluid was inoculated into pre-reduced thioglycolate and 2 SP media. The remaining fluid sample was transported to the laboratory in a sterile capped plastic syringe immediately after collection. Cultures for aerobic and anaerobic bacteria as well as genital mycoplasmas were performed according to methods previously described [13–18].

The diagnosis of bacterial vaginosis was made using a Gram stain of vaginal fluid using the criteria proposed by Nugent et al. [19] ECI was defined according to the presence of an increased number of polymorphonuclear leukocytes ($> 10/\text{field}$) at direct microscopic examination ($400\times$) of the endocervical secretions [20]. Vaginal inflammation was diagnosed when neutrophils were present in the Gram stain ($> 10/\text{field}$ at $400\times$). MIAC was defined as a positive amniotic fluid culture.

Allocation of patients to treatment group

Patients who met the inclusion criteria and consented to participate in the study were alternately assigned to receive either antibiotic administration or placebo. Amniotic fluid was used for microbiologic evaluation of the amniotic cavity and the assessment of lung maturity. Patients with a positive amniotic fluid culture for *S. agalactiae*, *Haemophilus influenzae*, *Fusobacterium nucleatum*, *Escherichia coli*, or *Neisseria gonorrhoeae* in the placebo group were considered serious enough to warrant notification to the clinician and initiate antibiotic treatment.

Tocolysis: All patients were given phenoterol 0.5 to 3 $\mu\text{g}/\text{min}$ IV in order to achieve uterine quiescence.

After 12 hours of intravenous administration, oral administration of the same agent was continued for 7 days. If uterine contractility increased after a period of uterine quiescence, intravenous tocolysis was reinitiated as described at the beginning of this paragraph. Tocolysis was discontinued if there was progression of cervical dilatation (more than 5 cm), rupture of membranes, or clinical chorioamnionitis. All patients received two doses of betamethasone 12 mg IM, 24 hours apart for induction of fetal lung maturity.

Antibiotic administration: Patients who were allocated to the antibiotic group were assigned to receive the following agents: clindamycin 600 mg IV every 8 hours for 48 hours and gentamycin 4 mg/kg/day IV for 48 hours, followed by clindamycin 300 mg orally every 6 hours for 5 days and gentamycin 2 mg/kg/day IM every 12 hours for 5 days. Patients in the placebo group were given placebos formulated in a similar manner as the antibiotics (IV solutions, IM injections, and oral capsules). After completion of the treatment regimen and in the absence of uterine contractions for 24 hours, patients were discharged from the hospital and followed at the high-risk outpatient clinic until 37 weeks of gestation.

Delivery was indicated if any of the following conditions occurred: clinical chorioamnionitis, positive amniotic fluid culture for *S. agalactiae*, *H. influenzae*, *F. nucleatum*, *E. coli*, or *N. gonorrhoeae*, fetal distress, and abruptio placentae. Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs and associates [21]. Patients with positive cultures for *S. agalactiae* were treated with ampicillin or penicillin during labor, regardless of group. Women allocated to the placebo group who had *C. trachomatis* in the endocervical secretions were not treated, but the microbiologic finding was communicated to the pediatricians who treated the neonates.

Puerperal endometritis was defined as a temperature $\geq 38^{\circ}\text{C}$ on two occasions four hours apart (excluding the day of delivery), associated with uterine tenderness, foul-smelling lochia, and no other apparent source of infection [21]. These patients were treated with antibiotic therapy according to the guidelines of our institution.

Neonatal complications and management

Pediatricians were informed of the microbiological state of the amniotic fluid and the administered therapy. All neonates under 2000 g received parenteral ampicillin and amikacin after the laboratory tests were obtained (blood cultures, C reactive protein, white blood cell count with differential count, and peripheral and gastric cultures). Antibiotics were discontinued in the absence of

clinical or laboratory signs consistent with neonatal sepsis.

Proven neonatal sepsis was diagnosed in the presence of a positive blood, urine, or cerebrospinal fluid culture. Probable neonatal sepsis was diagnosed on the basis of combined clinical and laboratory tests traditionally associated with neonatal sepsis. Pneumonia was diagnosed in the presence of definite clinical and radiologic findings, with or without a positive culture from tracheal aspirate, blood, or chest tube specimen. The diagnosis of respiratory distress syndrome (RDS) required the presence of respiratory grunting and thoracic retraction during the inspiratory phase, an increased oxygen requirement (forced inspiratory $\text{O}_2 > 0.4$), radiographic signs, and laboratory findings in the absence of other causes of respiratory disease. Necrotizing enterocolitis was diagnosed in the presence of abdominal distension and feeding intolerance for at least 24 hours with clear radiologic evidence of intramural air, perforation, meconium plug syndrome, or specific surgical or autopsy findings suggestive of necrotizing enterocolitis. Intraventricular hemorrhage was diagnosed by ultrasonographic examination of the neonatal head and grades III and IV were considered as significant lesions. Low Apgar score was defined as an Apgar score of less than 3 at 5 minutes.

Outcome measures

The main outcome was the frequency of neonatal complications determined by the composite neonatal morbidity/mortality outcome [22]. This composite neonatal outcome has been extensively used as the primary endpoint for clinical randomized trials in preterm labor and intact membranes and in preterm PROM.

Other outcome variables included the frequency of preterm delivery < 34 and < 37 weeks, rupture of membranes, maternal infectious-related morbidity (chorioamnionitis and puerperal endometritis), and admission-to-delivery interval.

A composite neonatal outcome was used because it was conceivable that antibiotic administration might reduce certain complications (e.g., neonatal sepsis) while increasing others (e.g., necrotizing enterocolitis). It was reasoned that combining the number of complications would more appropriately represent the overall effect of antibiotics on neonatal outcome than providing a single account of individual morbid events [22].

Statistical analysis

Chi-square and Fisher's exact tests were used for the comparison of proportions and Mann-Whitney

U-tests were used to compare continuous variables. A p value of <0.05 was considered significant. Survival analysis was used to examine the admission-to-delivery interval between groups. Statistical analyses were performed using SPSS v12.0 (SPSS Inc, Chicago, IL, USA).

Results

Study population profile

Eighty-four patients were enrolled into this trial. Three patients were excluded after enrollment: one patient in the treatment group (antibiotic use was discovered after allocation) and two patients in the placebo group (one case with an erroneous gestational age and the other due to pyelonephritis). Two patients in the treatment group were lost to follow-up. Seventy-nine patients were available for analysis: 39 patients in the antibiotic group and 40 in the placebo group (Figure 1).

Table I displays the general characteristics of the study population. There were no significant differences in maternal age, parity, gestational age at admission, and mode of delivery. The overall prevalence of ECI was 62% (49/79), which was similar in both study groups (antibiotics 61.5% (24/39); placebo 62.5% (25/40); $p > 0.05$). The overall frequency of MIAC was 20.3% (16/79); there were no differences in the frequency of MIAC in the two groups (antibiotics 20.5% (8/39); placebo 20%

(8/40); $p > 0.05$). MIAC or ECI was not present in 36.7% (29/79) of patients.

Table II displays the microorganisms isolated from the amniotic fluid of patients with MIAC according to the study groups. The most frequent isolates were *Ureaplasma urealyticum*, followed by *Gardnerella vaginalis*. No significant differences in the frequency of specific microorganisms between study groups were observed. The rate of polymicrobial MIAC in both groups was identical (antibiotic group 25% (2/8) and placebo group 25% (2/8)).

Bacterial vaginosis was diagnosed in 51.3% (20/39) of patients in the antibiotic group and 30% (12/40) in the placebo group ($p = 0.07$). The most frequent microorganism isolated from the cervix and vagina in the treatment and placebo groups were *U. urealyticum* (73.1% (19/26) and 88.9% (24/27), respectively), followed by *G. vaginalis* (76.9% (20/26) and 55.6% (15/27), respectively), *Mycoplasma hominis* (19.2% (5/26) and 29.6% (8/27), respectively), *Candida albicans* (19.2% (5/26) and 22.2% (6/27), respectively), and *E. coli* (11.5% (3/26) and 14.8% (4/27), respectively) (Table III).

Frequency of preterm delivery

There were no significant differences in the rate of preterm delivery between patients allocated to antibiotics and those allocated to placebo (Table IV). The admission-to-delivery interval was not significantly different between groups (Figure 2).

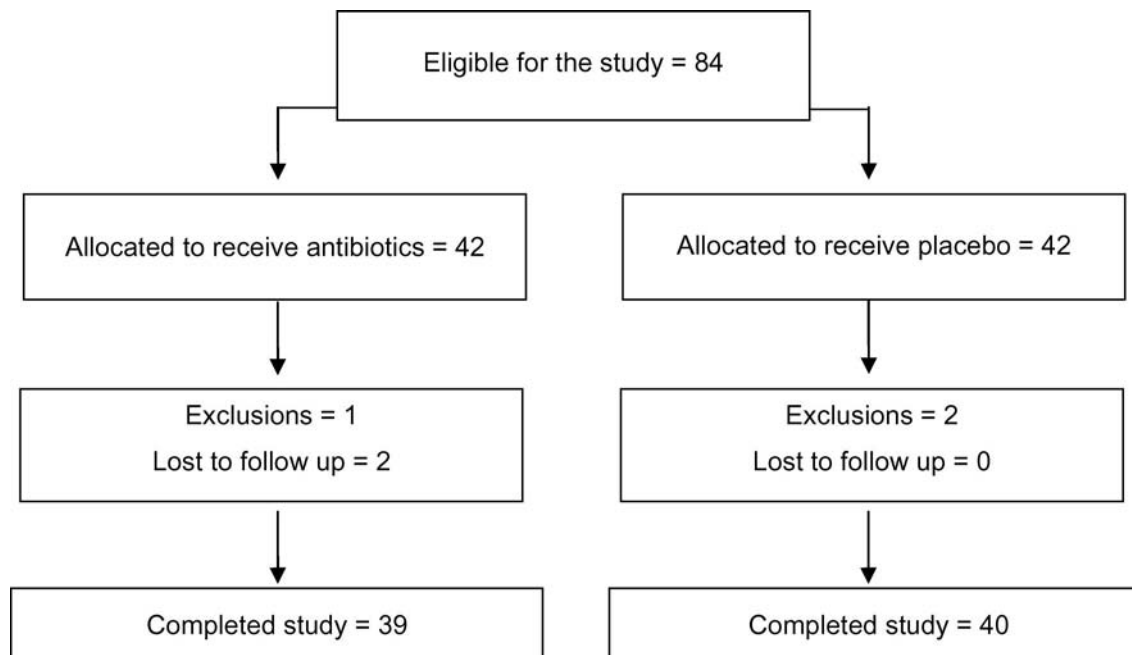


Figure 1. Trial design diagram and patient compliance according to study protocol. Three patients were excluded after enrollment: one patient in the treatment group (antibiotic use was discovered after allocation) and two patients in the placebo group (one case with an erroneous gestational age and the other due to pyelonephritis). Two patients in the treatment group were lost to follow-up.

Table I. Clinical and demographic characteristics according to study groups.

	Antibiotic group (n = 39)	Placebo group (n = 40)	p
Maternal age (years)			
Median (range)	23 (16–40)	23 (16–40)	NS
Parity			
Nulliparous	38.5% (15/39)	55% (22/40)	NS
Multiparous	61.5% (24/39)	45% (18/40)	NS
Gestational age at admission (weeks)			
Median (range)	31.4 (24.3–34.0)	31.2 (24.0–34.0)	NS
Mode of delivery			
Vaginal	71.8% (28/39)	80% (32/40)	NS
Cesarean section	28.2% (11/39)	20% (8/40)	NS
Birth weight (g)			
Median (range)	2980 (850–3900)	2685 (700–4300)	NS

NS, not significant.

Table II. Amniotic fluid microorganisms isolated by culture from patients with MIAC (N = 16), according to study group.

Microorganism	Antibiotic group (n = 8)	Placebo group (n = 8)
<i>Ureaplasma urealyticum</i>	3	7
<i>Gardnerella vaginalis</i>	2	1
<i>Mycoplasma hominis</i>	0	1
<i>Fusobacterium nucleatum</i>	1	2
<i>Peptostreptococcus anaerobius</i>	1	1
<i>Streptococcus</i> D non-enterococcus	1	0
<i>Streptococcus viridans</i>	1	0
<i>Staphylococcus coagulase</i> (–)	1	0

Mixed flora: 25% (2/8) in the antibiotic group and 25% (2/8) in the placebo group. MIAC, microbial invasion of the amniotic cavity.

Table III. Microorganisms isolated from cervico-vaginal samples (n = 52) according to study groups.

Microorganisms	Antibiotic group (n = 26) % (n)	Placebo group (n = 27) % (n)
<i>Ureaplasma urealyticum</i>	73.1% (19)	88.9% (24)
<i>Gardnerella vaginalis</i>	76.9% (20)	55.6% (15)
<i>Mycoplasma hominis</i>	19.2% (5)	29.6% (8)
<i>Candida albicans</i>	19.2% (5)	22.2% (6)
<i>Streptococcus agalactiae</i>	11.5% (3)	0% (0)
<i>Staphylococcus coagulase</i> (–)	11.5% (3)	3.7% (1)
<i>Escherichia coli</i>	11.5% (3)	14.8% (4)
<i>Klebsiella pneumoniae</i>	3.8% (1)	0% (0)
<i>Proteus mirabilis</i>	3.8% (1)	0% (0)
<i>Chlamydia trachomatis</i>	3.8% (1)	7.4% (2)
<i>Streptococcus viridans</i>	3.8% (1)	11.1% (3)
<i>Streptococcus</i> group D non-enterococcus	3.8% (1)	0% (0)
<i>Enterococcus</i> sp	3.8% (1)	0% (0)
<i>Trichomonas vaginalis</i>	3.8% (1)	0% (0)

No significant differences in the rate of preterm delivery were observed when the analysis was restricted to patients with MIAC, ECI without

MIAC, or patient without MIAC and without ECI (Table IV).

Maternal morbidity

The prevalence of spontaneous rupture of membranes was significantly lower in the antibiotic group than in the placebo group (2.6% (1/39) vs. 25% (10/40), respectively, $p = 0.007$). Similar results were observed in patients with MIAC (Table V). All patients with MIAC progressed to preterm delivery spontaneously while the results of the cultures were pending. This included two patients who developed clinical chorioamnionitis and two women with *F. nucleatum* in the amniotic fluid. Therefore, no patients had their labor induced or augmented because of MIAC.

The rate of maternal infection was low (two patients had clinical chorioamnionitis and one had puerperal endometritis: 3.8% (3/79)). There were no significant differences in the frequency of these complications between the two groups.

Neonatal morbidity and mortality

This was the primary endpoint of the trial. There was no significant difference in the overall rate of morbid events between patients allocated to receive antibiotics and those allocated to the placebo group (Table VI).

Neonatal death was observed only among patients in the placebo group (0% (0/39) vs. 10% (4/40), $p = 0.13$) (Table VII). Two neonatal deaths occurred during the first week of life: one neonate (birth weight 1020 g) developed pneumonia and sepsis and the other (birth weight 700 g) developed severe RDS and respiratory insufficiency. A third neonatal death occurred during the second week of life. This newborn weighed 1052 g at birth and acquired an infection with *Citrobacter* and *Klebsiella pneumoniae*. The last case was a 1490 g neonate who died during the third week of life due to necrotizing enterocolitis.

Neonatal infection-related morbidity was observed in two neonates born to mothers who received placebos (5%, (2/40)); one neonate developed pneumonia and the other pneumonia and sepsis.

In all cases, neonatal complications or neonatal death occurred in patients with either MIAC or ECI (Table VII). When each neonatal complication and neonatal death was compared between the study groups, no significant differences were observed (Table VI).

Outcome according to the presence or absence of ECI

To examine if antibiotic administration could be of benefit to a subgroup of women who present with

Table IV. Prematurity rates according to the microbiologic state of the amniotic cavity and ECI.

Microbiologic state	Prematurity <34 weeks		<i>p</i>	Prematurity <37 weeks		<i>p</i>
	Antibiotic group (<i>n</i> = 39)	Placebo group (<i>n</i> = 40)		Antibiotic group (<i>n</i> = 39)	Placebo group (<i>n</i> = 40)	
MIAC	50% (4/8)	100% (8/8)	NS	75% (6/8)	100% (8/8)	NS
ECI without MIAC	25.0% (4/16)	33.3% (6/18)	NS	37.5% (6/16)	50% (9/18)	NS
No ECI and No MIAC	13.3% (2/15)	14.3% (2/14)	NS	33.3% (5/15)	42.9% (6/14)	NS
Overall	25.6% (10/39)	40% (16/40)	NS	43.6% (17/39)	57.5% (23/40)	NS

NS, not significant; MIAC, microbial invasion of the amniotic cavity; ECI, endocervical inflammation.

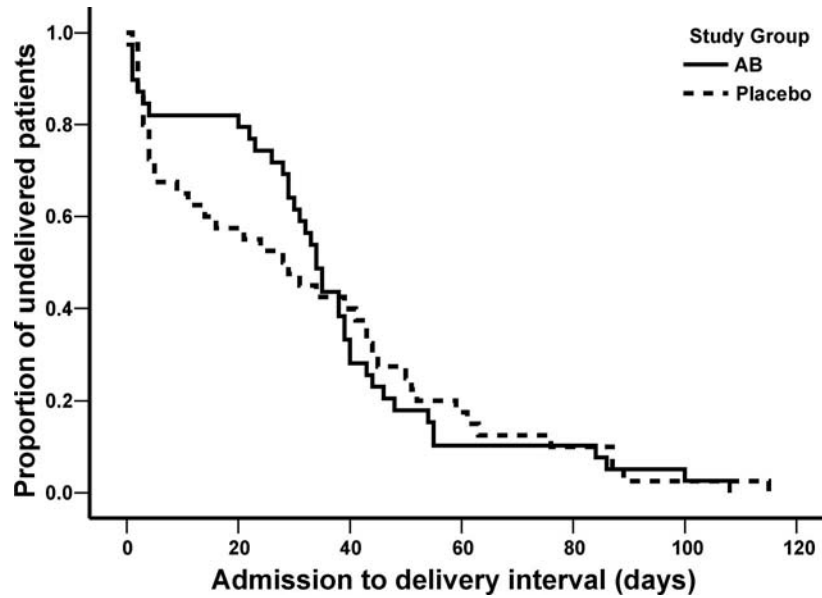


Figure 2. Admission-to-delivery interval according to study groups. Median admission-to-delivery interval for antibiotic and placebo groups, 34 days (95% CI: 34–38) vs. 28 days (95% CI: 13–43), respectively; *p*-value (log-rank test) = 0.89.

Table V. Frequency of subsequent rupture of membranes according to the microbiologic state of the amniotic cavity and ECI, in both study groups.

Microbiologic state	Antibiotic group (<i>n</i> = 39)	Placebo group (<i>n</i> = 40)	<i>p</i>
MIAC	12.5% (1/8)	87.5% (7/8)	0.01
ECI without MIAC	0% (0/16)	5.6% (1/18)	NS
No MIAC and No ECI	0% (0/15)	14.3% (2/14)	NS
Overall	2.6% (1/39)	25.0% (10/40)	0.007

NS, not significant; MIAC, microbial invasion of the amniotic cavity; ECI, endocervical inflammation.

preterm labor and intact membranes, we explored the outcome according to the presence or absence of ECI and MIAC. When the analysis was restricted to women with MIAC, there was no significant difference in the rate of preterm delivery, delivery within 48 hours, 7 days, interval-to-delivery, or neonatal morbidity/mortality. However, the rate of subsequent rupture of membranes was significantly

Table VI. Neonatal outcome according to study group.

	Antibiotic group (<i>n</i> = 39)	Placebo group (<i>n</i> = 40)	<i>p</i>
Admission to intensive care unit	23.1% (9/39)	27.5% (11/40)	NS
Respiratory distress syndrome	2.6% (1/39)	7.5% (3/40)	NS
Severe asphyxia	2.6% (1/39)	2.5% (1/40)	NS
Severe intraventricular hemorrhage	0% (0/39)	2.5% (1/40)	NS
Neonatal infection	0% (0/39)	5.0% (2/40)	NS
Necrotizing enterocolitis	0% (0/39)	2.5% (1/40)	NS
Neonatal death	0% (0/39)	10% (4/40)	NS
Composite neonatal outcome	2.6% (1/39)	17.5% (7/40)	NS

NS, not significant.

lower in patients with MIAC who received antibiotics than in those with MIAC who received placebo (12.5% (1/8) vs. 87.5% (7/8), respectively, *p* = 0.01).

Table VII. Description of cases with neonatal complications.

Case ID	Group	Parity	Gestational age at admission	ECI	Cervico-vaginal culture	AF culture	Admission-to-delivery interval (days)	Subsequent PPROM	Route of delivery	Gestational age at delivery	Maternal infectious complications	Birth weight	Apgar 5 min	Comments
68	Placebo	1	32.0	Yes	<i>Gardnerella vaginalis</i>	Negative	5	No	Vaginal	32.5	No	1550	3	Low Apgar score
46	Placebo	0	26.3	Yes	<i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i>	<i>Ureaplasma urealyticum</i>	4	Yes	Cesarean section	27.0	Endometritis	1020	8	Neonatal sepsis with severe RDS and neonatal death (first week)
47	Placebo	0	25.2	Yes	<i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i>	Negative	3	No	Vaginal	26.0	No	980	8	Severe RDS and IVH
63	Placebo	1	33.0	Yes	<i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i> / <i>Candida albicans</i>	Negative	21	No	Vaginal	36.0	No	2650	9	Pneumonia
78	Placebo	0	29.5	Yes	<i>Mycoplasma hominis</i> / <i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i>	Negative	9	No	Vaginal	31.0	No	1592	8	Neonatal death at day 17 secondary to necrotizing enterocolitis
6	Antibiotics	0	26.0	Yes	<i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i>	<i>Ureaplasma urealyticum</i> / <i>Fusobacterium nucleatum</i>	0	No	Vaginal	26.0	Clinical chorioamnionitis	850	3	Low Apgar score and severe RDS
44	Placebo	1	25.0	Yes	<i>Mycoplasma hominis</i> / <i>Ureaplasma urealyticum</i> / <i>Gardnerella vaginalis</i>	Negative	5	No	Vaginal	26.0	No	700	6	Severe RDS and neonatal death (first week)
50	Placebo	0	27.0	Yes	<i>Ureaplasma urealyticum</i> / <i>Streptococcus viridans</i> / <i>Candida albicans</i>	<i>Ureaplasma urealyticum</i> / <i>Fusobacterium nucleatum</i>	2	Yes	Cesarean section	27.2	No	1052	7	Bilateral pneumothorax with pneumonia and neonatal death at day 14

ECI, endocervical inflammation; AF, amniotic fluid; PPROM, preterm premature rupture of the membranes; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage.

It is noteworthy that patients with ECI and a negative amniotic fluid culture for microorganisms who received antibiotics had a lower rate of neonatal morbidity/mortality composite outcome than patients with ECI and a negative amniotic fluid culture who were assigned to the placebo group (0% (0/16) vs. 27.8% (5/18), respectively, $p = 0.046$) (Table VIII).

Discussion

Principal findings of the study

(1) Administration of antibiotics to patients with preterm labor and intact membranes did not reduce the frequency of preterm birth, admission-to-delivery interval, or neonatal morbidity/mortality. (2) A post-hoc analysis demonstrated that administration of antibiotics to patients with preterm labor and intact membranes who had ECI with a negative amniotic fluid culture was associated with a significantly lower frequency of neonatal morbidity/mortality composite outcome.

Antibiotic administration to patients with premature labor and intact membranes does not improve pregnancy outcome

The results of the current study are in agreement with the results of a recent systematic review from the Cochrane Database Library [8] as well as the results of major randomized clinical trials such as those reported by the NICHD Maternal Fetal Medicine Network, and the ORACLE trial [23,24]. Collectively, the evidence suggests that routine administration of antibiotics to patients with preterm labor and intact membranes does not result in a longer admission-to-delivery interval, or a reduction of the rates of preterm delivery or perinatal morbidity/mortality [23,24].

Is intrauterine infection associated with preterm labor?

A considerable body of evidence suggests that intrauterine infection is causally linked to preterm

labor and delivery. The authors have reviewed and updated the strengths of the evidence in support of this proposition several times over the years [5,6]. An association between intra-amniotic infection and spontaneous preterm labor and delivery has been consistently reported in the literature [5,16,25–28], and patients with a positive amniotic fluid culture for microorganisms are at increased risk for spontaneous preterm delivery, spontaneous rupture of membranes, and clinical chorioamnionitis. Moreover, there is evidence that subclinical intra-amniotic infection is a risk factor for the development of pulmonary edema during tocolysis [29]. The observations in the current study also confirm the association between preterm labor with intact membranes and MIAC. Approximately 20% of the patients had a positive microbiologic amniotic fluid culture. A temporal relationship between intra-amniotic infection and the subsequent development of preterm labor and preterm PROM has also been established [27]. Patients with a positive amniotic fluid culture at the time of genetic amniocentesis are at risk for spontaneous preterm birth [30–32]. Therefore, a relationship between intra-amniotic infection and spontaneous preterm labor and delivery has been well established, and there is evidence that it meets the postulates of causality [27].

If infection causes spontaneous preterm labor and delivery, why does antibiotic administration not prevent preterm birth or the neonatal complications associated with preterm delivery?

We have proposed that preterm labor is a syndrome and that infection is only one of the causes of this syndrome [6]. Accumulating evidence suggests that the frequency of MIAC is approximately 12 to 20% of patients with preterm labor and intact membranes [5,6]. In the current study, the frequency of MIAC was 20% for all patients enrolled and 35% for those who had a preterm delivery. Therefore, most women with preterm labor with intact membranes who delivered a preterm neonate do not have evidence of MIAC using conventional microbiologic techniques. The limitations of current microbiologic techniques in establishing microbial invasion, burden, and diversity have been described in a previous communication by the authors [7,33,34]. Specifically, it is now known that only a small fraction of the microbial world can be identified using standard culture techniques [35–37]. The role of other organisms (such as viruses or those yet to be discovered) remains to be established. Thus, it is likely that the rate of intrauterine infection is underestimated. Similarly, the microbial diversity involved is only partially known at this time. Therefore, it is

Table VIII. Frequency of neonatal composite outcome according to ECI and MIAC.

	Antibiotic group (n = 39)	Placebo group (n = 40)	p
ECI	4.2% (1/24)	28% (7/25)	0.048
ECI without MIAC	0% (0/16)	27.8% (5/18)	0.046
ECI with MIAC	12.5% (1/8)	28.6% (2/7)	NS
MIAC only	12.5% (1/8)	25% (2/8)	NS

NS, not significant; MIAC, microbial invasion of the amniotic cavity; ECI, endocervical inflammation.

possible that the antimicrobial agents that have been used in clinical trials may not be effective against many causative microorganisms.

Antibiotic treatment could be expected to benefit women with preterm labor and intact membranes in whom the primary mechanism of disease is intra-uterine infection. Yet, most trials (including the present one) have not focused on these patients, but rather have examined the role of antibiotics in patients presenting with preterm labor and intact membranes. Consequently, one of the potential explanations for the apparent failure of clinical trials of antibiotic administration is that they have focused on a population that is heterogeneous and contains many patients who cannot benefit from antibiotic administration.

Who are the patients with preterm labor and intact membranes who may not benefit from antibiotic administration?

We propose that there are several subgroups of patients who are unlikely to benefit from antibiotic administration. The first group is composed of women who do not have intrauterine infection/inflammation as a mechanism of disease. For example, the patient with uterine over-distension or an allergic-like mechanism for preterm labor [6] or those in whom the preterm labor is associated to a fetal growth disorder (deceleration or acceleration) may not accrue benefit from antibiotic administration. The second group is composed of women who had intra-amniotic infection but in whom the process is so advanced that antibiotics are ineffective. The typical example of this is the patient with septic shock and documented bacteremia. Antibiotic administration alone is often insufficient to prevent death, because the mechanism of disease involves not only microorganisms but also a deranged host response. This is the rationale for the use of biological response modifiers to improve the outcome of patients with septic shock [38,39]. Thus far, the administration of activated protein C has been shown to improve the outcome of septic shock [40]. It is also possible that in preterm labor associated with infection, the administration of biological response modifiers would be required to improve prognosis. Evidence of this comes from experimental studies in which interleukin-10 (IL-10), N-acetyl cysteine and other biological response modifiers have been administered to pregnant animals receiving either microbial products (e.g., endotoxin) or infection [41–43]. Recent evidence suggests that thrombin is activated in the course of preterm labor with intra-amniotic infection/inflammation [44,45]. Therefore it is possible that agents that block thrombin activity may have a beneficial effect on

the outcome of preterm labor associated with infection/inflammation.

Observations made in our study suggest that the administration of antibiotics by themselves to women with preterm labor and intact membranes with MIAC is unlikely to be effective. A sub-analysis in this particular population indicated that there was no difference in the interval-to-delivery, rate of preterm birth, and neonatal morbidity/mortality. A limitation of our study is its sample size for this particular comparison. Therefore, we do not exclude the possibility that antibiotic administration to women with proven MIAC could reduce the rate of neonatal sepsis or related complications. This has been the case in women with clinical chorioamnionitis near term who were randomized to antibiotics or placebo [46]. That trial was discontinued prior to the target enrollment because the rate of neonatal sepsis was higher in patients treated with antibiotics in the antepartum period than in those treated with antibiotics after birth [46]. It is difficult to imagine how antibiotics would be helpful in reducing the rate of neonatal sepsis in term neonates but not in preterm neonates who had been traditionally considered a relatively immune suppressed host. The issues surrounding the conduction of this trial have been addressed elsewhere by the authors [24].

Is there a subgroup of women with preterm labor and intact membranes who may benefit from antibiotic treatment?

We propose that women in the early phases of intrauterine infection are ideal candidates for antibiotic treatment. However, a major challenge is the identification of such patients. One approach is to select patients who have evidence of mild intra-amniotic inflammation in the amniotic cavity but negative amniotic fluid cultures for microorganisms. Another approach is to examine the lower genital tract for evidence of an inflammatory process (e.g., endocervicitis or vaginal inflammation) or changes in the microbial ecosystem (e.g., bacterial vaginosis).

Previous studies have indicated that women with a negative amniotic fluid culture for microorganisms but positive cultures from the material obtained from the chorioamniotic space have higher concentrations of amniotic fluid IL-6 than those with sterile amniotic fluid and negative cultures of the chorioamniotic space. This observation suggests that a mild elevation of amniotic fluid IL-6 may identify patients in whom there is an intrauterine infection but such process is extra-amniotic in nature. It remains to be determined if these patients can benefit from antibiotic administration.

Vaginal fluid was examined in a subset of patients for the presence of bacterial vaginosis. However, most patients with endocervical vaginitis had a Gram stain compatible with bacterial vaginosis. Given the recent changes in the understanding of the importance of changes in the microbial ecosystem of the vagina and vaginal inflammation, we have emphasized endocervical inflammation in this manuscript. Since patients enrolled in this study had endocervical material collected for the examination of infection/inflammation (endocervicitis), we conducted a sub-analysis to determine whether antibiotic administration had an effect on patients with and without endocervicitis but with negative amniotic fluid culture for microorganisms. We excluded patients with a positive amniotic fluid culture to rule out advanced MIAC, because these patients were unlikely to benefit from antibiotic administration. The results of such post-hoc analysis demonstrated that the rate of composite neonatal morbidity/mortality was lower in patients with endocervicitis treated with antibiotics than in those treated with placebo. We recognize that this analysis was conducted after the study was stopped, and therefore should be considered hypothesis-generating rather than a primary result.

Another interesting observation from this study was that patients with antibiotic administration had a lower rate of premature rupture of membranes. The mechanisms by which antibiotics may protect against rupture of membranes have not been defined. It is possible that this occurs through the control of microbial proliferation leading to less inflammation and eventual membrane rupture. Some antibiotics also have antiprotease activity. However, the relative importance of these two mechanisms is unknown.

Methodological issues pertinent to this study

This study began before the publication of the ORACLE trial II, but after the publication of several individual randomized controlled trials in which antibiotic administration had no demonstrable beneficial effects [24,47–52], had a beneficial effect in the interval-to-delivery [53–60] or neonatal outcome [54,60–62]. After the publication of the ORACLE trial II, which included over 6000 patients, we decided to stop this trial, conduct an interim analysis, and explore whether there was any evidence that patients may benefit from antibiotics.

We wish to bring to the attention of the readership a number of limitations of the current study. First, this trial used alternate allocation rather than randomization. The shortcomings of alternate allocation have been described in the literature in detail [63]. However, our results are similar to the largest previous clinical trials that have used random allocation. Second, the sample size of this trial is limited, and therefore we

cannot exclude a potential benefit of antibiotics in patients with MIAC. It is possible that antibiotic administration in the antenatal period may improve the outcome of congenital fetal sepsis. However, such a study would need to be targeted to patients with documented microbial invasion. Third, the antimicrobial agents used in this study (clindamycin and gentamycin) are not effective against *U. urealyticum*, which was the most common microorganism found in the amniotic fluid in this study. Therefore, it is possible that the results represent sub-optimal treatment of MIAC and congenital fetal sepsis. Future trials should consider antibiotics that cover *U. urealyticum*. Fourth, the analysis of the relationship between endocervical inflammation, antibiotic treatment, and pregnancy outcome was developed post-hoc. Fifth, cultures for *U. urealyticum* were not obtained from the newborn, and therefore the true prevalence of congenital sepsis with this microorganism is unknown.

Despite these limitations, we believe that investigators have an ethical obligation to report ‘negative trials’ so that the scientific community be informed of the existence of data that may be used as part of systematic reviews and perhaps inform the design of future studies in this important area of obstetrics.

Conclusion

The combination of antibiotics used in this study did not improve maternal or perinatal outcome in patients with preterm labor and intact membranes. Further studies are required to determine if women with endocervicitis presenting with preterm labor and intact membranes may benefit from antibiotic administration, and if the adjunctive administration of biological response modifiers may improve the outcome of patients with intra-amniotic inflammation or MIAC.

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