The frequency and clinical significance of intra-amniotic inflammation defined as an elevated amniotic fluid matrix metalloproteinase-8 in patients with preterm labor and low amniotic fluid white blood cell counts

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Objective
To determine the frequency and clinical significance of intra-amniotic inflammation (IAI) defined as an elevated amniotic fluid (AF) matrix metalloproteinase-8 (MMP-8) concentration in patients with preterm labor and intact membranes (PTL) and low AF white blood cell (WBC) counts.

Methods
Adverse pregnancy outcomes were compared according to the presence or absence of IAI in 220 singleton gestations who underwent amniocentesis due to PTL (gestational age<35.7 weeks) and had low AF WBC counts (<19 cells/mm³). Adverse pregnancy outcomes included preterm birth within 5 days of amniocentesis, acute histologic chorioamnionitis (acute-HCA) and positive AF culture. IAI was defined as an elevated AF MMP-8 concentration (≥23 ng/mL).

Results
IAI was present in 19% of study population. Adverse pregnancy outcomes were significantly more frequent in patients with IAI than in those without IAI (preterm birth within 5 days of amniocentesis, 88% vs. 41%; acute-HCA, 47% vs. 11%; positive AF culture, 10% vs. 2%; each for $P<0.05$). Patients with IAI had a significantly shorter median amniocentesis-to-delivery interval than those without IAI (7.8 hours [0.01–3,307.3 hours] vs. 310.3 hours [0.01–2,973.8 hours]; $P<0.001$ from survival analysis). Multiple logistic regression analysis demonstrated that only an IAI (odds ratio, 3.3; 95% confidence interval, 1.5–7.3; $P<0.005$) retained a statistical significance in the prediction of acute-HCA after other confounding variables were adjusted.

Conclusion
Approximately one-fifth of patients with PTL and low AF WBC counts have an evidence of IAI and are at risk for impending preterm delivery and acute-HCA when AF MMP-8 concentration is used.

Keywords: Amniotic fluid; Chorioamnionitis; Inflammation; Matrix metalloproteinase-8; Preterm labor; White blood cell

Introduction
Matrix metalloproteinase-8 (MMP-8), human neutrophil collagenase, is released from the secondary or specific granules of polymorphonuclear leukocytes stimulated by chemotactic cytokines [1], and has the potent capabilities for degrading extracellular matrix, in particular collagen type I [2,3]. The degradation of extracellular matrix of fetal membranes is an important biochemical process in chorio-decidua activation,
which is a part of the common terminal pathway of human parturition [4]. Indeed, MMP-8 has been documented in the amniotic cavity, fetal membranes and lower uterine segment in the context of preterm or term parturition [5-7].

Intra-amniotic inflammation (IAI) is associated with impending preterm delivery, acute histologic chorioamnionitis (acute-HCA), positive amniotic fluid (AF) culture and significant neonatal morbidity [8-19]. AF white blood cell (WBC) count has been generally used as a method for the rapid analysis of IAI, and a low AF WBC count has been thought to reflect the amniotic cavity without inflammation. However, AF MMP-8 has recently emerged as a reliable indicator of IAI [20-28]. We have previously observed that some patients with preterm labor and intact membranes (PTL) had low AF WBC counts but high AF MMP-8 concentrations. However, there is a paucity of data about the frequency and clinical significance of IAI defined as an elevated AF MMP-8 concentration in patients with PTL and low AF WBC counts. We hypothesized that only a low AF WBC count could not guarantee the continuation of pregnancy after 5 days of amniocentesis, placenta without any inflammation and a negative AF culture when AF MMP-8 concentration was elevated in patients with PTL. The objective of the study is to examine this hypothesis.

Materials and methods

1. Study design

Study population consisted of 220 singleton gestations who underwent amniocentesis due to PTL (gestational age [GA] <35.7 weeks) and had low AF WBC counts (defined as AF WBC count<19 cells/mm³) at Seoul National University Hospital between January 1993 and December 2007. Adverse pregnancy outcomes were compared according to the presence or absence of IAI. Adverse pregnancy outcomes included preterm birth within 5 days of amniocentesis, acute-HCA and a positive AF culture. Of adverse pregnancy outcomes, the relationship between IAI and acute-HCA was examined in 110 cases delivered within 7 days of amniocentesis. This criterion was used to preserve a meaningful temporal relationship between the results of AF studies and the placental pathologic findings at birth. At our institution, transabdominal amniocentesis for retrieval of AF and placental pathologic examination after delivery were routinely offered to all patients who were admitted with the diagnosis of PTL. AF was analyzed for microbiologic and inflammatory status and fetal lung maturity. Written informed consent was obtained from all these patients. 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 Protections of the Department of Health and Human Services of the United States. Many of patients in this study were included in our previous studies.

2. Clinical characteristics and pregnancy outcomes

The demographic and clinical characteristics of the mothers and their neonates were examined through a review of the medical records. We investigated parity (≥1), maternal age, antenatal use of corticosteroid, GA at amniocentesis, gender of newborn, route of delivery, GA at delivery, birth weight, 1 minute Apgar score and 5 minutes Apgar score.

3. Clinical chorioamnionitis

Clinical chorioamnionitis was diagnosed when maternal body temperature was elevated to 37.8°C and ≥2 of the following criteria were present according to the definitions previously described in detail [29]: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis (>15,000 cells/mm³), maternal tachycardia (>100 beats/min) and fetal tachycardia (>160 beats/min).

4. Placental pathology

Placental tissue samples obtained for pathologic evaluation included the chorio-decidua, amnion, chorionic plate and umbilical cord. These samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of prepared tissue blocks were stained with hematoxylin and eosin. Pathologists were masked to the clinical information. Acute-HCA was defined in the presence of acute inflammatory changes on examination of a membrane roll and chorionic plate of the placenta; inflammation of the chorio-decidua or amnion was diagnosed in the presence of at least 1 focus of >5 neutrophils in the chorio-decidua or amnion; inflammation of the chorionic plate was diagnosed in the presence of more than 1 focus of at least 10 neutrophilic collections or diffuse inflammation in subchorionic fibrin, or diffuse and dense inflammation, neutrophilic infiltration into connective