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Gastric fluid versus amniotic fluid analysis for the identification of intra-amniotic infection due to *Ureaplasma* species

Sun Min Kim^{1,2}, Roberto Romero^{3,4,5,6}, JoonHo Lee¹, Piya Chaemsaihong^{3,7}, Nikolina Docheva^{3,7}, and Bo Hyun Yoon¹

¹Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea

²Department of Obstetrics and Gynecology, Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul, Republic of Korea

³Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, Michigan, USA

⁴Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

⁵Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, Michigan, USA

⁶Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

⁷Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract

Objective—Early neonatal sepsis is often due to intra-amniotic infection. The stomach of the neonate contains fluid swallowed before and during delivery. The presence of bacteria as well as neutrophils detected by culture or Gram stain in the gastric fluid during the first day of life is suggestive of exposure to bacteria or inflammation. We undertook this study to determine the relationship between gastric fluid analysis and amniotic fluid obtained by transabdominal amniocentesis in the detection of *Ureaplasma* species, the most frequent microorganisms responsible for intra-amniotic infection.

Materials and Methods—The study population consisted of 100 singleton pregnant women who delivered preterm neonates (<35weeks) within 7 days of amniocentesis. Gastric fluid of newborns was obtained by nasogastric intubation on the day of birth. Amniotic fluid and gastric

Correspondence to: Roberto Romero, M.D., D.Med.Sci., Chief, Perinatology Research Branch, 3990 John R. Rd., 4 Brush, Detroit, MI, 48201, Tel: 313-993-2700; Fax: 313-993-2694; prbchiefstaff@med.wayne.edu. Bo Hyun Yoon, MD, PhD, Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, 110-744, Republic of Korea. Tel: 82-2-2702-2826; Fax: 82-2-765-3002; yoonbh@snu.ac.kr.

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Declaration of interest

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fluid were cultured for genital *Mycoplasmas* and polymerase chain reaction (PCR) for *Ureaplasma* species was performed. Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23ng/mL).

Results—1) *Ureaplasma* species were detected by culture or PCR in 18% (18/100) of amniotic fluid samples and in 5% (5/100) of gastric fluid samples; 2) among the amniotic fluid cases positive for *Ureaplasma* species, these microorganisms were identified in 27.8% (5/18) of gastric fluid samples; 3) none of the cases negative for *Ureaplasma* species in the amniotic fluid were found to be positive for these microorganisms in the gastric fluid; 4) patients with amniotic fluid positive for *Ureaplasma* species but with gastric fluid negative for these microorganisms had a significantly higher rate of intra-amniotic inflammation, acute histologic chorioamnionitis, and neonatal death than those with both amniotic fluid and gastric fluid negative for *Ureaplasma* species; and 5) no significant differences were observed in the rate of intra-amniotic inflammation, acute histologic chorioamnionitis, and neonatal death between patients with amniotic fluid positive for *Ureaplasma* species but with gastric fluid negative for these microorganisms and those with both amniotic fluid and gastric fluid positive for *Ureaplasma* species.

Conclusions—Gastric fluid analysis has 100% specificity in the identification of intra-amniotic infection with *Ureaplasma* species. However the detection of *Ureaplasma* species by culture or PCR in the gastric fluid of neonates at birth did not identify these microorganisms in two-thirds of cases with microbial invasion of the amniotic cavity. Thus, amniotic fluid analysis is superior to that of gastric fluid in the identification of intra-amniotic infection.

Keywords

acute histologic chorioamnionitis; amniocentesis; culture; gastric aspirate; genital *Mycoplasmas*; matrix metalloproteinase-8; neonatal sepsis; preterm birth

Introduction

Microbial invasion of the amniotic cavity is causally linked to preterm birth^{1–25} and neonatal morbidity and mortality^{13, 15, 19, 26–37}. Genital *Mycoplasmas*, and specifically *Ureaplasma* species, are the most common organisms isolated from the amniotic fluid of patients with preterm labor and intact membranes^{8, 20, 38–48}, preterm prelabor rupture of the membranes (preterm PROM)^{15, 19, 44, 47–67}, cervical insufficiency^{68–70}, a short cervix^{71–73}, idiopathic vaginal bleeding⁷⁴ and clinical chorioamnionitis at term⁷⁵. The presence of these organisms in the amniotic fluid is a risk factor for impending preterm delivery^{8, 25, 43, 45, 46, 48, 58–60, 65, 76–81} and adverse perinatal outcomes^{8, 36, 40, 43, 45, 57, 58, 63, 65, 74, 76, 77, 82–111}. Moreover, intra-amniotic infection with genital *Mycoplasmas* is associated with an intra-amniotic and maternal inflammatory responses^{64, 112, 113} more intense than those observed with other microorganisms in patients with preterm PROM⁶⁴.

The identification of preterm neonates born to mothers with intra-amniotic infection is a challenging clinical issue^{88, 114–121}. Many centers do not perform amniocentesis for the evaluation of patients with preterm labor who have intact membranes and preterm PROM^{122–125}. Moreover, most neonatal units do not perform routine cultures for genital

Mycoplasmas during the course of a septic workup. Therefore, the contribution of genital *Mycoplasma* infections to neonatal sepsis is unclear. This is important because the standard antibiotics used to treat neonates with suspected sepsis are not effective against *Mycoplasmas*, the most common organisms causing intra-amniotic infection^{91, 115–119, 126–132}.

The human fetus swallows amniotic fluid before birth^{133–137}. Consequently, examination of gastric fluid obtained immediately after birth could provide means to identify microbial invasion due to genital *Mycoplasmas*. Indeed, several investigators have reported the use of gastric fluid analysis in the identification of intra-amniotic infection/inflammation^{138–143}. Other reports suggest an association between the presence of organisms in gastric fluid and the subsequent development of bronchopulmonary dysplasia^{144, 145}. However, it is unclear whether the microbiologic status of gastric fluid obtained immediately after birth is similar to that of amniotic fluid. The objective of this study was to examine whether the detection of *Ureaplasma* species in the gastric fluid of preterm newborns at birth is of value in the identification of the neonate exposed to intra-amniotic infection with these microorganisms.

Materials and Methods

Study design

This was a retrospective cohort study of patients admitted to the Seoul National University Hospital who met the following criteria: (1) singleton gestation; (2) preterm delivery between 24⁺⁰ and 34⁺⁶ weeks; (3) amniotic fluid obtained by transabdominal amniocentesis for microbiologic studies; (4) delivery within 7 days of amniocentesis (to preserve a meaningful temporal relationship between the results of amniotic fluid studies and those of neonatal gastric fluid); and (5) gastric fluid obtained by nasogastric intubation on the day of birth.

Transabdominal amniocentesis was performed with patients who provided written informed consent, and the Institutional Review Board of Seoul National University Hospital, Seoul, Republic of Korea, approved the collection and application of the biological materials and clinical data for research purposes. Seoul National University has a Federalwide Assurance with the Office for Human Research Protections of the U. S. Department of Health and Human Services.

Amniotic fluid and gastric fluid analysis

Amniotic fluid was obtained by transabdominal amniocentesis and cultured for aerobic and anaerobic bacteria as well as for genital *Mycoplasmas* including *Ureaplasma* species (*Ureaplasma urealyticum* and *Ureaplasma parvum*) and *Mycoplasma hominis* using previously described methods^{19, 20, 61, 63, 64, 70, 75}. The remaining amniotic fluid was centrifuged, and the supernatant was stored at –70°C until assayed. After delivery, gastric fluid was collected by nasogastric intubation for microbiologic examinations of genital *Mycoplasmas* in the neonatal intensive care unit on the day of birth. Polymerase chain reaction (PCR) for *Ureaplasma* species was performed with the same specific primers in amniotic fluid and gastric fluid.

Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 (MMP-8) concentration (>23 ng/mL), as previously reported^{15, 146–155}. Other studies showed that amniotic fluid MMP-8 concentration is a sensitive and specific index of intra-amniotic inflammation^{151, 156–166} and correlates with an amniotic fluid white blood cell count^{156, 158, 159}. MMP-8 concentration in the stored amniotic fluid was measured with a commercially available, enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Little Chalfont, Buckinghamshire, UK) with a sensitivity of 0.3 ng/mL. Both inter- and intra- assay coefficients of variation were $< 10\%$.

Acute histologic chorioamnionitis, funisitis, and neonatal morbidity

Acute histologic chorioamnionitis was diagnosed when inflammatory changes were detected in any part of a placental tissue sample (amnion, chorion-decidua, and chorionic plate)^{149, 150, 152, 154, 167–171}; funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or the Wharton's jelly, according to the criteria outlined in previous reports^{26, 31, 146, 168, 169, 171–176}. Significant neonatal morbidities included: proven congenital neonatal sepsis, respiratory distress syndrome, congenital pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade II), and necrotizing enterocolitis. Criteria for the definition of each condition have been previously reported¹⁷⁷.

Statistical analysis

Comparison of the continuous variables was performed by using the Mann-Whitney U test. Proportions were compared to the Pearson chi-square test or Fisher's exact test. A probability value of $P < 0.05$ was considered to be of statistical significance.

Results

Rate of detection of *Ureaplasma* species in amniotic fluid and gastric fluid

Ureaplasma species were detected by culture or PCR in 18% (18/100) of the amniotic fluid samples but in only 5% (5/100) of the gastric fluid samples. Microorganisms isolated from the amniotic fluid by culture included *Ureaplasma* species (n=18), *Mycoplasma hominis* (n=4), *Escherichia coli* (n=3), and *Candida* species (n=2). None of the patients had *Ureaplasma* species detected in the amniotic fluid along with other microorganisms. Of the 18 cases with a positive culture or PCR for *Ureaplasma* species in the amniotic fluid, these microorganisms were identified in only 27.8% (5/18) of the samples of gastric fluid. However, among the 73 cases with a negative culture or PCR for *Ureaplasma* species in the amniotic fluid, none of these microorganisms were identified in the gastric fluid samples. Table I describes the clinical characteristics of patients according to the presence of *Ureaplasma* species in the amniotic fluid or gastric fluid.

Classification according to the detection of *Ureaplasma* species

Group 1 comprised patients with a negative culture or PCR for *Ureaplasma* species in both amniotic fluid and gastric fluid samples. Group 2 consisted of patients with a positive culture or PCR for *Ureaplasma* species in the amniotic fluid but negative in gastric fluid. Group 3 included patients with a positive culture or PCR for *Ureaplasma* species in both

amniotic fluid and gastric fluid. Group 4 included patients with a positive culture for other microorganisms but not *Ureaplasma* species in the amniotic fluid. The organisms identified were *Mycoplasma hominis* (n=4), *Escherichia coli* (n=3), and *Candida* species (n=2).

Comparison of pregnancy and neonatal outcomes among the groups

Table II shows the pregnancy and neonatal outcomes of the study population among the groups. Patients in Group 2 (patients with a culture or PCR result positive for *Ureaplasma* species in the amniotic fluid but negative in gastric fluid) had significantly higher median amniotic fluid MMP-8 concentrations and a higher rate of intra-amniotic inflammation, acute histologic chorioamnionitis, and neonatal death than those in Group 1 (patients with a culture or PCR result negative for *Ureaplasma* in both amniotic fluid and gastric fluid) ($P < 0.05$ for each). However, no significant difference in perinatal outcomes was observed between Group 2 (patients with a positive culture or PCR for *Ureaplasma* species in the amniotic fluid but negative in gastric fluid) and Group 3 (patients with a positive culture or PCR for *Ureaplasma* species in both amniotic fluid and gastric fluid samples) ($P > 0.05$ for each).

Discussion

Principal findings of the study: 1) *Ureaplasma* species were detected by culture or PCR in 18% (18/100) of the amniotic fluid samples and in 5% (5/100) of the gastric fluid samples; 2) among the positive amniotic fluid cases for *Ureaplasma* species, these microorganisms were identified in 27.8% (5/18) of the gastric fluid samples; 3) none of the cases that tested negative for *Ureaplasma* species in the amniotic fluid were found to be positive for these microorganisms in the gastric fluid; 4) patients with amniotic fluid positive for *Ureaplasma* species but with gastric fluid negative for these microorganisms had a significantly higher rate of intra-amniotic inflammation, acute histologic chorioamnionitis, and neonatal death than those with both amniotic fluid and gastric fluid results negative for *Ureaplasma* species; and 5) no significant differences were observed in the rate of intra-amniotic inflammation, acute histologic chorioamnionitis, and neonatal death between patients whose amniotic fluid tested positive and gastric fluid tested negative for *Ureaplasma* species and those with positive results for *Ureaplasma* species in the amniotic fluid and gastric fluid.

Clinical implications of this study

Collection of gastric fluid by nasogastric intubation at birth is easier and less invasive than transabdominal amniocentesis. Since microorganisms isolated from gastric fluid are associated with adverse neonatal outcomes^{139, 140, 142–145, 178–197}, we explored whether gastric fluid could be used as an indicator of the microbiology of amniotic fluid. We focused on *Ureaplasma* species because these are the most common microorganisms isolated from the amniotic cavity in patients with preterm labor and intact membranes^{8, 20, 38–48} and preterm PROM^{15, 19, 44, 47–67, 198}. This study demonstrates that the isolation of *Ureaplasma* species from the gastric fluid of preterm neonates at birth missed more than two-thirds of cases presenting microbial invasion of the amniotic cavity with these microorganisms. In other words, relying on gastric fluid examination can identify only one-third of all cases

with intra-amniotic infection. Therefore, amniotic fluid analysis is preferable for the reliable detection of microbial invasion of the amniotic cavity.

Why is the analysis of gastric fluid unable to detect microorganisms in amniotic fluid?

Neonatal gastric fluid results from the ingestion of amniotic fluid^{133–137}; therefore, it should reflect the microbiologic content of amniotic fluid. Several explanations can be invoked from our observations that gastric fluid does not reflect amniotic fluid microbial content. First, antibiotics were used between amniocentesis and delivery for the treatment of some patients with possible intra-amniotic infection. Group 2 consisted of patients with a culture or PCR result positive for *Ureaplasma* species in amniotic fluid but negative for these microorganisms in gastric fluid. As shown in Table II, fewer cases delivered within 24 hours of amniocentesis in Group 2 (35.8%; 5/13) than in Group 1 (63%; 46/73) or Group 3 (80%; 4/5) although we restricted the time interval between amniocentesis and delivery to within a week. Thus, longer exposure to antibiotics occurred in some patients in Group 2 as compared to those in Group 1 or Group 3. It is possible that *Ureaplasma* species were not detected in gastric fluid due to eradication by antibiotics before delivery or, alternatively, because anti-microbial administration prevented growth of the microorganisms in culture. However, the latter explanation cannot apply as molecular microbiologic techniques were used in this study. Previous studies have shown that eradication of *Ureaplasma urealyticum* with antibiotics in patients with preterm PROM^{199, 200}, preterm labor with intact membranes^{201, 202} or a sonographic short cervix is possible⁷². Second, the bactericidal effect of gastric fluid does not appear until the pH is less than 2.5^{203, 204}. The neonatal gastric pH is about 6.2 at birth in term neonates. Moreover, the pH of gastric fluid is higher (about 7.0) in preterm neonates regardless of the mode of delivery²⁰⁵. The gastric fluid pH gradually decreases within days after birth²⁰⁶. Therefore, the discrepancy between *Ureaplasma* species detection in the amniotic fluid and gastric fluid cannot be attributed to the effect of pH. Third, there were no cases with a culture or PCR result negative for *Ureaplasma* species in the amniotic fluid with a corresponding positive result in the gastric fluid. Theoretically, microorganisms acquired during passage through the birth canal can be detected in gastric fluid despite the absence of intra-amniotic infection. However, our data do not support the frequent occurrence of or a major role for this phenomenon.

Limitations of this study and proposals for future research

First, in this retrospective study, we analyzed data for only *Ureaplasma* species, and cultures of microorganisms other than genital *Mycoplasmas* were not performed in gastric fluid (since *Ureaplasma* species are the most common microorganisms isolated in intra-amniotic infection). Second, this study was confined to a comparative detection of microorganisms in the amniotic fluid and gastric fluid by culture or PCR. Future studies are required to evaluate whether inflammation in gastric fluid correlates with intra-amniotic inflammation. It will also be important to determine the relationship between inflammatory proteins or mediators in gastric fluid and adverse pregnancy outcomes among cases without microorganisms in gastric fluid.

Conclusions

Gastric fluid analysis has 100% specificity in the identification of intra-amniotic infection with *Ureaplasma* species. However the detection of *Ureaplasma* species by culture or PCR in the gastric fluid of neonates at birth did not identify two-thirds of cases with microbial invasion of the amniotic cavity and these microorganisms. Therefore, a culture or PCR result negative for *Ureaplasma* species in neonatal gastric fluid is not indicative of the absence of intra-amniotic infection with these bacteria. Patients who test positive for *Ureaplasma* species in amniotic fluid but negative for these bacteria in gastric fluid are at risk for adverse outcomes. Gastric fluid is easy to obtain and may reflect amniotic fluid composition; however, gastric fluid cannot replace amniotic fluid in the identification of intra-amniotic infection.

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Table I

Characteristics of the study population. Classification according to the detection of *Ureaplasma* species

	Group 1 (n=73)	Group 2 (n=13)	Group 3 (n=5)	Group 4 ^a (n=9)
Detection of <i>Ureaplasma</i> species in amniotic fluid	No	Yes	Yes	No
Detection of <i>Ureaplasma</i> species in gastric fluid	No	No	Yes	No

^aGroup 4: Amniotic fluid and gastric fluid negative for *Ureaplasma* species but positive for other microorganisms: *Mycoplasma hominis* (n=4), *Escherichia coli* (n=3), and *Candida* species (n=2)

Table II
Comparison of clinical characteristics and pregnancy outcomes of the study population

Characteristics	Group 1 (n=73)	p ^d -value	Group 2 (n=13)	p ^d -value	Group 3 (n=5)	Group 4 (n=9)
Age (years) ^c	32 (25–42)	0.952	32 (28–37)	0.804	31 (24–38)	35 (32–38)
Nulliparity (n)	46 (6%)	0.766	53 (8%)	0.596	80%	33 (3%)
Gestational age at amniocentesis (weeks) ^c	29.1 (23.1–34.4)	0.673	29.1 (24.6–33.9)	0.068	26.4 (24.0–26.6)	27.7 (24.6–34.0)
Amniocentesis to delivery interval (hours) ^c	7.8 (0–167.8)	0.149	34.5 (0–149.8)	0.256	4.2 (0–144.7)	12 (0–167.6)
• Nearly 0 (n)	33 (45.2%)	0.065	2 (15.4%)	>0.99	1 (20%)	2 (22.2%)
• < 24 hours (n)	46 (63%)	0.128	5 (38.5%)	0.294	4 (80%)	6 (66.7%)
Intra-amniotic inflammation (n/N)	24/72 (33.3%)	0.017	9/13 (69.2%)	0.278	5/5 (100%)	7/8 (87.5%)
Amniotic fluid MMP-8 (ng/mL) ^c	7.0 (0.1–5304.8)	0.035	415.2 (0.3–2818.1)	0.301	918.4 (103.3–2549.0)	625.2 (20.9–2106.9)
Amniotic fluid white blood cell count (WBC)	1 (0–9900)	0.033	1300 (0–10000)	0.804	423 (105–4300)	862 (51–50000)
Acute histologic chorioamnionitis (n/N)	27/72 (37.5%)	0.034	9/13 (69.2%)	>0.99	4/5 (80%)	9/9 (100%)
Funisitis (n/N)	4/72 (5.6%)	0.573	1/13 (7.7%)	0.172	2/5 (40%)	3/9 (33.3%)
Birthweight (grams) ^c	1230 (420–2690)	0.559	980 (630–2080)	0.554	980 (670–1120)	1120 (760–2080)
1-min Apgar score <7	72.6%	>0.99	76.9%	0.522	100%	77.8%
5-min Apgar score <7	39.7%	0.374	53.8%	>0.99	60%	44.4%
Neonatal survival (n/N)	71/72 ^d (98.6%)	0.037	11/13 (84.6%)	0.510	5/5 (100%)	8/9 (88.9%)
Significant morbidities (n/N)	41/71 ^e (57.7%)	0.805	7/13 (53.8%)	0.324	4/5 (80%)	5/9 (55.6%)
Bronchopulmonary dysplasia	29/71 ^e (40.8%)	0.668	5/12 ^f (41.7%)	0.278	4/5 (80%)	5/9 (55.6%)

MMP-8: matrix metalloproteinase-8.

Group 1: negative amniotic fluid and negative gastric fluid for *Ureaplasma* species; Group 2: positive amniotic fluid but negative gastric fluid for *Ureaplasma* species; Group 3: positive amniotic fluid and positive gastric fluid for *Ureaplasma* species; Group 4: negative amniotic fluid and gastric fluid for *Ureaplasma* species but positive for other microorganisms: *Mycoplasma hominis* (n=4), *Escherichia coli* (n=3), and *Candida* species (n=2).

Significant neonatal morbidities: proven congenital neonatal sepsis, respiratory distress syndrome, congenital pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade II), and necrotizing enterocolitis.

^aComparison between groups 1 and 2.

^bComparison between groups 2 and 3.

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^cValues are given as median (range).

^dA case of Down syndrome was excluded.

^eCases of neonatal death shortly after birth (< 1 day) and Down syndrome were excluded.

^fA case of perinatal death (< 1 month) was excluded.