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The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis in preterm gestations

Sun Min KIM[1], **Roberto ROMERO**[2], **Jeong Woo PARK**[1], **Kyung Joon OH**[1], **Jong Kwan JUN**[1], and **Bo Hyun YOON**[1]

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

[2]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD and Detroit, MI, USA

Abstract

OBJECTIVE—Acute histologic chorioamnionitis (HCA) is associated with increased risk of perinatal mortality and morbiditiy. The purpose of this study was to determine the relationship between the intensity of intra-amniotic inflammation (IAI) and the severity of acute HCA in preterm gestation.

METHODS—The relationship between the intensity of IAI and the presence and severity of acute HCA was examined in 412 singleton patients who delivered within 120 hours of transabdominal amniocentesis. The concentration of amniotic fluid (AF) metalloproteinase (MMP)-8 was assayed to determine the presence and intensity of IAI. Acute HCA was defined as the presence of inflammatory change in any tissue samples according to the criteria previously reported (AJOG 1995;172:960–70). The total grade of acute HCA was used to determine the severity of HCA.

RESULTS—1) Patients with IAI had a significantly higher rate of acute HCA than those without IAI [76.9% (133/173)] vs 20.9% (50/239), $P<0.001$]. The AF MMP-8 concentration was significantly higher in patients with acute HCA than in those without acute HCA (median[range]; 188.3ng/mL[0.3–6142.6] vs 1.8ng/mL[0.3–2845.5], $P<0.001$); 2) of 183 patients with acute HCA, AF MMP-8 concentration was positively correlated with severity of acute HCA($P<0.001$).

CONCLUSIONS—AF MMP-8 concentration was not only a predictor of presence of acute HCA, but its concentration also correlated with the severity of acute HCA. The higher the intensity of IAI, the worse the degree of acute HCA in preterm gestation.

Keywords

Preterm birth; chorioamnionitis; prematurity; amniotic fluid; matrix metalloproteinase-8

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: 82-2-762-3599, yoonbh@snu.ac.kr.

Declaration of Interests

The authors report no conflicts of interest.

Introduction

Acute histologic chorioamnionitis is an inflammatory lesion of placenta frequently observed after a preterm birth. The presence of acute histologic chorioamnionitis is associated with an increased risk of perinatal mortality and morbidity, including neonatal sepsis, bronchopulmonary dysplasia, intraventricular hemorrhage, periventricular leukomalacia and cerebral palsy. [1–22] However, not all newborns whose placentas had acute histologic chorioamnionitis have adverse outcomes. In a previous study, when placental inflammation was divided into anatomical segments (e.g. amnion, choriodecidua and chorionic plate), involvement of the amnion was the most advanced stage of the maternal inflammatory response and a good predictor of early-onset neonatal sepsis.[23]

Intra-amniotic inflammation and/or infection is present in about one-third of patients with preterm labor and intact membranes [24] and in about half of patients with preterm premature rupture of membranes, [25] and it is a risk factor for impending preterm delivery and adverse perinatal outcomes. [3, 7, 13, 24–89] Metalloproteinase-8 (MMP-8) is a matrix-degrading enzyme stored within specific granules of neutrophils and released during activation,[90] and an increased concentration of MMP-8 in the amniotic fluid has been extensively used to define intra-amniotic inflammation.[25, 56–58, 64, 71, 85, 87, 91–101] Thus, this study was performed to analyze the relationship between the concentration of amniotic fluid MMP-8 and the grading of histologic chorioamnionitis to examine the intensity of intra-amniotic inflammation and the severity of acute histologic chorioamnionitis.

Materials and Methods

Study population

The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis was examined in 412 cases delivered at the Seoul National University Hospital, Seoul, Korea between January 1993 and February 2009. Patients included met the following criteria: (1) singleton gestation; (2) preterm birth at gestational age between 24 and 35 weeks; (3) transabdominal amniocentesis for microbiologic studies in amniotic fluid or assessment of fetal lung maturity; (4) delivery within 120 hours of amniocentesis (to preserve a meaningful temporal relationship between the results of amniotic fluid studies and the histologic findings of placenta); and (5) placental histological examination after preterm delivery.

Amniocentesis was performed with written informed consent, and the Institutional Review Board of our institution (Seoul National University Hospital, Seoul, Korea) approved the collection and utilization of the biological materials and clinical data for research purposes. The Seoul National University has a Federal Wide Assurance (FWA) with the Office for Human Research Protections (OHRP) of the Department of Health and Human Services (DHHS) of the United States.

Retrieval of amniotic fluid and amniotic fluid MMP-8 concentration measurements

Amniotic fluid was obtained by transabdominal amniocentesis with ultrasound guide and aseptic technique. Amniotic fluid was cultured for aerobic, anaerobic bacteria and genital mycoplasmas (ureaplasmas [*Ureaplasma urealyticum & Ureaplasma parvum*] and *Mycoplasma hominis*) or used for assessment of fetal lung maturity. The remaining amniotic fluid was centrifuged, and supernatant was stored at −70°C until assayed.

MMP-8 concentrations were measured with commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Little Chalfont, Bucks, UK) with sensitivity of 0.3 ng/mL. Both inter-and intra- assay coefficients of variation were < 10%. Intra-amniotic inflammation was defined as an elevated amniotic fluid MMP-8 concentration (> 23 ng/mL) according to a previous study.[91]

Placental examination

Placental tissue samples were obtained from amnion, choriodecidua, umbilical cord and chorionic plate. These samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin. The degree of acute inflammation was classified as grade 1 or 2 in each tissue (amnion, choriodecidua, umbilical cord and chorionic plate) according to previously published criteria.[4] Grade 1 inflammation of the choriodecidua or amnion was diagnosed as the presence of at least 1 focus of > 5 neutrophils, and grade 2 inflammation of the choriodecidua or amnion was diagnosed as the presence of diffuse neutrophilic inflammation; in the chorionic plate, grade 1 inflammation was diagnosed in the presence of more than 1 focus of at least 10 neutrophilic foci or diffuse inflammation in subchorionic fibrin, and grade 2 inflammation was diagnosed as diffuse and dense inflammation, neutrophilic infiltration into connective tissue of placental plate or placental vasculitis. Acute histologic chorioamnionitis was defined as the presence of inflammatory change in any part of the tissue samples (amnion, choriodecidua and chorionic plate) and the highest total grade of acute histologic chorioamnionitis could be 6 if each score was 2 in all three sections. Funisitis was diagnosed as the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly. Funisitis was classified separately from acute histologic chorioamnionitis as it is a fetal (rather than maternal) inflammatory response.[102–104]

Statistical analysis

Comparison of the continuous variables that could not be assumed as normal distribution was performed by using the Mann-Whitney *U* test. Proportions were compared with the use of the Chi-square test or Fisher's exact test. Among three or more groups, Kruskal-Wallis test and Jonckheere-Terpstra test were used for comparison of continuous variables and linear-by-linear association was used for comparison of the proportions. Logistic regression analysis was used to examine the relationship between the presence of histologic chorioamnionitis and outcome of interest after adjusting for potential confounding factors. A probability value of < 0.05 was considered statistically significant.

Results

Four hundred and twelve patients met the inclusion criteria. The prevalence of acute histologic chorioamnionitis was 44.4% (183/412). The overall rate of intra-amniotic inflammation was 42.0% (173/412); proven intra-amniotic infection was found in 18.5% (74/400). The most common micro-organism cultured from amniotic fluid was genital mycoplasmas (ureaplasmas [*Ureaplasma urealyticum & Ureaplasma parvum*] and *Mycoplasma hominis*) (47/74). Other micro-organisms, such as *Candida spp., Streptococcus spp., Staphylococcus spp., Lactobacillus spp., Corynebacterium spp., Actinetobacter bauman, Klebsiella pneumonia, Burkholderia cepacia, Gardnerella vaginalis, Enterococcus faecalis* and *Escherichia coli* were also isolated.

Patients with intra-amniotic inflammation had a significantly higher rate of acute histologic chorioamnionitis than those without intra-amniotic inflammation (76.9% [133/173] vs 20.9% [50/239], P <0.001). Table 1 presents the characteristics of the study population according to the presence of acute histologic chorioamnionitis. The amniotic fluid MMP-8 concentration was significantly higher in patients with acute histologic chorioamnionitis than in those without histologic chorioamnionitis (median, 188.3 ng/ml [range, 0.3–6142.6] vs 1.8 ng/ml [range, 0.3–2845.5], P <0.001). Intra-amniotic inflammation and infection were also more common in patients with acute histologic chorioamnionitis than in those without histologic chorioamnionitis (72.7% [133/183] vs 17.5% [40/229] and 31.1% [56/180] vs 8.2% [18/220], P <0.001). This difference remained significant after adjusting the gestational age at amniocentesis by logistic regression analysis.

Tables 2 and 3 present the relationship between amniotic fluid MMP-8 concentration and the amniotic fluid white blood cell (WBC) count and prevalence of funisitis, amnionitis, proven intra-amniotic infection and intra-amniotic inflammation according to an increase in the total grade of acute histologic chorioamnionitis. The amniotic fluid concentration of MMP-8 and the amniotic fluid WBC count, the prevalence of funisitis, amnionitis, positive amniotic culture and intra-amniotic inflammation increased significantly as a function of the severity of acute histologic chorioamnionitis (total grade of histologic chorioamnionitis).

There were noticeable differences between acute histologic chorioamnionitis with a total grade 1 and histologic chorioamnionitis with total grade 2 or more. First, patients with a total grade 1 histologic chorioamnionitis accounted for over 40% (78/183) of all cases of acute histologic chorioamnionitis. The prevalence of funisitis was only 19.2% in patients with histologic chorioamnionitis (grade 1), while it was over 50% in patients with acute histologic chorioamnionitis (grade 2 or more). There were no cases of amnionitis in patients with a total grade 1 histologic inflammation. Amnionitis was present in patients with total grade 2 or more histologic chorioamnionitis, and increased as the total grade of histologic chorioamnionitis became higher. The prevalence of intra-amniotic inflammation was only 51.3% in patients with total grade 1 histologic chorioamnionitis, while it was over 80% in patients with total grade 2 or higher acute histologic chorioamnionitis. The median value of amniotic fluid MMP-8 concentrations and amniotic fluid WBC count was also higher in patients with a total grade 2 or more acute histologic chorioamnionitis than in those with grade 1.

When the placenta was examined by region, inflammation was most common in choriodecidua (43.9%), while the frequency of inflammation of the amnion and chorionic plate was 19.9% and 14.6% for each.

Figure 1 shows the amniotic fluid MMP-8 concentration according to the total grade of histologic chorioamnionitis; amniotic fluid MMP-8 concentration was positively correlated with the total grade of acute histologic chorioamnionitis for each (r^2=0.37, P<0.001 by Spearman's rho).

Discussion

Principal finding and strength of this study

The higher the amniotic fluid MMP-8 concentration, the worse the intensity of the inflammatory response in the placenta.

Our study demonstrated that amniotic fluid MMP-8 concentration is an indication of the likelihood and severity of inflammatory changes of the placenta before birth, although the placenta can only be obtained after delivery. Previous studies have suggested that there is an association between intra-amniotic infection and/or inflammation and acute histologic chorioamnionitis, [2, 4, 25, 65, 98, 105–117] but the current study analyzed the quantitative correlation between the intensity of intra-amniotic inflammation (expressed as amniotic fluid MMP-8 concentration) and the severity of histologic chorioamnionitis reflected by histopathologic grading.

Severity of the histologic chorioamnionitis

In a previous report, when placental inflammation was classified as affecting amnion, choriodecidua and chorionic plate, the involvement of amnion reflected the most advanced stage of the maternal inflammatory response and was a good predictor of early-onset neonatal sepsis.[23] In our study, there were no cases of amnionitis in patients with total grade 1 acute histologic chorioamnionitis, but amnionitis began to appear in placentas with a total grade 2 or more histologic chorioamnionitis and its frequency increased significantly as the degree of acute histologic chorioamnionitis worsened. These findings were consistent with the findings of a previous study[23] indicating that amnionitis reflects the most advanced form of inflammation of the extra-placental membranes.

Almost half of cases with histologic chorioamnionitis had a total grade 1 acute histologic chorioamnionitis. Funisitis and intra-amniotic inflammation were more common in patients with total grade 2 or higher of acute histologic chorioamnionitis than those with total grade 1 acute histologic chorioamnionitis, and the median amniotic fluid MMP-8 concentrations or median amniotic fluid WBC counts were significantly elevated in patients with total grade 2 or higher histologic chorioamnionitis. Therefore, we can categorize histologic chorioamnionitis into two groups: mild placental inflammation with a total grade 1 and severe placental inflammation with a total grade 2 or higher.

When placentas were examined by region, inflammation was most common in choriodecidua (43.9% of all cases). Considering that the overall frequency of histologic

chorioamnionitis was 44.4%, this means that inflammation in choriodecidua was present in most cases with acute histologic chorioamnionitis. All cases with total grade 1 histologic chorioamnionitis (except one with inflammation of chorionic plate) had the score for the inflammation because of choriodecidual involvement.

Development and progression of histologic chorioamnionitis

The pathway of intrauterine infection has not been fully elucidated. Two pathways have been proposed.[23] The first suggests that microorganisms from the lower genital tract traverse the cervix and gain access to the decidua. Bacteria can multiply at this site and cross the chorioamniotic membranes to invade the amniotic cavity. The second proposed pathway is that microorganisms traversing the cervix cross intact membranes or the rupture site in the case of preterm PROM to gain access to the amniotic cavity. In this pathway, there is no broad dissemination of the organisms in the decidua. The findings of this current study suggest that inflammation begins in the decidua. This may occur in either pathway. If the bacteria or microorganisms are located in the decidua, maternal inflammatory cells will concentrate there. On the other hand, if the organisms are in the amniotic cavity, a chemotactic gradient would be established that would bring neutrophils from the decidua to invade the chorion, and eventually, the amnion. Therefore, in either pathway, the presence of inflammatory cells in the amnion would represent the most advanced stage of inflammation. Examples of chemokines which may be responsible for this gradient include interleukin-8 [11, 50, 118–120] and others [4, 22–25, 38, 40, 41, 53, 58, 64, 65, 91, 94, 112, 121–125] which have been shown to be elevated in patients with preterm labor and intra-amniotic infection/inflammation with intact or ruptured membranes, or even during the course of spontaneous labor at term.[126]

Funisitis and amniotic fluid MMP-8 concentration

Funisitis was excluded in the analysis of this study, because it reflects fetal inflammation. While acute histologic chorioamnionitis reflects a maternal immune response, funisitis is a hallmark of the fetal inflammatory response syndrome and is correlated with the plasma concentration of inflammatory cytokines such as interleukin-6, interleukin-10, or C-reactive protein in umbilical cord blood.[102, 103, 127–131] Funisitis is also a risk factor for cerebral palsy or other neonatal morbidity, such as neonatal sepsis.[11, 52, 91, 102, 103, 132–134] A previous study[91] indicated that AF MMP-8 concentration is a better predictor of funisitis than AF WBC count or the presence of a positive culture for microorganisms. In this study, the frequency of funisitis increased as a function of the severity of histologic chorioamnionitis.

In conclusion, we have demonstrated that the higher the amniotic fluid MMP-8 concentration, the more severe the acute inflammatory process in the placenta. Since advanced stages of acute histologic chorioamnionitis are associated with worse perinatal outcome, we infer that the magnitude of the elevation of MMP-8 concentrations may have prognostic value.

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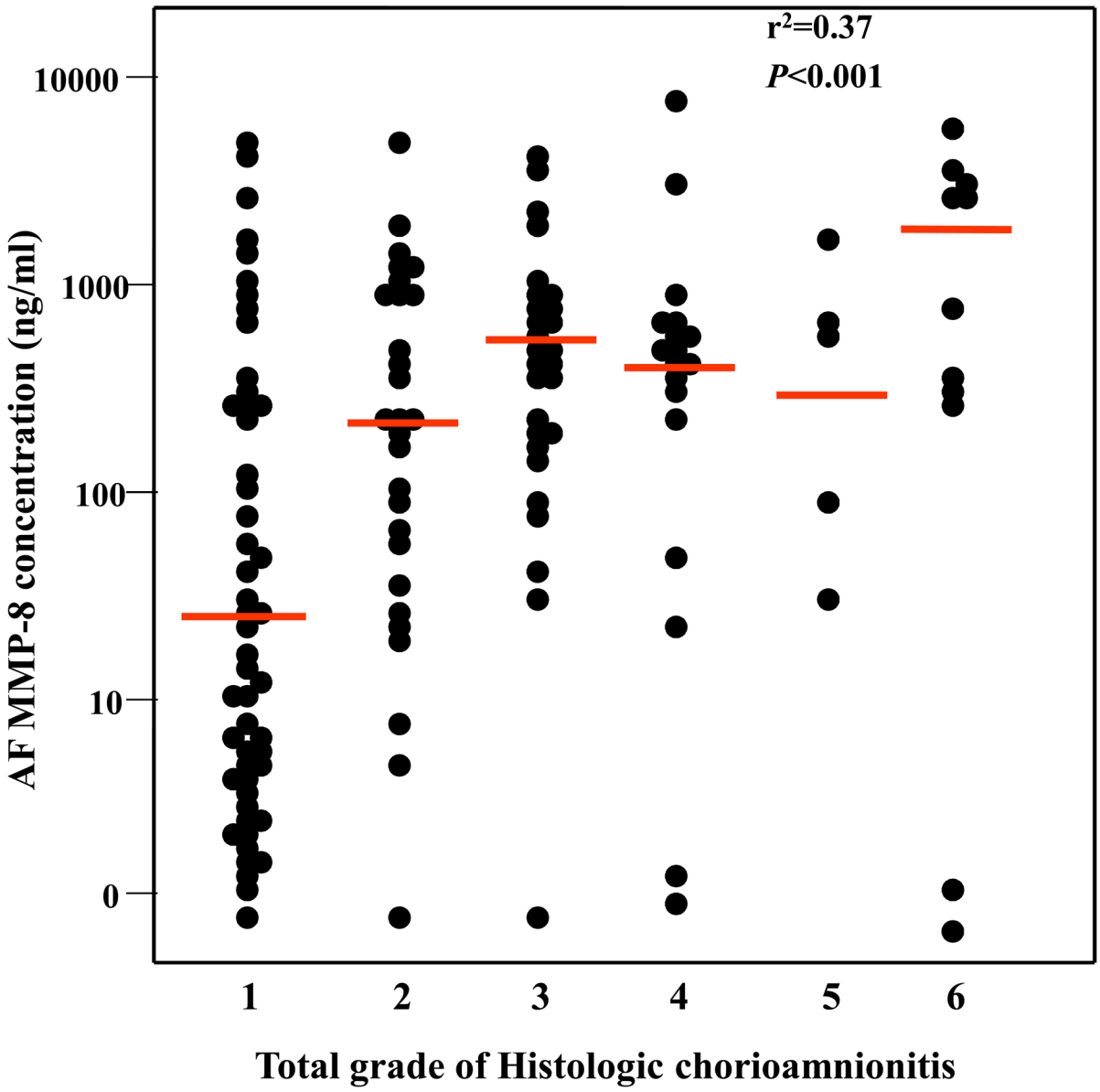

Figure 1.
Amniotic fluid MMP-8 concentration was positively correlated with the total grade of HCA (r^2=0.37, *P*<0.001 by Spearman's rho)

Table 1

Clinical characteristics of patients according to the presence or absence of acute histologic chorioamnionitis

Characteristics	Acute histologic chorioamnionitis		*P* value	
	Present (n=183)	Absent (n=229)	Unadjusted	Adjusted[d]
Maternal age (y) [a]	30.0 (21–43)	30.0 (21–41)	0.74	-
Nulliparity (n)	86 (47.0%)	109 (47.6%)	0.92	-
Gestational age at amniocentesis (wk)[a]	30.9 (23.7–34.9)	32.7 (24.0–34.9)	<0.001	-
Gestational age at delivery (wk)[a]	31.3 (24.0–35.0)	32.9 (24.0–35.0)	<0.001	0.002
Birth weight (g) [a]	1580 (500–3950)	1710 (450–3280)	0.025	<0.001
Amniocentesis to delivery interval (hr)[a]	18.0 (0.0–118.3)	4.2 (0.0–106.7)	<0.001	0.001
Positive AF culture (n/N)[b]	56/180 (31.1%)	18/220 (8.2%)	<0.001	<0.001
AF MMP-8 concentration(ng/mL)[a]	188.3 (0.3–6142.6)	1.8 (0.3–2845.5)	<0.001	<0.001
IAI (n)[c]	133 (72.7%)	40 (17.5%)	<0.001	<0.001

AF, amniotic fluid; MMP-8, matrix metalloproteinase-8; IAI, intra-amniotic inflammation

[a] Median value and range

[b] Total cases with AF culture results: 400

[c] AF MMP-8 concentration > 23 ng/mL

[d] Adjusted for gestational age at amniocentesis

Table 2

The prevalence of funisitis, amnionitis and positive amniotic fluid culture according to the total grade of acute histologic chorioamnionitis

Total grade of HCA	Prevalence of each grade (%)	Funisitis (n)	*p*[a]	Amnionitis (n)	*p*[a]	AF culture (n)	*p*[a]
0 (n=229)	55.6	4 (1.7%)	<0.001	0	<0.001	18 (8.2%)	<0.001
1 (n=78)	18.9	15 (19.2%)		0		19 (24.7%)	
2 (n=38)	9.2	21 (55.3%)		21 (55.3%)		16 (43.2%)	
3 (n=32)	7.8	20 (62.5%)		27 (84.4%)		8 (25.0%)	
4 (n=19)	4.6	17 (89.5%)		18 (94.7%)		6 (31.6%)	
5 (n=5)	1.2	4 (80.0%)		5 (100.0%)		3 (60.0%)	
6 (n=11)	2.7	10 (90.9%)		11 (100.0%)		4 (40.0%)	

HCA, acute histologic chorioamnionitis; AF, amniotic fluid

[a]Linear-by-Linear Association

Table 3

The prevalence of intra-amniotic inflammation and amniotic fluid matrix metalloproteinase-8 concentration, amniotic fluid white blood cell count according to the total grade of acute histologic chorioamnionitis

Total grade of HCA	IAI[c] (n)	*P*[a]	AF MMP-8 (ng/ml)[d]	*P*[b]	AF WBC [d]	*P*[b]
0 (n=229)	17.5% (40)	<0.001	1.8 (0.3–2845.5)	<0.001	2 (0–7200)	<0.001
1 (n=78)	51.3% (40)		25.9 (0.3–4202.7)		5 (0–50000)	
2 (n=38)	81.6% (31)		229.7 (0.3–3929.0)		405 (0–13248)	
3 (n=32)	96.9% (31)		486.2 (0.4–3392.0)		478 (0–15000)	
4 (n=19)	89.5% (17)		422.4 (0.6–6142.6)		892 (1–5800)	
5 (n=5)	100.0% (5)		257.0 (35.1–1007.3)		198 (17–>1000)	
6 (n=11)	81.8% (9)		1475.7 (0.5–3116.7)		1026 (1–8640)	

HCA, acute histologic chorioamnionitis; IAI, intra-amniotic inflammation; AF, amniotic fluid; MMP-8, matrix metalloproteinase-8; WBC, white blood cell

[a] Linear-by-Linear Association

[b] Kruskal-Wallis analysis test and Jonckheere-Terpstra test

[c] AF MMP-8 concentration > 23 ng/mL

[d] Values are given as median (range)