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Clinical chorioamnionitis is characterized by increased expression of the alarmin HMGB1 and One of Its Receptors, sRAGE

Roberto Romero, MD[1,2,3], **Tinnakorn Chaiworapongsa, MD**[1,3], **Zeynep Alpay Savasan, MD**[1,3], **Youssef Hussein, MD**[1], **Zhong Dong, MD, PhD**[1], **Juan Pedro Kusanovic, MD**[4], **Chong Jai Kim, MD, PhD**[1,5], and **Sonia S Hassan, MD**[1,3]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, Michigan and Bethesda, Maryland, USA

[2]Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan, USA

[3]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

[4]Department of Obstetrics and Gynecology, Pontificia Universidad Católica de Chile, Santiago, Chile and Center for Perinatal Research, Sótero del Río Hospital, Santiago, Chile

[5]Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract

Objective—High-mobility group box-1 (HMGB1) protein is an alarmin, a normal cell constituent, which is released into the extracellular environment upon cellular stress/damage, and is capable of activating inflammation and tissue repair. The receptor for advanced glycation end products (RAGE) can bind HMGB1. RAGE, in turn, can induce the production of pro-inflammatory cytokines; this may be modulated the soluble truncated forms of RAGE, including soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE). The objective of this study was to determine: 1) if clinical chorioamnionitis at term is associated with changes in amniotic fluid concentrations of HMGB1, sRAGE and esRAGE; and 2) if the amniotic fluid concentration of HMGB1 changes with labor or as a function of gestational age.

Methods—Amniotic fluid samples were collected from the following groups: 1) mid-trimester (MT) (n=45); 2) term with (n=48) and without labor (n=22) without intra-amniotic infection; and 3) term with clinical chorioamnionitis (n=46). Amniotic fluid concentrations of HMGB1, sRAGE and esRAGE concentrations were determined by ELISA.

Results—1) the median amniotic fluid HMGB1 concentration was higher in patients at term with clinical chorioamnionitis than that of those without this condition (clinical chorioamnionitis: median 3.8 ng/mL vs. term in labor: median 1.8 ng/mL, p=0.007; and vs. term not in labor median 1.1 ng/mL, p=0.003); 2) in contrast, patients with clinical chorioamnionitis had a lower median sRAGE concentration than those without this condition (clinical chorioamnionitis: median 9.3 ng/mL vs. term in labor: median 18.6 ng/mL, p=0.001; and vs. term not in labor median 28.4 ng/mL, p<0.001); 3) amniotic fluid concentrations of esRAGE did not significantly change in patients with clinical chorioamnionitis at term (clinical chorioamnionitis: median 5.4 ng/mL vs. term in

Address correspondence to: Roberto Romero, MD, Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/ Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694.

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labor: median 6.1 ng/mL, p=0.9; and vs. term not in labor median 9.5 ng/mL, p=0.06); and 4) there was no significant difference in the median AF HMGB1 concentration between women at term in labor and those not in labor (p=0.4) and between women in the mid-trimester and those at term not in labor (mid-trimester: median 1.5 ng/mL; p=0.2).

Conclusion—An increase in the amniotic fluid HMGB1 concentration and a decrease in sRAGE were observed in clinical chorioamnionitis at term. This finding provides evidence that an alarmin, HMGB1, and one of its receptors, sRAGE, are engaged in the process of clinical chorioamnionitis at term. These changes are quite different from those observed in cases of intra-amniotic infection/inflammation in preterm gestations.

Keywords

danger signal; intra-amniotic inflammation; sterile inflammation; pregnancy; neuroinflammation; neuro-immune reflects; amniotic fluid; DAMPs; damage-associated molecular patterns; intra-amniotic infection; term labor

INTRODUCTION

Clinical chorioamnionitis is diagnosed by the presence of maternal fever accompanied by signs and symptoms of intrauterine inflammation (i.e. foul smelling discharge, uterine tenderness, fetal tachycardia, etc.) [1–3]. Neonates born to mothers with clinical chorioamnionitis, even at term gestation, are at an increased risk for short- and long- term complications including low APGAR scores, umbilical cord pH<7, delivery room intubation, pneumonia, sepsis [4], neonatal encephalopathy [5,6], long-term cognitive impairment [7] and cerebral palsy [8–12].

Inflammation can be elicited in response to an infection or sterile injury such as trauma or ischemia/reperfusion [13–15]. Indeed, several physiologic processes such as implantation, ovulation [16] and parturition [17,18] utilize cells and mediators involved in the innate immune response. Classically, the innate immune system of multicellular organisms recognizes infection via specific molecular patterns (pathogen-associated molecular patterns or PAMPs) on microbes by specific receptors (pattern recognition receptors or PRRs) on host cells [19–21]. In cases of trauma or sterile injury, cells sense an endogenous “danger signal” through alarmins, normal cell constituents capable of inducing an inflammatory response upon release into the extracellular environment [14,15,22–24]. Currently, several endogenous proteins including S100 proteins [25,26], uric acid [27], interleukin (IL)-1α [28], heat shock protein [29,30], hepatoma-derived growth factor [31], and high mobility group box (HMGB)-1 have been proposed to be alarmins [14].

The specific characteristics of alarmins include the ability to: 1) undergo passive release following necrotic cell death or actively secrete through the non-classical pathway; 2) recruit and activate antigen presenting cells (eg: dendritic cells, macrophages) to induce adaptive immune responses; and 3) promote tissue repair [14,23,32–36].

HMGB-1, a nuclear protein, is considered to be the only alarmin that meets all the proposed criteria for a danger signal [14]. HMGB1 exerts its biological effects through specific receptors including Toll-Like Receptor (TLR)-2, 4 and 9 [32–36] as well as a receptor for advanced glycation end products (RAGE) [37,38]. Upon binding to RAGE, HMGB1 can induce and sustain the production of pro-inflammatory cytokines which may be modulated by soluble, truncated forms of RAGE including soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) [23,38–45].

We have examined the changes of amniotic fluid concentrations of pro-inflammatory cytokines [46–54], anti-inflammatory cytokines [55], chemokines [56–62], proteases/anti-proteases [63], matrix-metalloproteinase [64–71], pro- and anti-angiogenic factors [72–74], coagulation factors [75,76], adipocytokines [77–79], anti-microbial peptides [80] and prostaglandins [81–84] in patients with intra-amniotic infection /inflammation (IAI) both at term and preterm gestations. Amniotic fluid concentrations of sRAGE and esRAGE have been reported to be elevated in patients with IAI in preterm gestations and decreased in labor at term [85], whereas the amniotic fluid concentration of HMGB1 in patients at term with clinical chorioamnionitis has not yet been examined.

The objective of this study was to determine if: 1) clinical chorioamnionitis at term is associated with changes in amniotic fluid concentrations of HMGB1, sRAGE and esRAGE; and 2) amniotic fluid concentration of HMGB1 changes as a function of gestational age or labor at term, a condition generally considered as a sterile inflammatory response [17].

MATERIALS AND METHODS

Study design and population

A retrospective cross-sectional study was conducted by searching our clinical database and bank of biologic samples. Women with singleton pregnancies who had amniotic fluid samples obtained by trans-abdominal amniocentesis from the following groups were included: 1) those in the mid-trimester of pregnancy (14–18 weeks) who underwent amniocentesis for genetic indications and delivered at term (n=45); 2) women at term gestation and without intra-amniotic fluid infection with (n=22) and without labor (n=48); and 3) those at term with clinical chorioamnionitis (n=46).

All women provided written informed consent before the collection of amniotic fluid samples. The collection and utilization of the samples was approved by the Human Investigation Committee of the participating institutions and the IRB of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been used in previous studies of sRAGE and esRAGE in intra-amniotic infection.

Clinical definition

Clinical chorioamnionitis was diagnosed by the presence of a temperature elevation to 37.8°C or higher and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, fetal tachycardia (fetal heart rate >160 beats/min), maternal tachycardia (heart rate >100 beats/min) and maternal leukocytosis (leukocyte count >15,000 cells/mm^3) [1,86]. Spontaneous term labor was defined as the presence of regular uterine contractions with a frequency of at least one every 10 min and cervical changes after 37 weeks of gestation. Intra-amniotic infection was defined as a positive microbiological culture in amniotic fluid, and intra-amniotic inflammation as an amniotic fluid IL-6 concentration of 2.6ng/mL or more [87].

Sample collection

Amniotic fluid samples were obtained by transabdominal amniocentesis performed for genetic indications, evaluation of microbial status of the amniotic cavity and/or assessment of fetal lung maturity in patients approaching term. Women with clinical chorioamnionitis underwent amniocentesis to evaluate infection/inflammation status in the amniotic cavity. This information was used by obstetricians and neonatologists in the management of mothers and neonates in terms of treatment with antibiotics. Women at term in labor consisted of those who were admitted for suspected preterm labor because of uncertain dates

and had an amniocentesis for the assessment of fetal lung maturity. The criteria for considering whether these patients were at term in labor was derived retrospectively, if the following criteria were met: 1) spontaneous labor; 2) delivery within 24 hours of amniocentesis; 3) analysis of amniotic fluid was consistent with fetal lung maturity; 4) birthweight >2500 g; 5) absence of respiratory distress syndrome or other complications of prematurity; and 6) physical examination of the newborn by a pediatrician was consistent with a term neonate. Samples of amniotic fluid were transported to the laboratory in a sterile capped syringe and cultured for aerobic/anaerobic bacteria and genital mycoplasmas. White blood cell count [88], glucose concentration [89] and Gram stain [90] were also performed shortly after collection as previously described [88,89]. The results of these tests were used for clinical management. Amniotic fluid IL-6 concentrations were obtained in some patients and used only for research purposes. Amniotic fluid not required for clinical assessment was centrifuged at 1300*g* for 10 min at 4°C and the supernatant was stored at −70°C.

Determination of HMGB1, sRAGE and esRAGE in amniotic fluid

Concentrations of HMGB1, sRAGE, and esRAGE in amniotic fluid were determined by sensitive and specific enzyme immunoassays obtained from IBL International (Toronto, Canada); and IL-6 immunoassay from R&D Systems (Minneapolis, MN, USA). The initial assay validation was performed in our laboratory prior to the conduction of this study. Briefly, the immunoassay utilized the quantitative sandwich enzyme immunoassay technique and the concentrations were determined by interpolation from the standard curves. The inter-assay coefficients of variations for HMGB1, sRAGE, esRAGE and IL-6 were 3.1%, 3.2%, 4.8%, and 8.7% respectively. Intra-assay coefficients of variations for HMGB1, sRAGE, esRAGE and IL-6 were 4.4%, 4.2%, 2.1% and 4.6%, respectively. The sensitivities of the assays for HMGB1, sRAGE, esRAGE and IL-6 were 0.2 ng/mL, 33 pg/mL, 28 pg/mL and 0.09 pg/mL, respectively. The results of plasma sRAGE and esRAGE concentrations in patients in the mid-trimester and at term with and without labor have been previously reported, but were included in this manuscript in order to provide a comprehensive picture of HMGB1 and its soluble receptors

Statistical analysis

The Kolmogorov-Smirnov test and Shapiro-Wilk test were used to determine if the data were normally distributed. Kruskal-Wallis and post-hoc Mann-Whitney U tests were used to compare continuous nonparametric variables among and between groups. Comparisons between proportions were performed using Chi-square or Fisher's exact tests. A p-value <0.05 was considered statistically significant. Analysis was performed with SPSS, version 15 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics of the study population

Tables I and II display the demographic and clinical characteristics of patients in the mid-trimester, term not in labor, term in labor and term with clinical chorioamnionitis. Among patients with the clinical diagnosis of chorioamnionitis, 41% (19/46) had a positive microbial culture in amniotic fluid and 76% (35/46) had evidence of intra-amniotic fluid inflammation (defined as an amniotic fluid concentration of IL-6 of 2.6ng/mL or more). The most common organisms were *Ureaplasma urealyticum* (n=7), *Mycoplasma hominis* (n=3) and *Streptococcus agalactiae* (n=3).

Clinical chorioamnionitis is associated with high HMGB1 concentrations

Patients with clinical chorioamnionitis at term had a significantly higher median amniotic fluid HMGB1 concentration than women at term (clinical chorioamnionitis: median 3.8 ng/mL; range: 0–37.4 ng/mL vs. term without labor: median 1.1 ng/mL; range: 0–8.8 ng/mL; p=0.007; vs. term with labor: median 1.8 ng/mL; range: 0–21.5 ng/mL; p=0.003; Figure 1). Similar results were obtained when the analysis was restricted to patients with clinical chorioamnionitis with evidence of intra-amniotic infection/inflammation (clinical chorioamnionitis with IAI (n=35): median 5.1 ng/mL; range: 0–37.4 ng/mL vs. term without labor; p=0.001; vs. term with labor: p=0.001).

Amniotic fluid concentrations of sRAGE decreased in patients with clinical chorioamnionitis at term

Patients with clinical chorioamnionitis at term had a lower median sRAGE concentration than those without chorioamnionitis (clinical chorioamnionitis: median 9.3 ng/mL; range: 0–29.3 ng/mL vs. term not in labor: median 28.4 ng/mL; range: 1.1–56.8 ng/mL; p<0.001; vs. term in labor: median 18.6 ng/mL; range: 0–79.8 ng/mL; p=0.001; Figure 2). In contrast, there were no significant differences in the median amniotic fluid concentration of esRAGE between patients with clinical chorioamnionitis at term and patients at term (clinical chorioamnionitis: median: 5.4 ng/mL; range: 0–18.1 ng/mL vs. term not in labor median: 9.5 ng/mL; range: 0–22.6 ng/mL; p=0.06; vs. term in labor median: 6.1 ng/mL; range: 0–15.1 ng/mL; p=0.9; Figure 3).

When the analysis was restricted to patients with clinical chorioamnionitis with evidence of intra-amniotic infection/inflammation, similar findings were observed (clinical chorioamnionitis with IAI: sRAGE median 9.2 ng/mL; range: 0–29.3 ng/mL vs. term without labor; p<0.001; vs. term with labor: p=0.001 and clinical chorioamnionitis with IAI: esRAGE median 5.1 ng/mL; range: 0–18.1 ng/mL vs. term without labor; p=0.06; vs. term with labor: p=1.0).

Among patients with clinical chorioamnionitis, there was a significant relationship between amniotic fluid concentrations of HMGB1 and the soluble forms of its receptor (sRAGE: Spearman's Rho 0.53; p<0.001 and esRAGE Spearman's Rho 0.46; p<0.001) and between HMGB1 and markers of inflammation (WBC: Spearman's Rho 0.4; p=0.005 and IL-6: Spearman's Rho 0.49; p=0.001).

Amniotic fluid concentration of HMGB1 did not change with spontaneous labor at term

There was no significant difference in the median amniotic fluid HMGB1 concentration between women at term with and without labor (p=0.4; Figure 1). In contrast, similar to results previously reported, spontaneous labor at term was associated with a decrease in the median amniotic fluid concentration of sRAGE and esRAGE (p=0.007 and p= 0.02 respectively; Figures 2 and 3).

Amniotic fluid concentrations of HMGB1 did not change as a function of gestational age

There was no significant difference in the median amniotic fluid concentration of HMGB1 between women in the mid-trimester and those at term not in labor (mid-trimester median: 1.5 ng/mL; range: 0–8 ng/mL vs. term not in labor median: 1.1 ng/mL; range: 0–8.8 ng/mL; p=0.2; Figure 4).

DISCUSSION

Principal findings of the study

1) clinical chorioamnionitis at term was associated with an increase in amniotic fluid concentration of HMGB1, but a decrease in its soluble receptor (sRAGE); 2) amniotic fluid concentration of HMGB1 did not change with spontaneous labor at term, a condition in which amniotic fluid concentrations of sRAGE and esRAGE decreased; and 3) amniotic fluid concentration of HMGB1 did not change as a function of gestational age.

The Biology of HMGB1

HMGB1, a non-histone, chromatin-associated protein, was originally characterized to be involved in DNA organization and the regulation of transcription [24,39,40,44,91]. Subsequently, this protein has been proposed to be a late mediator of sepsis [42] and to play an important role in infection-induced lung injury and lethality [92,93].

HMGB1 is constitutively expressed in almost every cell type that has a nucleus [24]. This alarmin can be released actively or passively into the extracellular environment. The active release of HMGB1 outside the cell is accomplished through a nontraditional “leaderless” pathway (i.e. not through endoplasmic reticulum or Golgi apparatus) [14,94] upon stress (ischemia, oxidative stress) [95–97] or stimulation with bacterial products [98–101] or cytokines such as tumor necrosis factor (TNF)-α, IL-1, interferon-γ [101–103]. The passive release of HMGB1 out of the cells was observed during cellular necrosis [104,105]. Extra-cellular HMGB1 can potentially be associated with DNA, RNA, endotoxin, nucleosomes [24], thrombospondin [106–108] or CD24 [109,110] to augment or decrease the function of HMGB1 itself when they bind to its receptors [111].

HMGB1 exerts a wide variety of biological activities, including induction of the maturation and migration of dendritic cells, neutrophils and monocytes [43,112–115], stimulation of the production of reactive oxygen species [116], chemotaxis of neutrophils [117], secretion of inflammatory cytokines from immune cells [23,40,118–120], proliferation of T cells[43,121] and migration of stem cells for tissue repair [122–129]. These effects are accomplished through the binding of HMGB1 to its receptors, which include Toll-Like Receptors (TLR)-2, 4 and 9 [32–35] as well as RAGE [37,38,43,112,130,131].

The Biology of soluble RAGE and endogenous secretory RAGE

RAGE was first described as a transmembrane receptor for advanced glycation products (AGE), the product of nonenzymatic glycation and oxidation of proteins and lipids that accumulate under the condition of oxidative stress and hyperglycemia. AGE induces the expression of pro-inflammatory mediators through mitogen-activated protein kinase and nuclear factor (NF)-κB [132]. Other ligands of RAGE are amyloid-β peptides (accumulating in Alzheimer’s disease)[133–136], amyloid A (accumulating in systemic amyloidosis)[137], S100/calgranulins [138], surface molecules on bacteria [139], prions [140], leukocytes [141] and HMGB1 [131]. Engagement of RAGE and its ligand also results in a rapid and sustained activation of NF-κB with a positive feedback loop, in which ligand interaction increases expression of the receptor itself [142]. Thus, activation of NF-κB results in increased RAGE expression and the numbers of ligand binding sites, thereby prolonging NF-κB activation [37].

RAGE is expressed in vascular smooth muscle and endothelial cells, cardiac myocytes, neural tissues, macrophages [143], human pregnant myometrium [144], first trimester human chorionic villi [145] and human term placenta [146,147]. Animal experiments and studies in humans indicate that RAGE is involved in the pathophysiologic processes of

neurogenerative disorders [148], rheumatoid arthritis [149,150], chronic renal disease [151], inflammatory bowel disease and chronic vascular disorders, which include diabetic complications (i.e. neuropathy, nephropathy) and atherosclerosis [37,133,137,152–154]. Moreover, an intense immunostaining for RAGE in both myometrium and omental blood vessels of patients with preeclampsia [155] has been observed.

The soluble form of RAGE is composed of the extracellular ligand-binding domain without transmembrane and cytosolic regions. This protein is originally thought to function as a decoy receptor abrogating cellular activation [141,156–159]. Subsequently, sRAGE has also been found to have pro-inflammatory activity, depending on the cell types and conditions of target cells [160,161]. Recently, a novel splice variant of RAGE mRNA has been identified as esRAGE and reported to be released from human microvascular endothelial cells and pericytes [162,163].

Amniotic fluid concentrations of HMGB1 increased in term clinical chorioamnionitis

The finding that clinical chorioamnionitis at term was associated with an increase in amniotic fluid concentration of HMGB1 suggests that this alarmin participates in the inflammatory response. Moreover, a relationship between amniotic fluid concentrations of HMGB1 and IL-6 was observed. Bacterial endotoxin is capable of stimulating macrophages to release HMGB1 partly through CD14 and TNF-dependent mechanisms, since either genetic disruption of CD14 expression or neutralization of TNF activity blocks endotoxin-induced TNF production, but only partially attenuates HMGB1 release from macrophages [99]. However, either endotoxin or pro-inflammatory cytokines, individually, are capable of inducing HMGB1 release into the extracellular environment [39]. These observations are consistent with our findings in preterm gestations in which the amniotic fluid concentration of HMGB1 was elevated in patients with preterm labor or preterm PROM with IAI [164].

Amniotic fluid concentrations of sRAGE decreased in term clinical chorioamnionitis

Patients with clinical chorioamnionitis or those with IAI at term had lower amniotic fluid concentrations of sRAGE than those without clinical chorioamnionitis. These findings differ from our observation in preterm gestations, in which amniotic fluid concentrations of sRAGE and esRAGE were elevated in patients with intra-amniotic inflammation [85].

It is possible that a lower amniotic concentration of sRAGE observed herein reflects a different relationship between HMGB1 and sRAGE in women at term. A similar observation in sRAGE in synovial fluid and blood has been reported in patients with rheumatoid arthritis [150]. The increased HMGB1 concentration in these patients may be responsible, in part, for the concentration of sRAGE in the amniotic fluid of patients with clinical chorioamnionitis at term by stimulating RAGE receptor production. Consistent with this hypothesis, amniotic fluid concentrations of HMGB1 were correlated with sRAGE. This relationship was observed in IAI only at term, not preterm gestations. Similarly, amniotic fluid concentrations of HMGB1 also correlated with IL-6 in intra-amniotic inflammation only at term, but not in preterm gestations. Thus, HMGB1 and sRAGE may modulate the inflammatory response differently in term and preterm gestations. Future studies are required to elucidate the factors responsible for the differential response to intra-amniotic inflammation at different gestational ages.

Advancing gestational age or parturition at term did not change amniotic fluid concentrations of HMGB1

Amniotic fluid concentrations of HMGB1 did not change with advancing gestational age or with the presence of spontaneous labor at term without intra-amniotic fluid infection. In contrast, in our previous study, sRAGE increased with advancing gestational age and

decreased with parturition at term [85]. The difference in behavior of HMGB1 and sRAGE could be explained, in part, by the multi-ligand nature of both proteins [37,39]. sRAGE may modulate the physiologic inflammatory response of labor at term through different ligands other than HMGB1.

Strengths and limitations

This is the first study to evaluate amniotic fluid concentrations of HMGB1, an alarmin, in patients with clinical chorioamnionitis at term, a condition associated with short- and long-term adverse neonatal outcomes. Moreover, amniotic fluid concentrations of its soluble receptors, sRAGE and esRAGE, were also determined. However, due to the cross-sectional nature of the study, a temporal relationship of this alarmin as well as its soluble receptors and clinical chorioamnionitis at term could not be established.

Conclusion

A substantial increase in the amniotic fluid concentration of HMGB1 and a decrease in sRAGE were observed in clinical chorioamnionitis at term. This is evidence that an alarmin system (a “danger signal”), HMGB1, and one of its receptors, sRAGE, are operative in clinical chorioamnionitis at term. These observations are different from those made in preterm gestations with intra-amniotic fluid infection/inflammation. It is possible that HMGB1 and sRAGE may have different roles in the regulation of inflammatory responses in term and preterm gestations.

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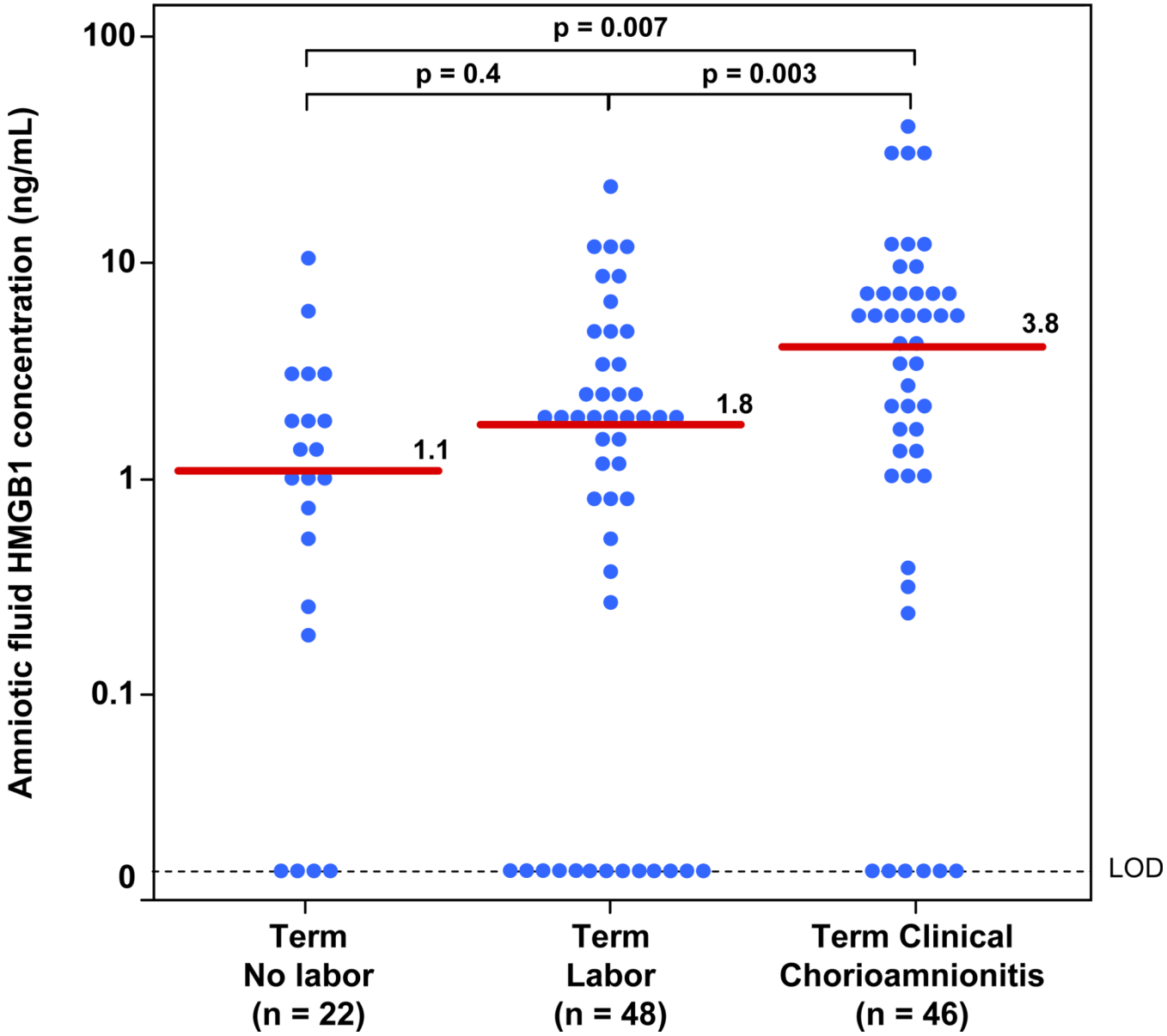

Figure 1. Amniotic fluid concentrations of high-mobility group box-1 (HMGB1) in women at term with and without labor and patients with clinical chorioamnionitis

Patients with clinical chorioamnionitis at term had a significantly higher median amniotic fluid HMGB1 concentration than women at term with and without labor (clinical chorioamnionitis: median 3.8 ng/mL; range: 0–37.4 ng/mL vs. term without labor: median 1.1 ng/mL; range: 0–8.8 ng/mL; p=0.007; vs. term with labor: median 1.8 ng/mL; range: 0–21.5 ng/mL; p=0.003).

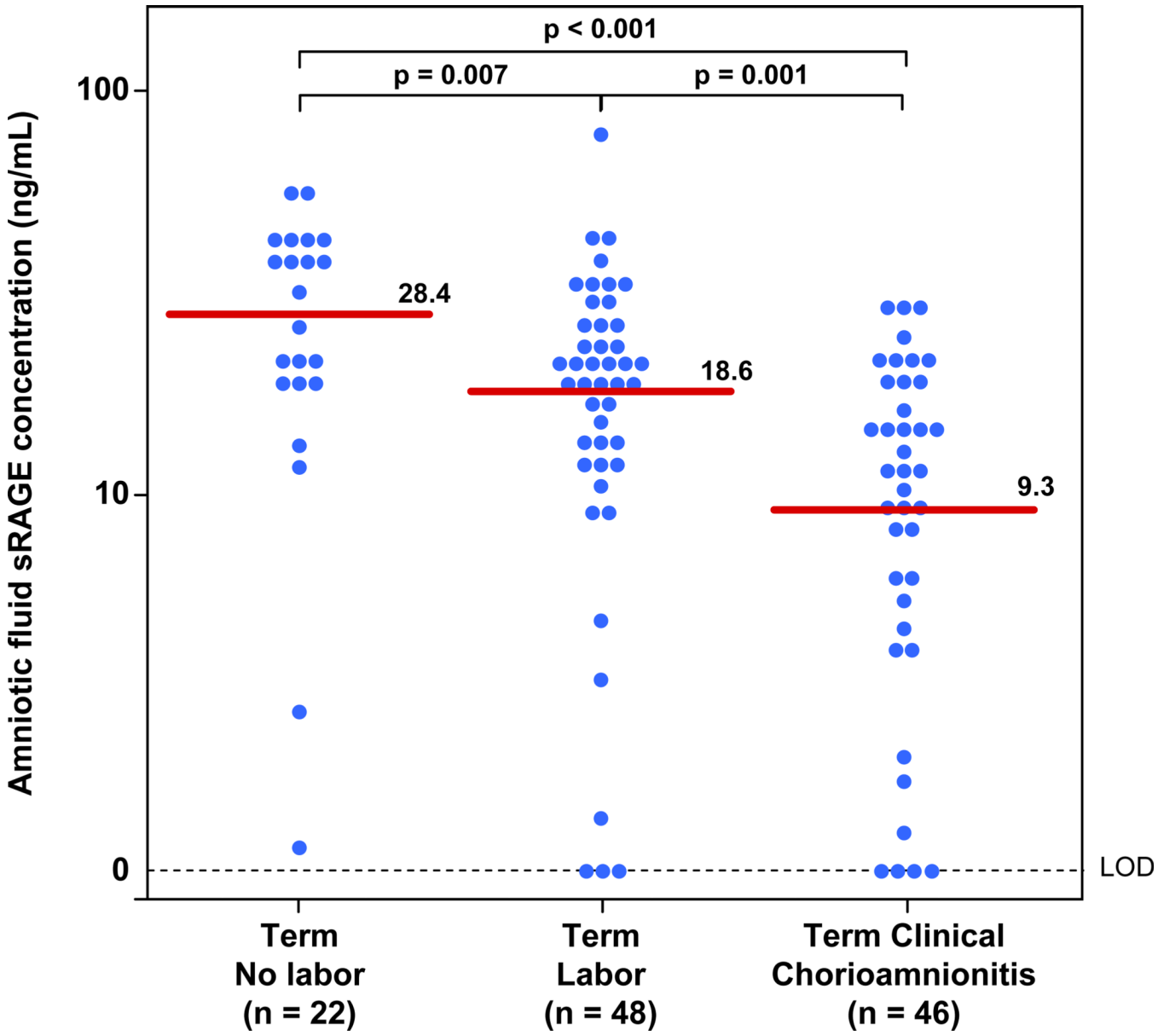

Figure 2. Amniotic fluid concentrations of soluble receptor for advanced glycation end products (sRAGE) in women at term with and without labor and patients with clinical chorioamnionitis

Patients with clinical chorioamnionitis at term had a lower median sRAGE concentration than those without chorioamnionitis regardless of labor status (clinical chorioamnionitis: median 9.3 ng/mL; range: 0–29.3 ng/mL vs. term not in labor: median 28.4 ng/mL; range: 1.1–56.8 ng/mL; $p<0.001$; vs. term in labor: median 18.6 ng/mL; range: 0–79.8 ng/mL; $p=0.001$).

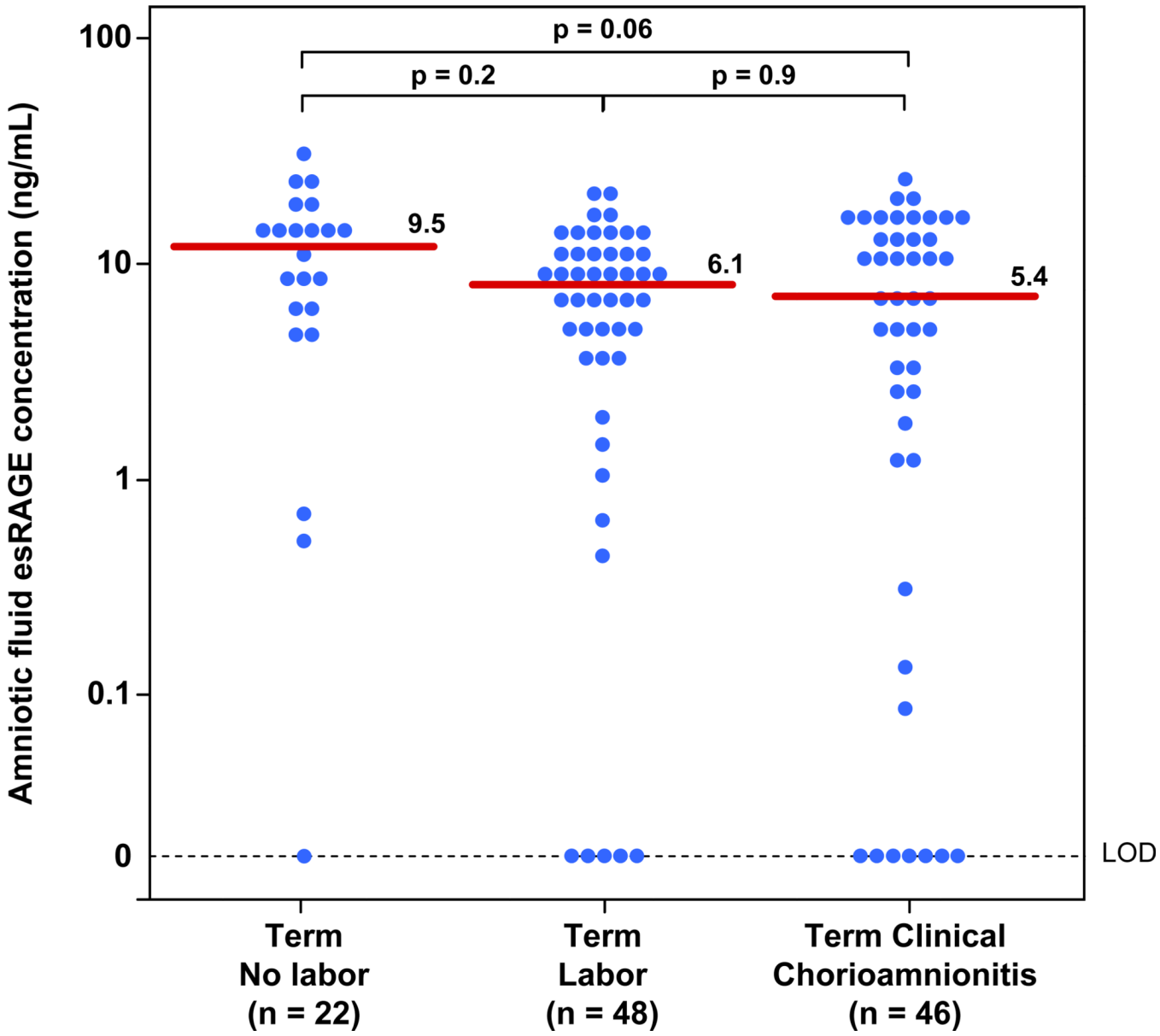

Figure 3. Amniotic fluid concentrations of endogenous secretory RAGE (esRAGE) in women at term with and without labor and patients with clinical chorioamnionitis

There were no significant difference in the median amniotic fluid concentration of esRAGE between patients with clinical chorioamnionitis at term and patients at term with and without labor (clinical chorioamnionitis: median: 5.4 ng/mL; range: 0–18.1 ng/mL vs. term not in labor median: 9.5 ng/mL; range: 0–22.6 ng/mL; p=0.06; vs. term in labor median: 6.1 ng/mL; range: 0–15.1 ng/mL; p=0.9).

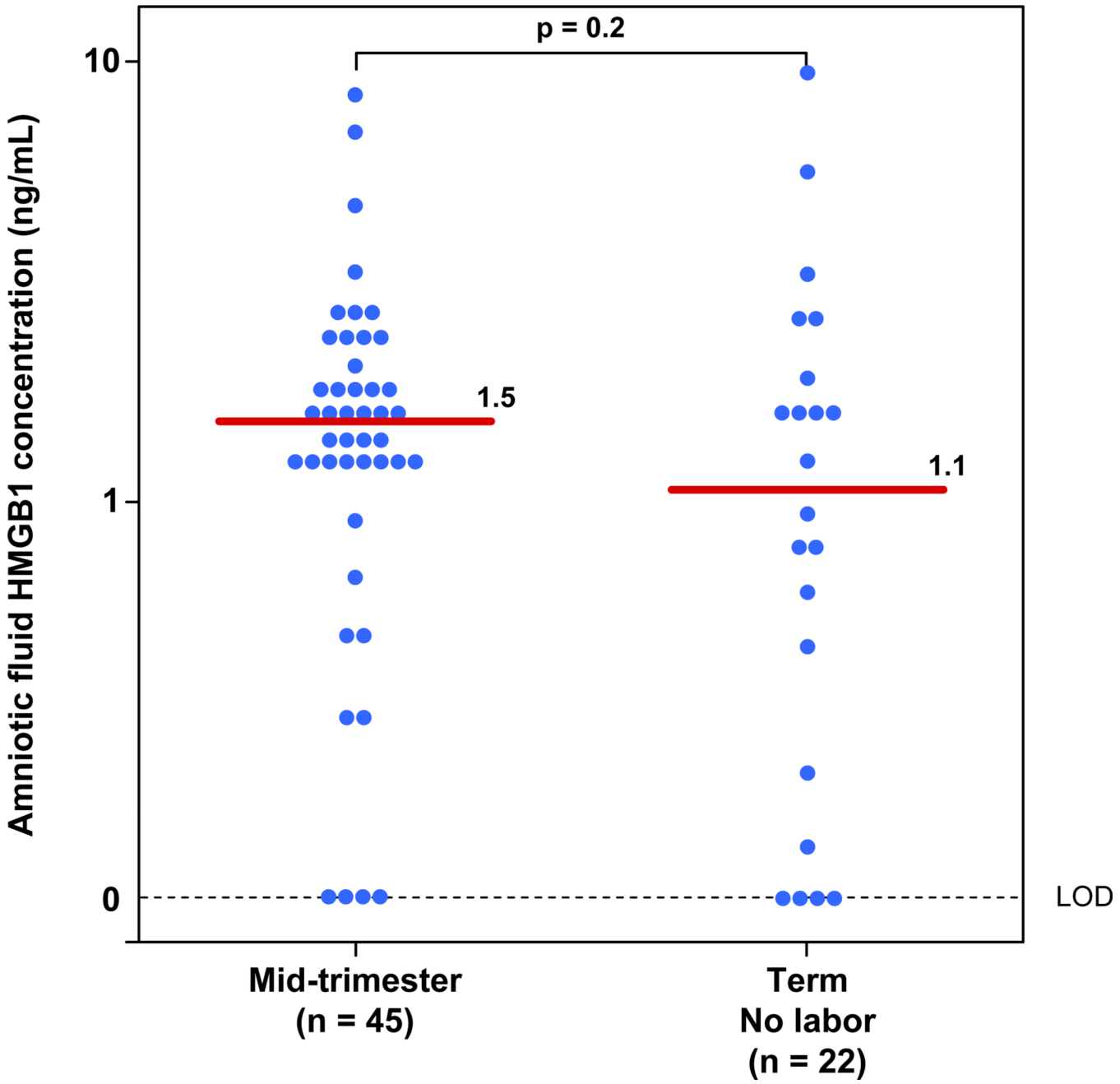

Figure 4. Amniotic fluid concentrations of high-mobility group box-1 (HMGB1) in women in the mid-trimester and those at term not in labor

There was no significant difference in the median amniotic fluid concentration of HMGB1 between patients in the mid-trimester and those at term not in labor (mid-trimester median: 1.5 ng/mL; range: 0–8 ng/mL vs. term not in labor median: 1.1 ng/mL; range: 0–8.8 ng/mL; p=0.2).

TABLE I

Clinical and obstetrical characteristics of women in the mid-trimester and those at term no labor

	Mid-trimester n=45	**Term no labor n=22**	**p**
Maternal age (years)	37 (24–42)	28 (17–40)	<0.001
GA at amniocentesis (weeks)	16 (14–18)	39.8 (38–42)	<0.001
GA at delivery (weeks)	39 (37–41)	39.8 (38–42)	0.4
Birthweight (grams)	3345 (2809–4180)	3405 (2810–4530)	0.9

GA: Gestational age Values are expressed as median (range)

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TABLE 2

Clinical and obstetrical characteristics of women at term with and without labor and term with clinical chorioamnionitis

	Term no labor n=22	**p***	**Term labor n=48**	**p****	**Term clinical chorioamnionitis n=46**	**p*****
Maternal age (years)	28 (17–40)	0.05	23 (16–37)	0.02	20 (14–38)	0.002
GA at amniocentesis (weeks)	39.8 (38–42)	0.3	39 (37–41.5)	0.04	39.8 (37.4–42.7)	0.5
GA at delivery (weeks)	39.8 (38–42)	0.3	39.7 (37–41.5)	0.03	39.8 (37.4–42.7)	0.4
Birthweight (grams)	3405 (2810–4530)	0.3	3265 (2540–4440)	0.08	3550 (2720–4750)	0.4

GA: Gestational age Values are expressed as median (range)

* comparison between term no labor and term in labor

** comparison between term in labor and term clinical chorioamnionitis

*** comparison between term no labor and term clinical chorioamnionitis