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INTRA-AMNIOTIC INFECTION WITH GENITAL MYCOPLASMAS EXHIBITS MORE INTENSE INFLAMMATORY RESPONSE THAN INTRA-AMNIOTIC INFECTION WITH OTHER MICROORGANISMS IN PATIENTS WITH PRETERM PREMATURE RUPTURE OF MEMBRANES

Kyung Joon OH, MD¹, Kyung A LEE, MD¹, Yoo-Kyung SOHN, MD¹, Chan-Wook PARK, MD¹, Joon-Seok HONG, MD¹, Roberto ROMERO, MD², and Bo Hyun YOON, MD, PhD¹

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

² Perinatology Research Branch, National Institute of Child Health & Human Development/ National Institutes of Health/Department of Health & Human services, Bethesda, Maryland, Detroit, Michigan

Abstract

OBJECTIVE: To compare the intensity of inflammatory responses between intra-amniotic infection with genital mycoplasmas and intra-amniotic infection with other microorganisms.

STUDY DESIGN: We examined the intensity of intra-amniotic and maternal inflammatory responses in 99 patients with preterm premature rupture of membranes (PROM) and a positive amniotic fluid (AF) culture. AF was obtained by transabdominal amniocentesis or at the time of cesarean delivery. Patients were divided according to the recovered microorganisms: 1) genital mycoplasmas (n=62); 2) other microorganisms (n=31); 3) mixed infection (n=6).

RESULTS: The median AF white blood cell (WBC) count, maternal blood WBC count and plasma C-reactive protein concentration were significantly higher in cases with intra-amniotic infection with genital mycoplasmas than in those with intra-amniotic infection with other microorganisms ($p < 0.05$ for each).

CONCLUSION: Intra-amniotic and maternal inflammatory responses are more intense in intra-amniotic infection with genital mycoplasmas than in intra-amniotic infection with other microorganisms in patients with preterm PROM.

Correspondence to: Bo Hyun Yoon, MD, PhD, Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, 110-744, Korea. Tel: 82-2-2702-2826, Fax: 82-2-765-3002, Yoonbh@snu.ac.kr.

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Keywords

intra-amniotic infection; genital mycoplasmas; ureaplasmas; intra-amniotic inflammation; preterm premature rupture of membranes

Introduction

Intra-amniotic infection is causally linked to spontaneous preterm labor/delivery and fetal injury.^{1,2} Moreover, neonates born with evidence of a fetal systemic inflammatory response syndrome are at increased risk for short-term and long-term morbidity.^{3–6}

Genital mycoplasmas (ureaplasmas and *Mycoplasma hominis*) are the most common organisms isolated from the amniotic fluid (AF) of women with preterm premature rupture of membranes (PROM)^{7,8} and preterm labor with intact membranes,^{9,10} as well as women with cervical insufficiency.^{11,12} Their presence in AF is associated with a shorter interval to delivery (in women with preterm labor and intact membranes^{9,13} as well as preterm PROM¹⁴) and a higher rate of neonatal morbidity^{8,9,13,14} than patients with a negative amniotic fluid for microorganisms. Of interest is the recent observation by Goldenberg et al.¹⁵ that approximately 20% of neonates born before 32 weeks of gestation have positive blood cultures for genital mycoplasmas. Viscardi et al.¹⁶ found that 23% of very low birthweight infants have ureaplasmas retrieved from venous blood or cerebrospinal fluid using polymerase chain reaction (PCR) techniques, and that the presence of microbial footprints is associated with an elevated concentration of interleukin-1 beta.

The traditional view has been that genital mycoplasmas are commensal in the lower genital tract of normal pregnant and non-pregnant women, and that these organisms have low virulence. Moreover, the mere presence of these organisms in the lower genital tract does not increase the risk for upper genital infection in non-pregnant women^{17,18} nor the risk for preterm delivery or adverse neonatal outcome in pregnant women.^{19,20} Indeed, some investigators^{21,22} suggested that the significance of ureaplasmas is mainly its association with other microorganisms.

Recent observations suggest that microbial products from ureaplasmas have a similar capacity than those of Gram-negative bacteria (lipopolysaccharide or bacterial endotoxin) to induce an inflammatory response in vitro in choriodecidual explants.²³ Indeed, AF interleukin-8 concentration at birth was higher in patients with positive AF cultures or evidence of placental invasion with ureaplasmas than in those with other microorganisms.²⁴

A major gap in knowledge is whether or not the inflammatory response of the amniotic cavity and/or the mother is different when intra-amniotic infection is due solely to genital mycoplasmas or with other microorganisms. The current study was designed to address this question in humans, and specifically in a population of patients with preterm PROM.

Material and Methods

Study Design:

This study population consisted of 99 patients admitted to Seoul National University Hospital between Jan 1993 and Mar 2008 with the diagnosis of preterm PROM (<37 weeks of gestation) who met the following criteria: (1) singleton pregnancy, (2) AF obtained for microbiologic studies by transabdominal amniocentesis or at the time of cesarean delivery, (3) Proven AF infection by culture. Patients were divided into three groups according to the microorganisms isolated from AF: group 1, cases with a positive AF culture for genital mycoplasmas including ureaplasmas (*U urealyticum* and *U parvum*) and *M hominis* (n=62); group 2, cases with a positive AF culture for other microorganisms (n=31); group 3, cases with mixed infection with genital mycoplasmas and other microorganisms (n=6). The intensity of the intra-amniotic inflammatory response was determined by the AF white blood cell (WBC) count, and that of maternal inflammatory response was by the concentration of C-reactive protein (CRP) and WBC count in maternal blood at the time of amniocentesis.

Amniocentesis is routinely offered for microbiologic studies and assessment of fetal lung maturity to all patients who are admitted with the diagnosis of preterm PROM. Retrieval of AF and maternal blood was performed after written informed consent was obtained. The Institutional Review Board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

AF studies:

After amniocentesis, amniotic fluid in a capped plastic tube and commercialized culture media was transported immediately to the clinical laboratory of our hospital. Amniotic fluid was cultured for genital mycoplasmas (ureaplasmas and *M hominis*) and for aerobic and anaerobic bacteria. An aliquot of AF was examined in a hemocytometer chamber to determine the white blood cell (WBC) count.

Maternal plasma C-reactive protein (CRP):

Maternal blood was collected into ethylenediaminetetraacetic acid-containing tubes by venipuncture at the time of amniocentesis. Samples were centrifuged, and supernatants were stored in polypropylene tubes at -70°C until assayed. CRP concentration was measured with a highly sensitive enzyme-linked immunoassay (Immunodiagnostik AG, Bensheim, Germany). The sensitivity of the assay was 0.3 ng/mL. Intra- and interassay coefficients of variation were <10%.

Diagnosis of histologic chorioamnionitis, funisitis and clinical chorioamnionitis:

Histologic chorioamnionitis was defined in the presence of acute inflammatory changes on examination of a membrane roll and/or chorionic plate of the placenta; funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly with the use of criteria previously reported.²⁵ Clinical chorioamnionitis was diagnosed when maternal temperature was elevated to 37.8°C and 2 of the following

criteria were present: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis ($> 15,000$ cells/mm³), maternal tachycardia (> 100 beats/minute), and fetal tachycardia (> 160 beats/minute).

Statistical analysis:

Proportions were compared with the Fisher's exact test. The Mann-Whitney *U* test and Kruskal-Wallis analysis were used for comparison of continuous variables. The generalized Wilcoxon test for survival analysis was used to compare the interval between amniocentesis and delivery. Statistical significance was defined as a probability value of $< .05$.

Results

Characteristics of the study population:

A total of 99 cases with a positive AF culture met the inclusion criteria of the study. Table 1 shows types of microorganisms that were isolated from AF. Ureaplasmas were the most common isolates (64/99). Table 2 compares characteristics and the outcomes of the study population. There were no significant differences in the clinical characteristics and pregnancy outcomes including age, parity, gestational age at amniocentesis, rupture of membranes-to-amniocentesis interval, gestational age at birth, birthweight and use of corticosteroids and antibiotics before amniocentesis between patients with intra-amniotic infection due solely to genital mycoplasmas (group 1) and those with intra-amniotic infection caused by other microorganisms (group 2).

Intra-amniotic and maternal inflammatory responses:

Table 3 shows the intra-amniotic and maternal inflammatory responses according to the microorganisms isolated from the amniotic cavity. Patients in group 1 had significantly higher median AF WBC count, maternal blood WBC count and maternal plasma CRP concentration than those in group 2 ($p < 0.05$, respectively). Patients in group 3 had a significantly higher median AF WBC count than those in groups 1 and 2 ($p < .01$ and $< .005$, respectively).

Figure 1 shows detailed information about the intra-amniotic inflammatory response according to the microorganisms isolated from the AF (data of cases with infection with genital mycoplasmas and other microorganisms were not shown). Even if other microorganisms were subdivided into other pathogens and skin contaminants as other studies,^{24,26} intra-amniotic infection with genital mycoplasmas had a significantly higher median AF WBC count than did intra-amniotic infection with other pathogens ($p < 0.05$). The median maternal blood WBC count and maternal plasma CRP concentration were higher in patients with intra-amniotic infection with genital mycoplasmas than in those with intra-amniotic infection with other pathogens excluding skin contaminants (Figure 2). Yet, this difference fell short of reaching statistical significance ($p = 0.051$ and 0.053 , respectively).

Table 4 displays the indices of an inflammatory response according to the microorganisms isolated from the AF in patients whose intervals between rupture of membranes and amniocentesis was shorter than 24 hours. Patients with intra-amniotic infection with genital

mycoplasmas had a significantly higher median AF WBC count and maternal plasma CRP concentration than those with intra-amniotic infection with other microorganisms ($p < 0.05$, respectively).

Comment

Principal finding of this study:

Intra-amniotic infection with genital mycoplasmas has a more intense intra-amniotic inflammatory response (determined by an AF WBC count) and maternal inflammatory response (determined by the maternal blood WBC count and plasma CRP concentration) than did intra-amniotic infection with other microorganisms in patients with preterm PROM.

Genital mycoplasmas and host inflammatory response in preterm PROM:

Our findings indicate that genital mycoplasmas, traditionally considered to be of low virulence, elicit a more intense host inflammatory response than did other microorganisms in patients with preterm PROM. Our previous study⁸ and that of other investigators⁷ have demonstrated that the AF WBC count is much higher in patients with intra-amniotic infection with genital mycoplasmas than in those without intra-amniotic infection in patients with preterm PROM. In these studies,^{7,8} the degree of elevation of the AF WBC count was not significantly different between women with intra-amniotic infection due to genital mycoplasmas and those with intra-amniotic infection caused by other microorganisms, though it was higher in intra-amniotic infection with genital mycoplasmas (these results were based on cultivation techniques). Possible explanations for this apparent discrepancy between the results of the current study and previous studies^{7,8} include the following: 1) previous studies have included genital mycoplasmas if other microorganisms are isolated from the amniotic cavity (an infection caused by *E coli* and a genital mycoplasma would be classified in the non-mycoplasma group). In the current study, we subdivided such cases into the group of mixed infection (genital mycoplasmas plus other microorganisms) to maintain the purity of those with genital mycoplasma infections; 2) the sample size of the current study is larger than that of previous studies.

Our results are partially consistent with those of Witt et al.²⁴ in which the AF interleukin-8 concentration, but not maternal blood WBC count and CRP concentration, was higher in patients with intrauterine infection with ureaplasmas than in intrauterine infection caused by other microorganisms. However, there are differences between the current study and that of Witt et al.²⁴ They defined intra-uterine infection as the presence of a positive culture of the AF and/or placental tissue.

Genital mycoplasmas and intra-amniotic inflammation:

In the present study, the AF WBC count was significantly higher in intra-amniotic infection caused by genital mycoplasmas than in intra-amniotic infection with other microorganisms. Our previous study²⁷ showed that the intensity of intra-amniotic inflammation determined by the AF WBC count correlates well with the severity of inflammation of amnion, chorion-decidua and umbilical cord. The occurrence of an intense inflammatory response may be a function of the inoculum size and/or the degree of host response to the stimulus. Findings of

other investigators^{7,28,29} demonstrate that the inoculum size plays a role in determining the intensity of the inflammatory response. Therefore, one potential explanation for the higher severity of intra-amniotic inflammation with genital mycoplasmas is that the microbial inoculum size is larger in these cases. The difference in the colony count or number of bacteria may be caused by variations in the duration of the intra-amniotic infection or the frequency of secondary infection after the occurrence of rupture of membranes.

Another possible explanation is that chronic exposure to genital mycoplasmas may modulate and accentuate the immune response to bacterial antigens. One recent study³⁰ about fetal lung inflammation in an animal model showed that repeated intra-amniotic injection of *U. parvum* caused a modest but persistent increase in the recruitment of inflammatory cells even though the colony count of ureaplasmas did not change over time. It is of interest that mycoplasmas are able not only to stimulate but also to suppress the host inflammatory response.³¹ It is unclear at this point how the chronic and repetitive exposure of the host by genital mycoplasmas can affect the natural history of the intra-amniotic inflammatory response.

Genital mycoplasmas and maternal systemic inflammation:

Our results showed that genital mycoplasmas induce more intense maternal systemic inflammation determined by the maternal blood WBC count and the plasma CRP concentration than did other microorganisms in patients with preterm PROM. In the case of intrauterine infection, cytokines produced within the uterus first mount a local inflammatory response and then may reach the maternal circulation and stimulate the liver to produce CRP by hepatocytes or bone marrow targets to produce leukocytes.³² Therefore, chronic infection by genital mycoplasmas may have a greater opportunity to gain access to a maternal systemic compartment than an intra-uterine infection caused by organisms which are more virulent and elicit a rapid and intense acute inflammatory response. This interpretation would be consistent with the findings of other studies in which genital mycoplasmas were found frequently in the bloodstream of obstetric patients.^{33,34}

Interval between amniocentesis and delivery:

In the current study, intra-amniotic infection with genital mycoplasmas elicited a more intense inflammatory response detected at the time of amniocentesis than that associated with microbial invasion by other microorganisms. However, the interval between amniocentesis and delivery was not statistically significant between groups. One possible explanation is that chemokines and cytokines associated with labor may be regulated differently according to the type of microorganisms involved. A recent in vitro study³⁵ examined whether prostaglandin (PG) synthesis is different between infections caused by mycoplasmas and those caused by other microorganisms. Menon et al.³⁵ reported that ureaplasmas had no detectable effect on PG concentrations, though it raised TNF- α concentration; whereas lipopolysaccharide and *E. coli* induced PG production. This may explain why ureaplasmas are frequently found in AF of asymptomatic mid-trimester patients and why ureaplasmas are more associated with preterm PROM than with preterm labor with intact membranes. Other explanations include the response to antibiotics and the rate of progression of the inflammatory response which may be different according to the

microorganisms involved. Indeed, ureaplasmas have a self-limited growth in the AF in an animal model.³⁶

Clinical implication, limitation and further consideration of this study:

The major clinical implication of our study is that intra-amniotic infection with genital mycoplasmas exhibits more intense intra-amniotic and maternal inflammatory responses than did intra-amniotic infection with other microorganisms. Our observations suggest that infections with genital mycoplasmas may have deleterious effects despite the conventional wisdom that these organisms have low virulence. It is tempting to postulate that this occurs because infections with genital mycoplasmas have a more chronic course, and therefore allow for long-term exposure of the fetus and amniotic fluid to the microbial products produced by mycoplasmas. However, this study could not determine whether intra-amniotic infection with genital mycoplasmas is associated with a different type of neonatal morbidity compared with that of other microorganisms. A large cohort investigation is required to determine whether neonatal outcome is different according to the microorganisms isolated from AF.

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Condensation

Intra-amniotic infection with genital mycoplasmas exhibits a more intense inflammatory response than intra-amniotic infection with other microorganisms in patients with preterm premature rupture of membranes.

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Figure 1.

Amniotic fluid (AF) white blood cell (WBC) counts according to each of the microorganisms isolated from AF (data of cases with mixed infection with genital mycoplasmas and other microorganisms were not shown). GBS; group B *Streptococcus*, SAG; *Streptococcus anginosus* group, BCC; *Burkholderia cepacia complex*, GDS; group D *Streptococcus*, CoNS; coagulase negative *Staphylococcus*, α -HS; alpha-hemolytic *Streptococcus*. AF WBC counts (intra-amniotic infection with genital mycoplasmas: median, 306 cells/mm³ [range 0 to >1000 cells/mm³]; intra-amniotic infection with other pathogens: median, 83 cells/mm³ [range 0 to >1000 cells/mm³]; intra-amniotic infection with skin contaminants: median, 69 cells/mm³ [range 0 to >1000 cells/mm³]; $p < 0.05$, by Kruskal-Wallis analysis of variance test).

Figure 2.

Maternal blood white blood cell (WBC) counts and maternal plasma C-reactive protein (CRP) concentrations according to each of the microorganisms isolated from amniotic fluid (AF) (data of cases with mixed infection with genital mycoplasmas and other microorganisms were not shown). GBS; group B *Streptococcus*, SAG; *Streptococcus anginosus* group, BCC; *Burkholderia cepacia* complex, GDS; group D *Streptococcus*, CoNS; coagulase negative *Staphylococcus*, α-HS; alpha-hemolytic *Streptococcus*. A, Maternal blood WBC counts (intra-amniotic infection with genital mycoplasmas: median,

11900 cells/mm³ [range, 3390–20800 cells/mm³]; intra-amniotic infection with other pathogens: median, 10410 cells/mm³ [range, 1300–16500 cells/mm³]; intra-amniotic infection with skin contaminants: median, 10570 cells/mm³ [range, 7400–15600 cells/mm³]; p=0.10, by Kruskal-Wallis analysis of variance test). B, Maternal plasma CRP concentrations (intra-amniotic infection with genital mycoplasmas: median, 0.69 mg/dL [range 0.01–4.42 mg/dL]; intra-amniotic infection with other pathogens: median, 0.17 mg/dL [range 0.04–9.39 mg/dL]; intra-amniotic infection with skin contaminants: median, 0.29 mg/dL [range 0.03–2.60 mg/dL]; p=0.11, by Kruskal-Wallis analysis of variance test).

Table 1.

Types of microorganisms that were isolated from amniotic fluid

Microorganism	Number of cases
Group 1 (intra-amniotic infection with genital mycoplasmas)	
Ureaplasmas	57
<i>Mycoplasma hominis</i>	3
Ureaplasmas and <i>M hominis</i>	2
Group 2 (intra-amniotic infection with other microorganisms)	
<i>Candida</i> species	8
Coagulase-negative <i>Staphylococcus</i> (CoNS)	5
<i>Streptococcus anginosus</i> group	3
Group B <i>Streptococcus</i>	2
<i>Klebsiella pneumoniae</i>	2
<i>Burkholderia cepacia</i> complex	2
<i>Corynebacterium</i> species	2
<i>Escherichia coli</i>	1
<i>Peptostreptococcus</i> species	1
Group D <i>Streptococcus</i>	1
<i>Lactobacillus</i> species	1
Alpha hemolytic <i>Streptococcus</i>	1
<i>E coli</i> , <i>Acinetobacter baumannii</i> and CoNS	1
<i>Candida</i> species, <i>Staphylococcus aureus</i> and <i>A baumannii</i>	1
Group 3 (mixed infection with genital mycoplasmas and other microorganisms)	
Ureaplasmas and <i>E coli</i>	1
Ureaplasmas and <i>S anginosus</i> group	1
Ureaplasmas and alpha hemolytic <i>Streptococcus</i>	1
Ureaplasmas, <i>Candida</i> species and <i>Streptococcus urevis</i>	1
Ureaplasmas, <i>S anginosus</i> group and CoNS	1
<i>M hominis</i> and <i>E coli</i>	1

Clinical characteristics and pregnancy outcomes of the study population according to the microorganisms isolated from amniotic fluid (AF)

Table 2.

Characteristics	Positive AF culture for genital mycoplasmas (group 1; n=62)	<i>P^a</i>	Positive AF culture for other microorganisms (group 2; n=31)	<i>P^b</i>	Positive AF culture for genital mycoplasmas and other microorganisms (group 3; n=6)	<i>P^c</i>
Maternal age (y) ^d	30.2 ± 4.2	NS	30.7 ± 4.7	NS	29.2 ± 5.0	NS
Nulliparity (n)	27 (44%)	NS	12 (39%)	NS	3 (50%)	NS
Gestational age at amniocentesis (weeks) ^d	30.6 ± 4.4	NS	29.4 ± 4.8	NS	29.6 ± 3.2	NS
Gestational age at birth (weeks) ^{de}	31.5 ± 4.1	NS	30.5 ± 4.9	NS	29.9 ± 2.8	NS
Interval between rupture of membranes and amniocentesis (hours) ^d	65.7 ± 102.3	NS	119.9 ± 174.5	NS	41.0 ± 36.7	NS
Interval between amniocentesis and delivery (hours) ^{de}	129.8 ± 165.9	NS	183.1 ± 475.2	.09	51.6 ± 92.8	<.05
Corticosteroids use before amniocentesis (n)	9 (15%)	.06	10 (32%)	NS	0	NS
Antibiotics use before amniocentesis (n)	30 (48%)	NS	19 (61%)	NS	4 (67%)	NS
Body temperature at amniocentesis (°C) ^d	36.9 ± 0.5	NS	36.8 ± 0.5	<.05	37.2 ± 0.3	<.05
Clinical chorioamnionitis at amniocentesis (n)	2 (3%)	NS	1 (3%)	NS	0	NS
Birthweight (g) ^{df}	1739 ± 643	NS	1561 ± 767	NS	1432 ± 434	NS
Histologic chorioamnionitis (n/N)	43/51 (84%)	NS	15/21 (71%)	NS	5/5 (100%)	NS
Funisitis (n/N)	29/51 (57%)	NS	12/22 (55%)	NS	3/5 (60%)	NS

NS, not significant.

^aComparison between groups 1 and 2.^bComparison between groups 2 and 3.^cComparison between groups 1 and 3.^dValues are given as mean ± SD.^eOne patient lost to follow-up was excluded.^fTwo neonates whose birthweights were unknown were excluded.

Table 3.

The intra-amniotic and maternal inflammatory responses according to the microorganisms isolated from amniotic fluid (AF)

Characteristics	Positive AF culture for genital mycoplasmas (group 1; n=62)	P^a	Positive AF culture for other microorganisms (group 2; n=31)	P^b	Positive AF culture for genital mycoplasmas and other microorganisms (group 3; n=6)	P^c
AF WBC count (cells/mm ³)	306 (0–>1000)	<.05	83 (0–>1000)	<.005	>1000 (405–>1000)	<.01
Maternal blood WBC count (cells/mm ³)	11900 (3390–20800)	<.05	10440 (1300–16500)	NS	14670 (6900–23800)	NS
Maternal plasma CRP concentration (mg/dL)	0.69 (0.01–4.42)	<.05	0.20 (0.03–9.39)	<.05	1.07 (0.28–4.79)	NS

Values are given as median (range).

NS, not significant; WBC, white blood cell; CRP, C-reactive protein.

^aComparison between groups 1 and 2.

^bComparison between groups 2 and 3.

^cComparison between groups 1 and 3.

Table 4.

The intra-amniotic and maternal inflammatory responses according to the microorganisms isolated from amniotic fluid (AF) among patients whose interval between rupture of membranes and amniocentesis was shorter than 24 hours

Characteristics	Positive AF culture for genital mycoplasmas (group 1; n=34)	P^a	Positive AF culture for other microorganisms (group 2; n=14)	P^b	Positive AF culture for genital mycoplasmas and other microorganisms (group 3; n=3)	P^c
AF WBC count (cells/mm ³)	306 (0-→1000)	<.05	52 (0-864)	<.05	>1000 (612-→1000)	NS
Maternal blood WBC count (cells/mm ³)	11605 (3390-17910)	NS	10440 (6900-15930)	NS	13410 (6900-15930)	NS
Maternal plasma CRP concentration (mg/dL)	0.69 (0.06-4.42)	<.05	0.18 (0.03-2.48)	NS	0.66 (0.28-4.79)	NS

Values are given as median (range).

NS, not significant; WBC, white blood cell; CRP, C-reactive protein.

^aComparison between groups 1 and 2.

^bComparison between groups 2 and 3.

^cComparison between groups 1 and 3.