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CXCL10 and IL-6: Markers of Two Different Forms of Intra-Amniotic Inflammation in Preterm Labor

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Abstract

Problem: To determine whether amniotic fluid (AF) CXCL10 concentration is associated with histologic chronic chorioamnionitis in patients with preterm labor (PTL) and preterm prelabor rupture of the membranes (PPROM).

Methods os Study: This study included 168 women who had an an episode of PTL or PPROM. AF IL-6 and CXCL10 concentrations were determined by immunoassay.

Results: 1) Increased AF CXCL10 concentration was associated with chronic (OR 4.8; 95% CI 1.7-14), but not acute chorioamnionitis; 2) Increased AF IL-6 concentration was associated with an acute (OR 4.2; 95% CI 1.3-13.7), but not chronic chorioamnionitis; and 3) an increase of AF CXCL10 concentration was associated with placental lesions consistent with maternal anti-fetal rejection (OR 3.7; 95% CI 1.3-10.4). 4) all patients with elevated AF CXCL10 and IL6 delivered preterm.

Conclusions: Increased AF CXCL10 concentration is associated with chronic chorioamnionitis or maternal anti-fetal rejection, whereas increased AF IL-6 concentration is associated with acute histologic chorioamnionitis.

Keywords: amniocentesis, allograft, biomarker, chorioamnionitis, chronic chorioamnionitis, cytokine, maternal anti-fetal rejection

Introduction

Preterm labor is a syndrome characterized by the combination of increased uterine contractility, cervical remodeling (i.e. ripening and dilatation), and decidual membrane activation, caused by multiple pathologic processes[1-12]. One of the mechanisms of disease implicated in preterm parturition is a breakdown immune tolerance, which may evolve into maternal anti-fetal rejection[13-31].

The fetus and placenta express both maternal and paternal antigens; therefore, they are semiallografts[32-43]. The placenta is considered to be the most successful transplant in nature, a biological adaptation accomplished by immune tolerance[44-49]. Tolerance is a specific immunological term that refers to "the active state of antigen specific non-responsivenness"[50] leading to diminished reactivity to paternal antigens expressed by the placenta and/or fetus, and is considered key for successful pregnancy[32, 34, 40, 41, 51-54]. The mechanisms responsible for tolerance in pregnancy include the following: 1) T cell chemokine gene silencing in the decidual cells[55]; 2) a suppressive role of regulatory T- cells[53, 56-65]; 3) expression of non-classical major histocompatibility complex (MHC) molecules on trophoblast cells which do not elicit a maternal immune response[66-71]; 4) changes in tryptophan catabolisms[72-76]; 5) T cell apoptosis[77, 78]; 6)

complement[79-91]; and 7) co-stimulatory molecules such as the programmed death ligand[92-94]. Other mechanisms for tolerance are not currently understood. The interested reader is referred to recent contributions by Sing Sing Way's laboratory[28, 40, 95-97], Adrian Erlebacher[32, 43, 54].

In transplantation medicine, failure of tolerance is responsible for graft rejection which is characterized by the infiltration of recipient's CD8+ (cytotoxic) T cells into the graft, and overexpression of C-X-C motif chemokine 10 (CXCL10), a marker of allograft rejection[98-103]. In obstetrics, rejection as a mechanism of disease has been largely overlooked. However, recent evidence suggests that maternal anti-fetal rejection is operative in a subset of patients with spontaneous preterm labor[15, 16, 20, 29, 104], preterm prelabor rupture of membranes (PROM)[20], fetal death[17, 25], recurrent abortion[19] and other obstetrical syndromes[14, 18, 21-24, 104]. Maternal lymphocytes (akin to a transplant recipient) can infiltrate the chorioamniotic membranes (fetal tissue or semiallograft), lead to chronic chorioamnionitis[15, 26] and induce trophoblast apoptosis, which when excessive, can in turn result in graft failure (e.g. membrane rupture or activation of membrane decidua and the initiation of labor)[15, 105]. The chemotactic signal inducing the migration of maternal T lymphocytes into the chorioamniotic membranes appears to be present in the amniotic cavity. One of such chemokines is CXCL10[15, 105, 106] and an increased concentration of this chemokine in the amniotic fluid has been characterized by our group to represent a distinct form of intra-amniotic inflammation, which is associated with chronic inflammatory lesions of the placenta and a novel form of a fetal inflammatory response syndrome (FIRS) or FIRS type 2[21].

This distinct form of intra-amniotic inflammation differs from the intra-amniotic inflammatory process observed in patients with preterm labor due to a microbial invasion of the amniotic cavity. Microorganisms and their products can induce a robust intra-amniotic inflammatory response characterized by an elevation of amniotic fluid IL-6 concentration[107-131], and neutrophil chemokines, such as IL-8[112-114, 132-141], as well as other inflammatory mediators capable of inducing the onset of labor[119, 136, 142-181]. Recently, we have provided an analysis of the protein inflammatory network on this condition[182]. The histologic hallmark of microbial invasion of the amniotic cavity is acute histologic chorioamnionitis, which is defined by the infiltration of maternal neutrophils into the chorioamniotic membranes[110, 183-197]. Related lesions are chorionic vasculitis[198] and the spectrum of lesions obverved in cases of funisitis[156, 199-206]. Therefore, at this time, at least two major types of intra-amniotic inflammation appear to occur in the context of spontaneous preterm labor – one associated with microbial invasion of the

amniotic cavity or induced by danger signals[125, 207-211], and the other associated with chronic inflammatory lesions of the placenta (often attributed to maternal anti-fetal rejection).

The concentrations of the T cell chemokine CXCL10 (IP-10) is considered a marker for chronic inflammatory lesions associated with allograft rejection and chronic chorioamnionitis in the case of pregnancy. In contrast, IL-6, IL-8, IL-1 and TNFα are examples of cytokines involved in acute inflammatory lesions of the placenta[110, 129, 131, 197, 212].

The objective of this study was to determine the prevalence and clinical significance of an elevated CXCL10 concentration in the amniotic fluid of patients with preterm labor with intact membranes and preterm PROM and whether an elevation of CXCL10 concentration is associated with chronic chorioamnionitis. Since an increased concentration of IL-6 and CXCL10 are frequently observed in patients with intra-amniotic infection and acute histologic chorioamnionitis.

Materials and Methods

Study population

A nested retrospective cohort study was conducted by searching the clinical database and bank of Biological samples of Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch of the Eunice Kennedy Shriver National Institutes of Child Health and Human Development (NICHD) (Detroit, MI) in order to identify patients with a diagnosis of spontaneous preterm labor with intact membranes or preterm PROM. Patients were included if they met the following criteria: 1) singleton gestation; 2) episode of preterm labor and intact or ruptured membranes; and 3) trans-abdominal amniocentesis performed between 20 and 35 weeks of gestation for microbiological studies. Patients were excluded if chromosomal or structural fetal anomalies or placenta previa were present. All patients provided written informed consent, and the use of biological specimens and clinical data for research purposes was approved by the Institutional Review Boards of NICHD and Wayne State University.

Biological samples and analysis

Amniotic fluid was transported in a capped sterile syringe to the clinical laboratory where it was cultured for aerobic and anaerobic bacteria, including genital Mycoplasmas. Evaluation of white blood cell (WBC) count, glucose concentration, and Gram stain of amniotic fluid were performed shortly after collection. Amniotic fluid was centrifuged at 1,300g for 10 minutes at 4°C shortly after collection, and stored at -70°C until analysis. Amniotic fluid IL-6 and CXCL10

concentrations (ng/mL) were determined by enzyme-linked immunosorbent assay (ELISA) using immunoassays obtained from R&D Systems (Minneapolis, MN, USA). The assay time, volume and other characteristics for each method have been previously described[15, 124, 125, 170, 207-209].

Clinical Definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination, or by ultrasound examination alone if the sonographic determination of gestational age was not consistent with menstrual dating[213]. Preterm labor was diagnosed by the presence of at least two regular uterine contractions every 10 minutes in association with cervical changes in patients with a gestational age between 20 and 36 6/7 weeks which led to preterm delivery (defined as birth prior to the 37th week of gestation). Preterm PROM was diagnosed by a sterile speculum examination with documentation of pooling of amniotic fluid in the vagina in association with a positive nitrazine test and/or positive ferning test when necessary. Elevated amniotic fluid IL-6 concentration (≥2.6 ng/mL) was used to define Intra-amniotic inflammation[177, 207-210, 214-217]. Microbial invasion of the amniotic cavity (MIAC) was defined as a positive amniotic fluid culture. Intra-amniotic infection was defined as the combination of MIAC and intra-amniotic inflammation. An elevated amniotic fluid CXCL10 concentration as a marker of subclinical intraamniotic inflammation was defined as ≥2.2 ng/mL, which is above the 95th percentile among patients with uncomplicated term deliveries[170].

The diagnosis of acute histologic chorioamnionitis was based on the presence of acute inflammatory changes in the extra-placental chorioamniotic membrane roll and/or chorionic plate of the placenta using criteria previously described[189-191, 193, 197, 218, 219]. The grading and staging of placental lesions consistent with amniotic fluid infection was defined according to the Amniotic Fluid Infection Nosology Committee of the Perinatal Section of the Society for Pediatric Pathology as reported by Redline et al[189]. Acute funisitis was defined as the presence of neutrophils in the wall of umbilical vessels and/or Wharton's jelly[189, 197, 198]. Chronic placental inflammatory lesions included: 1) chronic chorioamnionitis; 2) villitis of unknown etiology (VUE) and; 3) chronic deciduitis. Chronic chorioamnionitis was diagnosed when lymphocytic infiltration into the chorionic trophoblast layer or chorioamniotic connective tissue was observed[14, 15, 26, 220]. VUE was defined as the presence of lymphohistiocytic infiltrate in varying proportion of the placental villous tree[14, 221]. Chronic deciduitis was diagnosed as the presence of lymphocytic infiltration into the decidua of the basal plate[222]. Lesions consistent with maternal anti-fetal

rejection proposed by our group included chronic chorioamnionitis, VUE or chronic deciduitis with plasma cells[14, 16].

Study groups

Participants were grouped according to whether they had an increase of amniotic fluid CXCL10 concentration and/or an increase of amniotic fluid IL-6 concentration into the following four study groups: 1) normal amniotic fluid IL-6 and CXCL10 concentrations; 2) an isolated increase of amniotic fluid IL-6 concentration; 3) an isolated increase of amniotic fluid CXCL10 concentration; and 4) an increase of both amniotic fluid IL-6 and CXCL10 concentrations. The cutoff has been derived from previous studies[110, 112, 170].

Study outcomes

The primary outcome of this study was the presence or absence of acute or chronic chorioamnionitis, which were defined as: 1) the absence of both acute and chronic chorioamnionitis; 2) acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; 3) chronic chorioamnionitis in the absence of acute histologic chorioamnionitis≥stage 2; and 4) the presence of both acute (≥stage 2) and chronic chorioamnionitis. The presence of placental lesions associated with maternal anti fetal-rejection was examined as a secondary outcome[219].

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess normality of arithmetic data distributions. The Kruskal-Wallis and the Mann-Whitney U tests were used to make comparisons among and between groups for arithmetic variables. Chi-square or Fisher's exact test were used for comparisons of proportions. Multinomial logistic regression models were fit to examine magnitudes of association with primary and secondary outcomes, adjusting for gestational age at amniocentesis. Statistical analysis was performed using SAS 9.4 (Cary, NC). Confidence intervals (95%) that do not include the null hypothesis (i.e., an odds ratio- OR- of '1.0') are considered statistically significant.

Results

Clinical Characteristics

One hundred and sixty eight women with either preterm labor with intact membranes (72%) or preterm PROM (28%) were included in this study. Table 1 shows the clinical

characteristics. Most women (88%) were African-American, 34% were nulliparous, and 83% delivered preterm (<37 weeks of gestation). The median gestational age at amniocentesis was 30 weeks (inter-quartile range: 27-32 weeks) and amniotic fluid cultures were positive in 20% of the study participants. Placental lesions associated with acute and chronic histologic chorioamnionitis and maternal anti-fetal rejection were observed in 49% (82/168), 27% (45/168) and 41% (69/168) of the study population, respectively.

Amniotic fluid CXCL10 and IL-6 concentrations according to placental pathologic lesions and outcome of pregnancy

Amniotic fluid CXCL10 concentrations were highest in patients with chronic chorioamnionitis (Figure 1), whereas amniotic fluid IL-6 concentrations were highest in patients with acute chorioamnionitis≥stage 2 (Figure 2). Clinical characteristics and the prevalence of acute and chronic inflammatory placental lesions for the four study groups defined according to the amniotic fluid CXCL10 and amniotic fluid IL-6 concentrations are shown in Table 2.

An elevation of both amniotic fluid CXCL10 (≥2.2 ng/mL) and amniotic fluid IL-6 (≥2.6 ng/mL) concentrations was observed in 35% (59/168) of the study participants, whereas 18% (30/168) had an isolated elevation of amniotic fluid CXCL10 concentration, 15% (26/168) had an isolated elevation of amniotic fluid IL-6 concentration, and 32% (53/168) did not have an elevation of amniotic fluid concentrations of either CXCL10 or IL-6. All patients with both elevated amniotic fluid CXCL10 and IL-6 concentrations delivered before 37 weeks of gestation, while 93% (24/26) of patients with an isolated elevation of amniotic fluid IL-6 concentration and 77% (23/30) of those with an isolated elevation of amniotic fluid CXCL10 concentration delivered preterm.

Acute and chronic chorioamnionitis in relationship to amniotic fluid concentrations of CXCL10 and IL-6

The prevalence of chronic chorioamnionitis was highest in patients with an isolated elevation of amniotic fluid CXCL10 concentration (46.7%; 14/30), and was lowest in those with an isolated elevation of amniotic fluid IL-6 concentration (7.7%; 2/26) (Table 2). In contrast, the prevalence of acute chorioamnionitis ≥stage 2 was highest in patients with an isolated elevation of both amniotic fluid IL-6 and CXCL10 concentrations (52.5%; 31/59) followed by in patients with an isolated elevation of amniotic fluid IL-6 concentration (42.3%; 11/26). The prevalence of such placental lesions was observed in 13% (4/30) of patients with an isolated elevation of

amniotic fluid CXCL10 concentration. Interestingly, almost all patients with acute and chronic chorioamnionitis (83.3%; 5/6) had an elevation of both amniotic fluid IL-6 and CXCL10 concentrations (Table 2).

The magnitude of the associations between the study groups according to amniotic fluid CXCL10 and IL-6 concentrations and the presence or absence of acute or chronic chorioamnionitis are described in Figure 3. Patients with an isolated elevation of CXCL10 concentration were significantly more likely to have chronic, but not acute chorioamnionitis (OR 4.8; 95%CI 1.7-14 and OR 2.1; 95%CI 0.5-8.9, respectively) than those with normal amniotic fluid CXCL10 and IL-6 concentrations, after adjusting for gestational age at amniocentesis. In contrast, patients with an isolated elevation of amniotic fluid IL-6 concentration were significantly more likely to have acute (≥stage 2), but not chronic chorioamnionitis (OR 4.2; 95%CI 1.3-13.7 and OR 0.5; 95%CI 0.1-2.8, respectively) than those with normal amniotic fluid CXCL10 and IL-6 concentrations. An elevation of amniotic fluid concentrations of both CXCL10 and IL-6 was associated with acute (≥stage 2) and chronic chorioamnionitis (OR 9.6; 95%CI 3.1-30 and OR 3.8; 95%CI 1.3-11.6, respectively). None of the patients whose placentas had both acute (≥stage 2) and chronic chorioamnionitis (n=6) had normal amniotic fluid CXCL10 and IL-6 concentrations.

The magnitude of the associations between the study groups according to amniotic fluid CXCL10 and IL-6 concentrations and placental lesions associated with maternal anti-fetal rejection are shown in Figure 4. Patients with an elevation of amniotic fluid CXCL10 concentration were significantly more likely to have placental lesions associated with maternal anti-fetal rejection, but not acute chorioamnionitis (≥stage 2) than those with normal amniotic fluid CXCL10 and IL-6 concentrations, adjusting for gestational age at amniocentesis (OR 3.7; 95%CI 1.3-10.4 and OR 1.6; 95%CI 0.3-8.3, respectively).

The combination of both acute chorioamnionitis (≥stage 2) and placental lesions associated with maternal anti-fetal rejection was not observed in patients without an elevation of amniotic fluid IL-6 and CXCL10 concentrations (Figure 4).

Discussion

Principal findings of the study: 1) An isolated elevation of amniotic fluid CXCL10 concentration is associated with chronic, but not acute (≥stage 2) histologic chorioamnionitis; 2) in contrast, an isolated elevation of amniotic fluid IL-6 concentration was associated with an

acute (≥stage 2), but not chronic histologic chorioamnionitis; 3) similar findings were observed in relation to placental lesions associated with maternal anti-fetal rejection (chronic chorioamnionitis, VUE and/or chronic deciduitis with plasma cells). Specifically, an isolated elevation of amniotic fluid CXCL10 concentration is associated with the subsequent delivery of placentas with lesions consistent with maternal anti-fetal rejection, but not acute histologic chorioamnionitis (≥stage 2); and 4) elevation of both CXCL10 and IL-6 is associated with acute and chronic inflammatory lesions of the placenta, as well as the combination of lesions suggesting that a complex pathologic state representing a mixture of maternal anti-fetal rejection and infection may lead to early preterm delivery in these cases. 5) all patients with elevated AF concentrations of both CXCL10 and IL6 delivered prematurely.

Two types of intra-amniotic inflammation in preterm labor

Microbial-associated and sterile intra-amniotic inflammation

Preterm parturition is a syndrome caused by multiple etiologies[2, 4, 6-8, 12]. Intra-amniotic infection is present in one of every three preterm deliveries, and is even more frequent in cases of spontaneous preterm labor with intact membranes[223-228]. Microorganisms are detected in the amniotic cavity in 25-40% of patients with preterm labor and intact membranes who deliver preterm[134, 223-236], and in 50-75% of those with preterm PROM at the time of labor onset[237]. The earlier the gestational age at presentation, the greater the risk of microbial invasion of the amniotic cavity or intra-amniotic infection[6, 12, 227, 228, 235, 238-240].

The current study found that 79.8% (134/168) of patients with preterm labor/PROM have no evidence of microbial invasion of the amniotic cavity, suggesting the important role of sterile inflammation of the amniotic cavity. Using a combination of cultivation and molecular techniques, we have previously reported that only a fraction of all patients with an intra-amniotic inflammation (defined as an increase of amniotic fluid IL-6 concentration) have microorganisms present in the amniotic cavity and therefore, sterile intra-amniotic fluid inflammation has emerged as an important mechanism of disease for preterm labor[125, 207-209, 211]. “Danger signals” released during the course of cellular stress, necrosis, pyroptosis and senescence as well as other non-microbial injury can trigger an inflammatory response in the absence of microorganisms[216, 241-258]. “Danger signals” may also participate in the sterile inflammatory response associated with spontaneous labor at term, and are probably mediated by activation of the inflammasomes[246, 259-263]. Recent evidence suggests that the intra-amniotic administration of alarmins such as HMGB1 can induce preterm parturition in mice[264] and that this cytokine can

induce a robust immune response characterized by secretion of IL-6 and IL-1β from human fetal membranes[265], suggesting a role for the inflammasomes in the mechanisms leading to premature labor in cases of sterile inflammation[125, 216, 252]. Thus, this mechanism may be involved in patients with sterile intra-amniotic inflammation that is characterized by elevated amniotic fluid IL-6 concentrations and acute histologic chorioamnionitis. Moreover, a fraction of patients included in this study have elevated amniotic fluid IL-6, as well as CXCL-10 concentrations. All these patients delivered preterm and had an odds ratio of 10.9 for acute histologic chorioamnionitis and 4.3 for placental lesions consistent with maternal anti-fetal rejection. The role of the interaction between the acute inflammatory processes which activate the inflammasome and that involved in fetal rejection are yet to be discovered.

When bacteria and other microorganisms are present in the amniotic cavity and elicit an inflammatory response, a wide range of chemokines and cytokines, such as IL-8[112-114, 132-141], IL-6[107-114, 116-122, 124-127, 129-131], monocyte chemotactic protein (MCP)-1[165, 166], CXCL10 (IP-10)[129, 170], macrophage inflammatory protein (MIP)-1α[266, 267], growth regulated oncogene (GRO)-alpha[136], and other inflammatory related proteins[119, 142-164, 167-169, 171-177, 180, 268] are produced, and this can result in the chemotaxis of inflammatory cells to the chorioamniotic membranes. Among these inflammatory related proteins, IL-6 has become the key cytokine for the diagnosis of intra-amniotic inflammation, because its increase in concentration has been associated with a shorter interval to delivery and an increased rate of neonatal morbidity and mortality[110, 125, 269, 270]. Recently, an in-depth analysis of the chemokine network in preterm labor with and without inflammation, sterile inflammation, and intra-amniotic infection have been reported[271]. Network analysis provides a greater level of insight into the biology of the process, given that the protein inflammatory process operates through a network rather than single molecules[271]. Collectively, amniotic fluid IL-6 is a pragmatic marker of microbial-associated or sterile intra-amniotic inflammation. We anticipate that with the development of high fidelity assays that allow multiplex analysis of biological fluids, it will be possible to characterize with greater detail the biology of the immune response, timetable, response to therapy, and other important clinical characteristics.

A novel form of intra-amniotic inflammation characterized by CXCL10

We have previously identified a form of intra-amniotic inflammation characterized by an increase of CXCL10 concentration[15, 170] which is associated with chronic chorioamnionitis, the most common placental lesion in late spontaneous preterm delivery[22]. This form of intra-

amniotic inflammation is considered a manifestation of maternal anti-fetal rejection[15, 22, 26, 170] as an infectious cause has not been identified using cultivation and molecular methods.

Compelling evidence suggests that CXCL10 plays an important role in the pathogenesis of graft failure and rejection in other organ systems[98-103]. Overexpression of this T cell chemokine has been demonstrated in serum/plasma[272, 273], urine[274, 275] and tissue biopsies[100, 276-278] in patients with rejection in cases of kidney[272, 279-284], heart[285-287], lung[273, 276] and vascular transplantation[288-290]. Moreover, there is a significant correlation between serum/plasma CXCL10 concentrations and the timing and severity of allograft rejection[101, 272, 273, 281, 287].

In chronic chorioamnionitis, which can be considered a form of allograft rejection, there is an upregulation of CXCL9, CXCL10 and CXCL11 mRNA expression in the chorioamniotic membranes[15]. Upregulation of CXC chemokines for CXCR3+ (receptor for T cell cheomokines) cells in the chorioamniotic membranes is associated with a higher median amniotic fluid T cell chemokine (CXCL10) concentration, and also chronic chorioamnionitis, presumably by stimulating amniotrophic maternal T cell migration to the chorioamniotic membranes[15]. This placental lesion represents a manifestation of maternal anti-fetal rejection as demonstrated by: 1) higher maternal anti-fetal human leukocyte antigen (HLA) sensitization[18] in patients with chronic chorioamnionitis than in those without this lesion; 2) complement deposition (C4d), a surrogate marker of antibody-mediated rejection, in the umbilical vein[16, 23, 24]; and 3) the presence of a novel form of fetal systemic inflammation (FIRS type 2) in the setting of chronic chorioamnionitis. The transcriptome of the umbilical cord blood in FIRS type 2 is different from that of FIRS type 1, indicating that this is a different condition[21]. Moreover, a proteomic analysis of the amniotic fluid of patients with chronic chorioamnionitis demonstrated that these patients have lower amniotic fluid concentrations of glycodelin-A[291], a protein implicated in the maintenance of maternal tolerance against a semiallogeneic fetus[292].

Interestingly, approximately 40% of placentas with chronic chorioamnionitis in patients with preterm labor or preterm PROM have concomitant VUE and chronic deciduitis with plasma cells[15]. We have demonstrated a systemic derangement in chemokine concentrations, both in maternal and fetal circulations, in patients with VUE which were distinct from those observed in the setting of acute chorioamnionitis[14]. The mRNA expression of a subset of chemokines and their receptors (CXCL9, CXCL10, CXCL11, CXCL13 and CXCR3) was also higher in VUE placentas than in normal placentas[14]. Moreover, the median concentrations of CXCL9, CXCL10, and CXCL11 in maternal and fetal plasma were higher in patients with VUE than in those

without this lesion[14]. Therefore, we also consider VUE as a manifestation of maternal anti-fetal rejection unless a microorganism can be identified.

In summary, intra-amniotic inflammation associated with maternal anti-fetal rejection is different from microbial-associated intra-amniotic inflammation and it is characterized by an elevation of T cell chemokine concentration in the amniotic fluid and chorioamniotic membranes as well as the presence of chronic inflammatory lesions of the placenta.

CXCL10: a biomarker for chronic placental inflammatory lesion

The results of the study herein support the view that CXCL10 is a marker for chronic inflammatory lesions of the placenta. Our findings are consistent with those of Gervasi et al., who reported that mid-trimester amniotic fluid CXCL10 concentrations >502 pg/mL were associated with late (>32 weeks) spontaneous preterm delivery (OR 3.9; 95% CI 1.6-9.9), whereas elevated amniotic fluid IL-6 concentrations (>1,740 pg/mL) were associated with higher risk of spontaneous preterm delivery at ≤32 weeks of gestation (OR 9.5; 95% CI 2.9-31.1)[170]. Our study differs, in that we examined the relationship between an isolated elevation of amniotic fluid CXCL10 or amniotic fluid IL-6 concentration and its association with acute and chronic histologic chorioamnionitis.

What is the significance of an elevation of both amniotic fluid IL-6 and CXCL10 concentrations?

Thirty five percent (59/168) of patients in this study had elevated amniotic fluid concentrations of both IL-6 and CXCL10. All and almost 80% of them had spontaneous preterm delivery <37 and <34 weeks of gestation, respectively, suggesting a severe inflammatory process associated with preterm delivery. Moreover, patients with an elevation of both amniotic fluid IL-6 and CXCL10 concentrations had significantly higher frequency of spontaneous preterm delivery within 48 hours of amniocentesis than those with an isolated elevation of amniotic fluid CXCL10 concentration (Table 2). Indeed, a systemic fetal inflammatory response (defined as the presence of funisitis or chorionic vasculitis)[198] was detected in 54.2% (32/59) of patients with an elevation in both of amniotic fluid CXCL10 and IL-6 concentrations, but in only 34.6% (9/26) and 20% (6/30) of patients with an isolated elevation of amniotic fluid IL-6 or CXCL10 concentration, respectively (Table 2). One interpretation of this is that patients with a combination of increased amniotic fluid IL-6 and CXCL10 concentrations had a more severe form of intra-amniotic inflammation than those with an isolated elevation of either CXCL10 or IL-

6 concentration, in whom the clinical course leading to preterm delivery may be more indolent in nature. This could explain the trend towards a more frequent involvement of the fetus in patients with both an elevation in amniotic fluid CXCL10 and IL-6 concentrations.

CXCL10 has been implicated in the pathophysiology of sepsis by recruiting neutrophils, macrophages and T cells[293, 294]. Previous studies have demonstrated that during infection and inflammation, there is an upregulation of CXCL10 leading to subsequent activation of its receptor (CXCR3)[295, 296]. In an experimental model of septic shock induced by cecal ligation and puncture, it has been shown that plasma and peritoneal fluid CXCL10 concentrations increase[297]. In addition, CXCL10 knockout mice and the wild-type mice treated with anti-CXCL10 IgG antibody had less cytokine production and increased survival[298]. Similar observations are found for the role of CXCR3 during sepsis; it regulates NK-and T-cell trafficking. Moreover, in a septic shock model for mice, the blockade of CXCR3 decreases systemic inflammation and improves survival[298, 299]. In addition, CXCL10 and CXCR3 play a role in human sepsis and plasma CXCL10 is a predictor of septic shock[300-302]. Collectively, these data suggests that CXCL10 is an inflammatory mediator involved in the response to microorganisms and bacterial products, and therefore, some cases of advanced infections could have elevated concentrations of both IL-6 and CXCL10. An elevated amniotic fluid concentration of CXCL10 would be more meaningful to identify that patient at risk for chronic inflammatory lesions of the placenta if the amniotic fluid concentration of IL-6 is not elevated.

Strengths and Limitations

The major strengths of this study include blinding of pathologists to obstetrical diagnoses and outcomes, the use of standardized protocols for placental examination, and our consideration of isolated rather than any increase of either amniotic fluid CXCL10 or amniotic fluid IL-6 concentrations. Limitations relate to the sample size to perform additional analyses separating early and late spontaneous preterm delivery with and without chronic lesions. Further studies are required to characterize the temporal relationship between exposure to microbial products or other insults and the amniotic fluid changes in cytokines and chemokines. Moreover, large studies are necessary to determine the diagnostic indices of CXCL10 elevations to identify the patient at risk for chronic placental inflammation.

Conclusions

An isolated elevation of amniotic fluid CXCL10 concentration (without a concomitant elevation of IL-6 concentration) is associated with the delivery of a placenta with histologic chronic chorioamnionitis or lesions consistent with maternal anti-fetal rejection, whereas an isolated increase of amniotic fluid IL-6 concentration is associated with the delivery of placenta with acute histologic chorioamnionitis.

Figure Legend

Figure 1: The median concentration of amniotic fluid C-X-C motif chemokine (CXCL) 10 concentrations in patients according to the presence of absence of acute (≥stage 2) or chronic chorioamnionitis. The median (interquartile range: IQR) amniotic fluid concentration of CXCL10 (ng/mL) was highest in patients with chronic chorioamnionitis. The median (IQR) amniotic fluid concentration of CXCL10 (ng/mL) was 1.6 (0.9-3.4), 3.1 (1.1-6.9), 4.1 (1.9-7.7) and 3.9 (3.0-9.7) in patients with neither acute nor chronic chorioamnionitis, acute chorioamnionitis (≥stage 2), chronic chorioamnionitis as well as acute and chronic chorioamnionitis, respectively.

AF: amniotic fluid; CXCL: C-X-C motif chemokine; IL: interleukin; Acute chorioamnionitis: the presence of acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; chronic chorioamnionitis: the presence of chronic chorioamnionitis in the absence of acute chorioamnionitis ≥stage 2; *p value <0.05

Figure 2: The median concentration of amniotic fluid interleukin (IL)-6 concentrations in patients with acute chorioamnionitis ≥stage 2 and/or chronic chorioamnionitis. The median (interquartile range: IQR) amniotic fluid concentration of IL-6 (ng/mL) was highest in patients with acute chorioamnionitis ≥stage 2. The median (IQR) amniotic fluid concentration of IL-6 (ng/mL) was 1.5 (0.6-4.2), 41.6 (5.3-20.7), 1.6 (0.7-5.4) and 17.6 (3.9-167.4) in patients with neither acute nor chronic chorioamnionitis, acute chorioamnionitis, chronic chorioamnionitis as well as acute and chronic chorioamnionitis, respectively.

AF: amniotic fluid; CXCL: C-X-C motif chemokine; IL: interleukin; Acute chorioamnionitis: the presence of acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; chronic chorioamnionitis: the presence of chronic chorioamnionitis in the absence of acute chorioamnionitis ≥stage 2; *p value <0.05

Figure 3: Magnitudes of association between the study groups according to amniotic fluid IL-6 and CXCL10 concentrations and the presence or absence of acute (≥stage 2) or chronic chorioamnionitis. Results obtained by fitting a multinomial logistic regression model adjusting for gestational age at amniocentesis;

AF: amniotic fluid; CXCL: C-X-C motif chemokine; IL: interleukin;
Normal AF IL-6 and CXCL10 concentrations: IL-6 <2.6 ng/mL and CXCL10 <2.2 ng/mL; Isolated increase of AF IL-6 concentration: IL-6 ≥2.6 ng/mL; Isolated increase of AF CXCL10 concentration: CXCL10 ≥2.2 ng/mL; Increase of both AF IL-6 and CXCL10 concentrations: IL-6 ≥2.6 ng/mL and CXCL10 ≥2.2 ng/mL;
Acute chorioamnionitis: the presence of acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; chronic chorioamnionitis: the presence of chronic chorioamnionitis in the absence of acute chorioamnionitis ≥stage 2

*None of the patients in normal AF IL-6 and CXCL10 concentrations group had both acute and chronic placental inflammatory lesions, therefore the computation of odds ratios relative to the common reference cannot be performed

Figure 4: Magnitudes of association between the study groups according to amniotic fluid IL-6 and CXCL10 concentrations and the presence or absence of acute chorioamnionitis (≥stage 2) or placental lesions associated with maternal anti-fetal rejection. Results obtained by fitting a multinomial logistic regression model adjusting for gestational age at amniocentesis;
AF: amniotic fluid; CXCL: C-X-C motif chemokine; IL: interleukin;
Normal AF IL-6 and CXCL10 concentrations: IL-6 <2.6 ng/mL and CXCL10 <2.2ng/mL; Isolated increase of AF IL-6 concentration: IL-6 ≥2.6 ng/mL; Isolated increase of AF CXCL10 concentration: CXCL10 ≥2.2 ng/mL; Increase of both AF IL-6 and CXCL10 concentrations: IL-6 ≥2.6 ng/mL and CXCL10 ≥2.2 ng/mL;
Acute chorioamnionitis: the presence of acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; chronic chorioamnionitis: the presence of chronic chorioamnionitis in the absence of acute chorioamnionitis ≥stage 2; Placental lesions associated with maternal anti-fetal rejection: Lesions included chronic chorioamnionitis, villitis of unknown etiology and chronic deciduitis with plasma cells

*None of the patients in normal AF IL-6 and CXCL10 concentrations group had both acute and chronic placental inflammatory lesions, therefore the computation of odds ratios relative to the common reference cannot be performed

References

1 Wilkins I, Creasy RK: Preterm labor. Clin Obstet Gynecol 1990;33:502-514.

2 Romero R, Mazor M, Munoz H, Gomez R, Galasso M, Sherer DM: The preterm labor syndrome. Ann N Y Acad Sci 1994;734:414-429.

3 Mazor M, Chaim W, Romero R: [Preterm labor syndrome]. Harefuah 1995;128:111-116.

4 Romero R, Gomez R, Mazor M, Ghezzi F, Yoon BH: The preterm labor syndrome. In *Preterm labor*, MG Elder, R Romero, RF Lamont (eds). New York, Churchill Livingstone, 1997, pp 29-49.

5 Dudley DJ: Pre-term labor: an intra-uterine inflammatory response syndrome? J Reprod Immunol 1997;36:93-109.

6 Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M: The preterm parturition syndrome. BJOG 2006;113 Suppl 3:17-42.

7 Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, Kramer M, Culhane JF, Barros FC, Conde-Agudelo A, Bhutta ZA, Goldenberg RL: The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol 2012;206:119-123.

8 Kramer MS, Papageorghiou A, Culhane J, Bhutta Z, Goldenberg RL, Gravett M, Iams JD, Conde-Agudelo A, Waller S, Barros F, Knight H, Villar J: Challenges in defining and classifying the preterm birth syndrome. Am J Obstet Gynecol 2012;206:108-112.

9 Goldenberg RL, Gravett MG, Iams J, Papageorghiou AT, Waller SA, Kramer M, Culhane J, Barros F, Conde-Agudelo A, Bhutta ZA, Knight HE, Villar J: The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol 2012;206:113-118.

10 Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, Kinney M, Lawn J: Born too soon: the global epidemiology of 15 million preterm births. Reprod Health 2013;10 Suppl 1:S2.

11 Guimaraes Filho HA, Araujo Junior E, Pires CR, Nardozza LM, Moron AF: Short cervix syndrome: current knowledge from etiology to the control. Archives of gynecology and obstetrics 2013;287:621-628.

12 Romero R, Dey SK, Fisher SJ: Preterm labor: one syndrome, many causes. Science 2014;345:760-765.

13 Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, Bencini E, Menicucci A, Baricordi OR: HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 2002;32:311-315.

14 Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, Lee DC, Draghici S, Gotsch F, Kusanovic JP, Hassan SS, Kim JS: Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feto-maternal and placental compartments: implications for

conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. J Immunol 2009;182:3919-3927.

15 Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, Gotsch F, Yoon BH, Chi JG, Kim JS: The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. Mod Pathol 2010;23:1000-1011.

16 Lee J, Romero R, Xu Y, Kim JS, Topping V, Yoo W, Kusanovic JP, Chaiworapongsa T, Hassan SS, Yoon BH, Kim CJ: A signature of maternal anti-fetal rejection in spontaneous preterm birth: chronic chorioamnionitis, anti-human leukocyte antigen antibodies, and C4d. PLoS One 2011;6:e16806.

17 Lee J, Romero R, Dong Z, Xu Y, Qureshi F, Jacques S, Yoo W, Chaiworapongsa T, Mittal P, Hassan SS, Kim CJ: Unexplained fetal death has a biological signature of maternal anti-fetal rejection: chronic chorioamnionitis and alloimmune anti-human leucocyte antigen antibodies. Histopathology 2011;59:928-938.

18 Lee J, Romero R, Xu Y, Kim JS, Park JY, Kusanovic JP, Chaiworapongsa T, Hassan SS, Kim CJ: Maternal HLA panel-reactive antibodies in early gestation positively correlate with chronic chorioamnionitis: evidence in support of the chronic nature of maternal anti-fetal rejection. Am J Reprod Immunol 2011;66:510-526.

19 Romero R, Whitten A, Korzeniewski SJ, Than NG, Chaemsaithong P, Miranda J, Dong Z, Hassan SS, Chaiworapongsa T: Maternal floor infarction/massive perivillous fibrin deposition: a manifestation of maternal antifetal rejection? Am J Reprod Immunol 2013;70:285-298.

20 Lee J, Romero R, Xu Y, Miranda J, Yoo W, Chaemsaithong P, Kusanovic JP, Chaiworapongsa T, Tarca AL, Korzeniewski SJ, Hassan SS, Than NG, Yoon BH, Kim CJ: Detection of anti-HLA antibodies in maternal blood in the second trimester to identify patients at risk of antibody-mediated maternal anti-fetal rejection and spontaneous preterm delivery. Am J Reprod Immunol 2013;70:162-175.

21 Lee J, Romero R, Chaiworapongsa T, Dong Z, Tarca AL, Xu Y, Chiang PJ, Kusanovic JP, Hassan SS, Yeo L, Yoon BH, Than NG, Kim CJ: Characterization of the fetal blood transcriptome and proteome in maternal anti-fetal rejection: evidence of a distinct and novel type of human fetal systemic inflammatory response. Am J Reprod Immunol 2013;70:265-284.

22 Lee J, Kim JS, Park JW, Park CW, Park JS, Jun JK, Yoon BH: Chronic chorioamnionitis is the most common placental lesion in late preterm birth. Placenta 2013;34:681-689.

23 Lee KA, Kim YW, Shim JY, Won HS, Lee PR, Kim A, Kim CJ: Distinct patterns of C4d immunoreactivity in placentas with villitis of unknown etiology, cytomegaloviral placentitis, and infarct. Placenta 2013;34:432-435.

24 Rudzinski E, Gilroy M, Newbill C, Morgan T: Positive C4d immunostaining of placental villous syncytiotrophoblasts supports host-versus-graft rejection in villitis of unknown etiology. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society 2013;16:7-13.

25 Lannaman K, Romero R, Chaemsaithong P, Ahmed AI, Yeo L, Hassan S, Yoon BH, chaiworapongsa T: Abstract No. 497 Fetal death: an extreme form of maternal anti-fetal rejection. Am J Obstet Gynecol 2015;212:S251.

26 Kim CJ, Romero R, Chaemsaithong P, Kim J: Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. Am J Obstet Gynecol 2015;213:S53-S69.

27 Clark GF: The role of glycans in immune evasion: the human fetoembryonic defence system hypothesis revisited. Mol Hum Reprod 2014;20:185-199.

28 Jiang TT, Chaturvedi V, Ertelt JM, Kinder JM, Clark DR, Valent AM, Xin L, Way SS: Regulatory T cells: new keys for further unlocking the enigma of fetal tolerance and pregnancy complications. J Immunol 2014;192:4949-4956.

29 Kim CJ, Romero R, Chaemsaithong P, Kim JS: Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. Am J Obstet Gynecol 2015;213:S53-69.

30 Lee YC, Lin SJ: Natural killer cell in the developing life. J Perinat Med 2015;43:11-17.

31 Schefold JC, Porz L, Uebe B, Poehlmann H, von Haehling S, Jung A, Unterwalder N, Meisel C: Diminished HLA-DR expression on monocyte and dendritic cell subsets indicating impairment of cellular immunity in pre-term neonates: a prospective observational analysis. J Perinat Med 2015;43:609-618.

32 Erlebacher A: Why isn't the fetus rejected? Curr Opin Immunol 2001;13:590-593.

33 Koch CA, Platt JL: Natural mechanisms for evading graft rejection: the fetus as an allograft. Springer Semin Immunopathol 2003;25:95-117.

34 Trowsdale J, Betz AG: Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol 2006;7:241-246.

35 Leslie M: Immunology. Fetal immune system hushes attacks on maternal cells. Science 2008;322:1450-1451.

36 Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, Lee TH, Nixon DF, McCune JM: Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008;322:1562-1565.

37 Burlingham WJ: A lesson in tolerance--maternal instruction to fetal cells. N Engl J Med 2009;360:1355-1357.

38 Chaouat G, Petitbarat M, Dubanchet S, Rahmati M, Ledee N: Tolerance to the foetal allograft? Am J Reprod Immunol 2010;63:624-636.

39 Bluestone JA: Mechanisms of tolerance. Immunol Rev 2011;241:5-19.

40 Rowe JH, Ertelt JM, Xin L, Way SS: Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature 2012;490:102-106.

41 Betz AG: Immunology: Tolerating pregnancy. Nature 2012;490:47-48.

42 Williams Z: Inducing tolerance to pregnancy. N Engl J Med 2012;367:1159-1161.

43 Erlebacher A: Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol 2013;13:23-33.

44 Le Moine A, Goldman M, Abramowicz D: Multiple pathways to allograft rejection. Transplantation 2002;73:1373-1381.

45 Colvin RB, Smith RN: Antibody-mediated organ-allograft rejection. Nat Rev Immunol 2005;5:807-817.

46 Alegre ML, Florquin S, Goldman M: Cellular mechanisms underlying acute graft rejection: time for reassessment. Curr Opin Immunol 2007;19:563-568.

47 Kim IK, Bedi DS, Denecke C, Ge X, Tullius SG: Impact of innate and adaptive immunity on rejection and tolerance. Transplantation 2008;86:889-894.

48 Wood KJ, Goto R: Mechanisms of rejection: current perspectives. Transplantation 2012;93:1-10.

49 Ali JM, Bolton EM, Bradley JA, Pettigrew GJ: Allorecognition pathways in transplant rejection and tolerance. Transplantation 2013;96:681-688.

50 Krensky AM: Immunologic tolerance. Pediatr Nephrol 2001;16:675-679.

51 Sacks G, Sargent I, Redman C: An innate view of human pregnancy. Immunol Today 1999;20:114-118.

52 Szekeres-Bartho J: Immunological relationship between the mother and the fetus. Int Rev Immunol 2002;21:471-495.

53 Aluvihare VR, Kallikourdis M, Betz AG: Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004;5:266-271.

54 Erlebacher A: Immunology of the maternal-fetal interface. Annu Rev Immunol 2013;31:387-411.

55 Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A: Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science 2012;336:1317-1321.

56 Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT: Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology 2004;112:38-43.

57 Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S: Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 2004;10:347-353.

58 Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, Kotsch K, Leber J, Volk HD: Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol 2005;166:811-822.

59 Lee JH, Ulrich B, Cho J, Park J, Kim CH: Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. J Immunol 2011;187:1778-1787.

60 Ramhorst R, Fraccaroli L, Aldo P, Alvero AB, Cardenas I, Leiros CP, Mor G: Modulation and recruitment of inducible regulatory T cells by first trimester trophoblast cells. Am J Reprod Immunol 2012;67:17-27.

61 Quinn KH, Parast MM: Decidual regulatory T cells in placental pathology and pregnancy complications. Am J Reprod Immunol 2013;69:533-538.

62 Wilczynski JR, Kalinka J, Radwan M: The role of T-regulatory cells in pregnancy and cancer. Front Biosci 2008;13:2275-2289.

63 Schumacher A, Zenclussen AC: Regulatory T cells: regulators of life. Am J Reprod Immunol 2014;72:158-170.

64 Collier A, Cook H, Loewendorf A, Yesayan M, Kahn D: Abstract No.438 Disruption of maternal tolerance during pregnancy leads to treg repopulation of the antigenic UPI. Am J Obstet Gynecol 2015;212:S226-227.

65 Saifi B, Aflatoonian R, Tajik N, Erfanian Ahmadpour M, Vakili R, Amjadi F, Valizade N, Ahmadi S, Rezaee SA, Mehdizadeh M: T regulatory markers expression in unexplained recurrent spontaneous abortion. J Matern Fetal Neonatal Med 2015:1-6.

66 Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R: A class I antigen, HLA-G, expressed in human trophoblasts. Science 1990;248:220-223.

67 McMaster MT, Librach CL, Zhou Y, Lim KH, Janatpour MJ, DeMars R, Kovats S, Damsky C, Fisher SJ: Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. J Immunol 1995;154:3771-3778.

68 Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H, Geraghty DE: Protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal-placental immune recognition. J Immunol 2003;171:1376-1384.

69 Hunt JS, Petroff MG, McIntire RH, Ober C: HLA-G and immune tolerance in pregnancy. FASEB J 2005;19:681-693.

70 Larsen MH, Hviid TV: Human leukocyte antigen-G polymorphism in relation to expression, function, and disease. Hum Immunol 2009;70:1026-1034.

71 Ritsick DR, Bommer C, Braverman J: Abstract: The role of fetomaternal MHC class II histoincompatibility in regulating tolerance of the semi-allogenic fetus. Am J Reprod Immunol 2014;71:37-38.

72 Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL: Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998;281:1191-1193.

73 Kudo Y, Boyd CA: Human placental indoleamine 2,3-dioxygenase: cellular localization and characterization of an enzyme preventing fetal rejection. Biochimica et biophysica acta 2000;1500:119-124.

74 Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH: Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. Nat Immunol 2001;2:64-68.

75 Mellor AL, Chandler P, Lee GK, Johnson T, Keskin DB, Lee J, Munn DH: Indoleamine 2,3-dioxygenase, immunosuppression and pregnancy. J Reprod Immunol 2002;57:143-150.

76 Kudo Y: The role of placental indoleamine 2,3-dioxygenase in human pregnancy. Obstet Gynecol Sci 2013;56:209-216.

77 Hunt JS, Vassmer D, Ferguson TA, Miller L: Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. J Immunol 1997;158:4122-4128.

78 Uckan D, Steele A, Cherry, Wang BY, Chamizo W, Koutsonikolis A, Gilbert-Barness E, Good RA: Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. Mol Hum Reprod 1997;3:655-662.

79 Holmes CH, Simpson KL, Wainwright SD, Tate CG, Houlihan JM, Sawyer IH, Rogers IP, Spring FA, Anstee DJ, Tanner MJ: Preferential expression of the complement regulatory protein decay accelerating factor at the fetomaternal interface during human pregnancy. J Immunol 1990;144:3099-3105.

80 Hsi BL, Hunt JS, Atkinson JP: Differential expression of complement regulatory proteins on subpopulations of human trophoblast cells. J Reprod Immunol 1991;19:209-223.

81 Altemani AM, Norato D, Baumel C: Immunological studies in placentas with villitis of unknown etiology: complement components and immunoglobulins in chorionic villi. J Perinat Med 1992;20:129-134.

82 Holmes CH, Simpson KL, Okada H, Okada N, Wainwright SD, Purcell DF, Houlihan JM: Complement regulatory proteins at the feto-maternal interface during human placental development: distribution of CD59 by comparison with membrane cofactor protein (CD46) and decay accelerating factor (CD55). Eur J Immunol 1992;22:1579-1585.

83 Tedesco F, Narchi G, Radillo O, Meri S, Ferrone S, Betterle C: Susceptibility of human trophoblast to killing by human complement and the role of the complement regulatory proteins. J Immunol 1993;151:1562-1570.

84 Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H: A critical role for murine complement regulator crry in fetomaternal tolerance. Science 2000;287:498-501.

85 Richani K, Romero R, Soto E, Espinoza J, Nien JK, Chaiworapongsa T, Refuerzo J, Blackwell S, Edwin SS, Santolaya-Forgas J, Mazor M: Unexplained intrauterine fetal death is accompanied by activation of complement. J Perinat Med 2005;33:296-305.

86 Soto E, Romero R, Richani K, Espinoza J, Nien JK, Chaiworapongsa T, Santolaya-Forgas J, Edwin SS, Mazor M: Anaphylatoxins in preterm and term labor. J Perinat Med 2005;33:306-313.

87 Girardi G, Bulla R, Salmon JE, Tedesco F: The complement system in the pathophysiology of pregnancy. Mol Immunol 2006;43:68-77.

88 Girardi G: Complement inhibition keeps mothers calm and avoids fetal rejection. Immunol Invest 2008;37:645-659.

89 Mittal P, Romero R, Tarca AL, Gonzalez J, Draghici S, Xu Y, Dong Z, Nhan-Chang CL, Chaiworapongsa T, Lye S, Kusanovic JP, Lipovich L, Mazaki-Tovi S, Hassan SS, Mesiano S, Kim CJ:

Characterization of the myometrial transcriptome and biological pathways of spontaneous human labor at term. J Perinat Med 2010;38:617-643.

90 Chaiworapongsa T, Romero R, Whitten A, Tarca AL, Bhatti G, Draghici S, Chaemsaithong P, Miranda J, Hassan SS: Differences and similarities in the transcriptional profile of peripheral whole blood in early and late-onset preeclampsia: insights into the molecular basis of the phenotype of preeclampsiaa. J Perinat Med 2013;41:485-504.

91 Madan I, Than NG, Romero R, Chaemsaithong P, Miranda J, Tarca AL, Bhatti G, Draghici S, Yeo L, Mazor M, Hassan SS, Chaiworapongsa T: The peripheral whole-blood transcriptome of acute pyelonephritis in human pregnancya. J Perinat Med 2014;42:31-53.

92 Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, Noelle RJ, Coyle A, Mellor AL, Khoury SJ, Sayegh MH: A critical role for the programmed death ligand 1 in fetomaternal tolerance. J Exp Med 2005;202:231-237.

93 Habicht A, Dada S, Jurewicz M, Fife BT, Yagita H, Azuma M, Sayegh MH, Guleria I: A link between PDL1 and T regulatory cells in fetomaternal tolerance. J Immunol 2007;179:5211-5219.

94 D'Addio F, Riella LV, Mfarrej BG, Chabtini L, Adams LT, Yeung M, Yagita H, Azuma M, Sayegh MH, Guleria I: The link between the PDL1 costimulatory pathway and Th17 in fetomaternal tolerance. J Immunol 2011;187:4530-4541.

95 Xin L, Ertelt JM, Rowe JH, Jiang TT, Kinder JM, Chaturvedi V, Elahi S, Way SS: Cutting edge: committed Th1 CD4+ T cell differentiation blocks pregnancy-induced Foxp3 expression with antigen-specific fetal loss. J Immunol 2014;192:2970-2974.

96 Kinder JM, Jiang TT, Ertelt JM, Xin L, Strong BS, Shaaban AF, Way SS: Cross-Generational Reproductive Fitness Enforced by Microchimeric Maternal Cells. Cell 2015;162:505-515.

97 PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, Fisher S, Golos T, Matzuk M, McCune JM, Mor G, Schulz L, Soares M, Spencer T, Strominger J, Way SS, Yoshinaga K: Immune mechanisms at the maternal-fetal interface: perspectives and challenges. Nat Immunol 2015;16:328-334.

98 Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S: CXC chemokines: the regulatory link between inflammation and angiogenesis. Trends Immunol 2004;25:201-209.

99 Lazzeri E, Romagnani P: CXCR3-binding chemokines: novel multifunctional therapeutic targets. Curr Drug Targets Immune Endocr Metabol Disord 2005;5:109-118.

100 Tan J, Zhou G: Chemokine receptors and transplantation. Cell Mol Immunol 2005;2:343-349.

101 Romagnani P: From basic science to clinical practice: use of cytokines and chemokines as therapeutic targets in renal diseases. J Nephrol 2005;18:229-233.

102 Romagnani P, Crescioli C: CXCL10: a candidate biomarker in transplantation. Clin Chim Acta 2012;413:1364-1373.

103 Zhang Q, Liu YF, Su ZX, Shi LP, Chen YH: Serum fractalkine and interferon-gamma inducible protein-10 concentrations are early detection markers for acute renal allograft rejection. Transplant Proc 2014;46:1420-1425.

104 Kim YM, Chaemsaithong P, Romero R, Shaman M, Kim CJ, Kim JS, Qureshi F, Jacques SM, Ahmed AI, Chaiworapongsa T, Hassan SS, Yeo L, Korzeniewski SJ: Placental lesions associated with acute atherosis. J Matern Fetal Neonatal Med 2015;28:1554-1562.

105 Gomez-Lopez N, Hernandez-Santiago S, Lobb AP, Olson DM, Vadillo-Ortega F: Normal and premature rupture of fetal membranes at term delivery differ in regional chemotactic activity and related chemokine/cytokine production. Reprod Sci 2013;20:276-284.

106 Gong X, Chen Z, Liu Y, Lu Q, Jin Z: Gene expression profiling of the paracrine effects of uterine natural killer cells on human endometrial epithelial cells. Int J Endocrinol 2014;2014:393707.

107 Romero R, Avila C, Santhanam U, Sehgal PB: Amniotic fluid interleukin 6 in preterm labor. Association with infection. J Clin Invest 1990;85:1392-1400.

108 Romero R, Sepulveda W, Kenney JS, Archer LE, Allison AC, Sehgal PB: Interleukin 6 determination in the detection of microbial invasion of the amniotic cavity. Ciba Found Symp 1992;167:205-220; discussion 220-203.

109 Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB: Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. Am J Reprod Immunol 1993;30:167-183.

110 Yoon BH, Romero R, Kim CJ, Jun JK, Gomez R, Choi JH, Syn HC: Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. Am J Obstet Gynecol 1995;172:960-970.

111 Cox SM, Casey ML, MacDonald PC: Accumulation of interleukin-1beta and interleukin-6 in amniotic fluid: a sequela of labour at term and preterm. Hum Reprod Update 1997;3:517-527.

112 Yoon BH, Romero R, Jun JK, Park KH, Park JD, Ghezzi F, Kim BI: Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. Am J Obstet Gynecol 1997;177:825-830.

113 Arntzen KJ, Kjollesdal AM, Halgunset J, Vatten L, Austgulen R: TNF, IL-1, IL-6, IL-8 and soluble TNF receptors in relation to chorioamnionitis and premature labor. J Perinat Med 1998;26:17-26.

114 Hsu CD, Meaddough E, Aversa K, Hong SF, Lu LC, Jones DC, Copel JA: Elevated amniotic fluid levels of leukemia inhibitory factor, interleukin 6, and interleukin 8 in intra-amniotic infection. Am J Obstet Gynecol 1998;179:1267-1270.

115 Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, Jun JK: Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol 2001;185:1130-1136.

116 Yoon BH, Romero R, Moon J, Chaiworapongsa T, Espinoza J, Kim YM, Edwin S, Kim JC, Camacho N, Bujold E, Gomez R: Differences in the fetal interleukin-6 response to microbial invasion of the amniotic cavity between term and preterm gestation. J Matern Fetal Neonatal Med 2003;13:32-38.

117 Jacobsson B, Mattsby-Baltzer I, Hagberg H: Interleukin-6 and interleukin-8 in cervical and amniotic fluid: relationship to microbial invasion of the chorioamniotic membranes. BJOG 2005;112:719-724.

118 Holst RM, Mattsby-Baltzer I, Wennerholm UB, Hagberg H, Jacobsson B: Interleukin-6 and interleukin-8 in cervical fluid in a population of Swedish women in preterm labor: relationship to microbial invasion of the amniotic fluid, intra-amniotic inflammation, and preterm delivery. Acta Obstet Gynecol Scand 2005;84:551-557.

119 Holst RM, Laurini R, Jacobsson B, Samuelsson E, Savman K, Doverhag C, Wennerholm UB, Hagberg H: Expression of cytokines and chemokines in cervical and amniotic fluid: relationship to histological chorioamnionitis. J Matern Fetal Neonatal Med 2007;20:885-893.

120 Menon R, Camargo MC, Thorsen P, Lombardi SJ, Fortunato SJ: Amniotic fluid interleukin-6 increase is an indicator of spontaneous preterm birth in white but not black Americans. Am J Obstet Gynecol 2008;198:77 e71-77.

121 Marconi C, de Andrade Ramos BR, Peracoli JC, Donders GG, da Silva MG: Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. Am J Reprod Immunol 2011;65:549-556.

122 Cobo T, Palacio M, Martinez-Terron M, Navarro-Sastre A, Bosch J, Filella X, Gratacos E: Clinical and inflammatory markers in amniotic fluid as predictors of adverse outcomes in preterm premature rupture of membranes. Am J Obstet Gynecol 2011;205:126 e121-128.

123 Combs CA, Gravett C, Garite T, Hickok D, Porreco R, Lapidus J, Grove T, Rael J, Miller H, Clewell W, Luthy D, Pereira L, Nageotte M, Robilio D, Fortunato S, Simhan H, Baxter J, Amon E, Franco A, Trofatter K, Heyborne K: Abstract No.73: Intraamniotic inflammation may be more important than the presence of microbes as a determinant of perinatal outcome in preterm labor. Am J Obstet Gynecol 2013;208:S44.

124 Romero R, Kadar N, Miranda J, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, Rogers W, Soto E, Gotsch F, Yeo L, Hassan SS, Chaiworapongsa T: The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not superior to amniotic fluid interleukin-6. J Matern Fetal Neonatal Med 2014;27:757-769.

125 Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS, Kim CJ, Yeo L: Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Reprod Immunol 2014;72:458-474.

126 Kacerovsky M, Musilova I, Andrys C, Hornychova H, Pliskova L, Kostal M, Jacobsson B: Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. Am J Obstet Gynecol 2014;210:325 e321-325 e310.

127 Kacerovsky M, Musilova I, Hornychova H, Kutova R, Pliskova L, Kostal M, Jacobsson B: Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes. Am J Obstet Gynecol 2014.

128 Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R, Rael J, Grove T, Morgan TK, Clewell W, Miller H, Luthy D, Pereira L, Nageotte M, Robilio PA, Fortunato S, Simhan H, Baxter JK, Amon E, Franco A, Trofatter K, Heyborne K: Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol 2014;210:125 e121-125 e115.

129 Chaemsaithong P, Romero R, Korzeniewski SJ, Dong Z, Yeo L, Hassan SS, Kim YM, Yoon BH, Chaiworapongsa T: A point of care test for the determination of amniotic fluid interleukin-6 and the chemokine CXCL-10/IP-10. J Matern Fetal Neonatal Med 2015;28:1510-1519.

130 Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, Hassan SS, Chaiworapongsa T, Yeo L: A point of care test for interleukin-6 in amniotic fluid in preterm prelabor rupture of membranes: a step toward the early treatment of acute intra-amniotic inflammation/infection. J Matern Fetal Neonatal Med 2015:1-8.

131 Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, Hassan SS, Chaiworapongsa T, Yeo L: A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. J Matern Fetal Neonatal Med 2015:1-11.

132 Romero R, Ceska M, Avila C, Mazor M, Behnke E, Lindley I: Neutrophil attractant/activating peptide-1/interleukin-8 in term and preterm parturition. Am J Obstet Gynecol 1991;165:813-820.

133 Cherouny PH, Pankuch GA, Romero R, Botti JJ, Kuhn DC, Demers LM, Appelbaum PC: Neutrophil attractant/activating peptide-1/interleukin-8: association with histologic chorioamnionitis, preterm delivery, and bioactive amniotic fluid leukoattractants. Am J Obstet Gynecol 1993;169:1299-1303.

134 Gomez R, Ghezzi F, Romero R, Munoz H, Tolosa JE, Rojas I: Premature labor and intra-amniotic infection. Clinical aspects and role of the cytokines in diagnosis and pathophysiology. Clin Perinatol 1995;22:281-342.

135 Ghezzi F, Gomez R, Romero R, Yoon BH, Edwin SS, David C, Janisse J, Mazor M: Elevated interleukin-8 concentrations in amniotic fluid of mothers whose neonates subsequently develop bronchopulmonary dysplasia. Eur J Obstet Gynecol Reprod Biol 1998;78:5-10.

136 Hsu CD, Meaddough E, Aversa K, Copel JA: The role of amniotic fluid L-selectin, GRO-alpha, and interleukin-8 in the pathogenesis of intraamniotic infection. Am J Obstet Gynecol 1998;178:428-432.

137 Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Wennerholm UB, Hagberg H: Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women in preterm labor. Acta Obstet Gynecol Scand 2003;82:120-128.

138 Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Nikolaitchouk N, Wennerholm UB, Hagberg H: Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. Acta Obstet Gynecol Scand 2003;82:423-431.

139 Figueroa R, Garry D, Elimian A, Patel K, Sehgal PB, Tejani N: Evaluation of amniotic fluid cytokines in preterm labor and intact membranes. J Matern Fetal Neonatal Med 2005;18:241-247.

140 Witt A, Berger A, Gruber CJ, Petricevic L, Apfalter P, Husslein P: IL-8 concentrations in maternal serum, amniotic fluid and cord blood in relation to different pathogens within the amniotic cavity. J Perinat Med 2005;33:22-26.

141 Cobo T, Kacerovsky M, Palacio M, Hornychova H, Hougaard DM, Skogstrand K, Jacobsson B: Intra-amniotic inflammatory response in subgroups of women with preterm prelabor rupture of the membranes. PLoS One 2012;7:e43677.

142 Romero R, Brody DT, Oyarzun E, Mazor M, Wu YK, Hobbins JC, Durum SK: Infection and labor. III. Interleukin-1: a signal for the onset of parturition. Am J Obstet Gynecol 1989;160:1117-1123.

143 Mitchell MD, Edwin SS, Silver RM, Romero RJ: Potential agonist action of the interleukin-1 receptor antagonist protein: implications for treatment of women. J Clin Endocrinol Metab 1993;76:1386-1388.

144 Romero R, Manogue KR, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC, Cerami A: Infection and labor. IV. Cachectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. Am J Obstet Gynecol 1989;161:336-341.

145 Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, Williams J: Tumor necrosis factor in preterm and term labor. Am J Obstet Gynecol 1992;166:1576-1587.

146 Sadowsky DW, Adams KM, Gravett MG, Witkin SS, Novy MJ: Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. Am J Obstet Gynecol 2006;195:1578-1589.

147 Athayde N, Romero R, Maymon E, Gomez R, Pacora P, Yoon BH, Edwin SS: Interleukin 16 in pregnancy, parturition, rupture of fetal membranes, and microbial invasion of the amniotic cavity. Am J Obstet Gynecol 2000;182:135-141.

148 Pacora P, Romero R, Maymon E, Gervasi MT, Gomez R, Edwin SS, Yoon BH: Participation of the novel cytokine interleukin 18 in the host response to intra-amniotic infection. Am J Obstet Gynecol 2000;183:1138-1143.

149 Greig PC, Herbert WN, Robinette BL, Teot LA: Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. Am J Obstet Gynecol 1995;173:1223-1227.

150 Gotsch F, Romero R, Kusanovic JP, Erez O, Espinoza J, Kim CJ, Vaisbuch E, Than NG, Mazaki-Tovi S, Chaiworapongsa T, Mazor M, Yoon BH, Edwin S, Gomez R, Mittal P, Hassan SS, Sharma S: The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic

infection/inflammation, and spontaneous parturition at term: a role for interleukin-10. J Matern Fetal Neonatal Med 2008;21:529-547.

151 Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, Yoon BH: Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol 2000;183:94-99.

152 Maymon E, Romero R, Chaiworapongsa T, Berman S, Conoscenti G, Gomez R, Edwin S: Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. Am J Obstet Gynecol 2001;185:1149-1155.

153 Angus SR, Segel SY, Hsu CD, Locksmith GJ, Clark P, Sammel MD, Macones GA, Strauss JF, 3rd, Parry S: Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. Am J Obstet Gynecol 2001;185:1232-1238.

154 Nien JK, Yoon BH, Espinoza J, Kusanovic JP, Erez O, Soto E, Richani K, Gomez R, Hassan S, Mazor M, Edwin S, Bahado-Singh R, Romero R: A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. Am J Obstet Gynecol 2006;195:1025-1030.

155 Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, Yoon BH: A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. Am J Obstet Gynecol 2007;197:292 e291-295.

156 Park CW, Lee SM, Park JS, Jun JK, Romero R, Yoon BH: The antenatal identification of funisitis with a rapid MMP-8 bedside test. J Perinat Med 2008;36:497-502.

157 Park CW, Yoon BH, Kim SM, Park JS, Jun JK: 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. Obstet Gynecol Sci 2013;56:167-175.

158 Maymon E, Romero R, Pacora P, Gervasi MT, Bianco K, Ghezzi F, Yoon BH: Evidence for the participation of interstitial collagenase (matrix metalloproteinase 1) in preterm premature rupture of membranes. Am J Obstet Gynecol 2000;183:914-920.

159 Maymon E, Romero R, Pacora P, Gomez R, Mazor M, Edwin S, Chaiworapongsa T, Kim JC, Yoon BH, Menon R, Fortunato S, Berry SM: A role for the 72 kDa gelatinase (MMP-2) and its inhibitor (TIMP-2) in human parturition, premature rupture of membranes and intraamniotic infection. J Perinat Med 2001;29:308-316.

160 Park KH, Chaiworapongsa T, Kim YM, Espinoza J, Yoshimatsu J, Edwin S, Gomez R, Yoon BH, Romero R: Matrix metalloproteinase 3 in parturition, premature rupture of the membranes, and microbial invasion of the amniotic cavity. J Perinat Med 2003;31:12-22.

161 Maymon E, Romero R, Pacora P, Gervasi MT, Edwin SS, Gomez R, Seubert DE: Matrilysin (matrix metalloproteinase 7) in parturition, premature rupture of membranes, and intrauterine infection. Am J Obstet Gynecol 2000;182:1545-1553.

162 Locksmith GJ, Clark P, Duff P, Schultz GS: Amniotic fluid matrix metalloproteinase-9 levels in women with preterm labor and suspected intra-amniotic infection. Obstet Gynecol 1999;94:1-6.

163 Maymon E, Romero R, Pacora P, Gervasi MT, Gomez R, Edwin SS, Yoon BH: Evidence of in vivo differential bioavailability of the active forms of matrix metalloproteinases 9 and 2 in parturition, spontaneous rupture of membranes, and intra-amniotic infection. Am J Obstet Gynecol 2000;183:887-894.

164 Harirah H, Donia SE, Hsu CD: Amniotic fluid matrix metalloproteinase-9 and interleukin-6 in predicting intra-amniotic infection. Obstet Gynecol 2002;99:80-84.

165 Jacobsson B, Holst RM, Wennerholm UB, Andersson B, Lilja H, Hagberg H: Monocyte chemotactic protein-1 in cervical and amniotic fluid: relationship to microbial invasion of the amniotic cavity, intra-amniotic inflammation, and preterm delivery. Am J Obstet Gynecol 2003;189:1161-1167.

166 Esplin MS, Romero R, Chaiworapongsa T, Kim YM, Edwin S, Gomez R, Mazor M, Adashi EY: Monocyte chemotactic protein-1 is increased in the amniotic fluid of women who deliver preterm in the presence or absence of intra-amniotic infection. J Matern Fetal Neonatal Med 2005;17:365-373.

167 Kacerovsky M, Celec P, Vlkova B, Skogstrand K, Hougaard DM, Cobo T, Jacobsson B: Amniotic fluid protein profiles of intraamniotic inflammatory response to Ureaplasma spp. and other bacteria. PLoS One 2013;8:e60399.

168 Jacobsson B, Holst RM, Andersson B, Hagberg H: Monocyte chemotactic protein-2 and -3 in amniotic fluid: relationship to microbial invasion of the amniotic cavity, intra-amniotic inflammation and preterm delivery. Acta Obstet Gynecol Scand 2005;84:566-571.

169 Mittal P, Romero R, Kusanovic JP, Edwin SS, Gotsch F, Mazaki-Tovi S, Espinoza J, Erez O, Nhan-Chang CL, Than NG, Vaisbuch E, Hassan SS: CXCL6 (granulocyte chemotactic protein-2): a novel chemokine involved in the innate immune response of the amniotic cavity. Am J Reprod Immunol 2008;60:246-257.

170 Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, Yeo L, Yoon BH, Chaiworapongsa T: Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. J Perinat Med 2012;40:329-343.

171 Nhan-Chang CL, Romero R, Kusanovic JP, Gotsch F, Edwin SS, Erez O, Mittal P, Kim CJ, Kim MJ, Espinoza J, Friel LA, Vaisbuch E, Than NG, Mazaki-Tovi S, Hassan SS: A role for CXCL13 (BCA-1) in pregnancy and intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med 2008;21:763-775.

172 Keelan JA, Wang K, Chaiworapongsa T, Romero R, Mitchell MD, Sato TA, Brown DA, Fairlie WD, Breit SN: Macrophage inhibitory cytokine 1 in fetal membranes and amniotic fluid from pregnancies with and without preterm labour and premature rupture of membranes. Mol Hum Reprod 2003;9:535-540.

173 Chaiworapongsa T, Romero R, Espinoza J, Kim YM, Edwin S, Bujold E, Gomez R, Kuivaniemi H: Macrophage migration inhibitory factor in patients with preterm parturition and microbial invasion of the amniotic cavity. J Matern Fetal Neonatal Med 2005;18:405-416.

174 Athayde N, Romero R, Maymon E, Gomez R, Pacora P, Araneda H, Yoon BH: A role for the novel cytokine RANTES in pregnancy and parturition. Am J Obstet Gynecol 1999;181:989-994.

175 Keelan JA, Yang J, Romero RJ, Chaiworapongsa T, Marvin KW, Sato TA, Mitchell MD: Epithelial cell-derived neutrophil-activating peptide-78 is present in fetal membranes and amniotic fluid at increased concentrations with intra-amniotic infection and preterm delivery. Biol Reprod 2004;70:253-259.

176 Cohen J, Ghezzi F, Romero R, Ghidini A, Mazor M, Tolosa JE, Goncalves LF, Gomez R: GRO alpha in the fetomaternal and amniotic fluid compartments during pregnancy and parturition. Am J Reprod Immunol 1996;35:23-29.

177 Pacora P, Romero R, Chaiworapongsa T, Kusanovic JP, Erez O, Vaisbuch E, Mazaki-Tovi S, Gotsch F, Jai Kim C, Than NG, Yeo L, Mittal P, Hassan SS: Amniotic fluid angiopoietin-2 in term and preterm parturition, and intra-amniotic infection/inflammation. J Perinat Med 2009;37:503-511.

178 Andrys C, Kacerovsky M, Drahosova M, Musilova I, Pliskova L, Hornychova H, Prochazka M, Jacobsson B: Amniotic fluid soluble Toll-like receptor 2 in pregnancies complicated by preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2013;26:520-527.

179 Stampalija T, Chaiworapongsa T, Romero R, Tarca AL, Bhatti G, Chiang PJ, Than NG, Ferrazzi E, Hassan SS, Yeo L: Soluble ST2, a modulator of the inflammatory response, in preterm and term labor. J Matern Fetal Neonatal Med 2014;27:111-121.

180 Park SP, Kim SA: Abstract No 322: The value of the genedia MMP-8 rapid test for diagnosing intraamniotic infection/inflammation and predicting adverse pregnancy outcomes in women with preterm premature rupture of membranes. Am J Obstet Gynecol 2015;212:S174.

181 Park JY, Romero R, Lee J, Chaemsaithong P, Chaiyasit N, Yoon BH: An elevated amniotic fluid prostaglandin F2a concentration is associated with intra-amniotic inflammation/infection, clinical and histologic chorioamnionitis as well as impending preterm delivery in patients with preterm labor and intact membranes. J Matern Fetal Neonatal Med (Accepted) 2015.

182 Romero R, Grivel JC, Tarca AL, Chaemsaithong P, Xu Z, Fitzgerald W, Hassan SS, Chaiworapongsa T, Margolis L: Evidence of perturbations of the cytokine network in preterm labor. Am J Obstet Gynecol 2015;213:836 e831-836 e818.

183 Blanc WA: Pathology of the placenta and cord in ascending and in haematogenous infection. Ciba Found Symp 1979:17-38.

184 Russell P: Inflammatory lesions of the human placenta: clinical significance of acute chorioamnionitis. Am J Diagn Gynecol Obstet 1979;2:127-137.

185 Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA: A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-978.

186 Salafia CM, Weigl C, Silberman L: The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. Obstet Gynecol 1989;73:383-389.

187 Salafia CM, Vogel CA, Vintzileos AM, Bantham KF, Pezzullo J, Silberman L: Placental pathologic findings in preterm birth. Am J Obstet Gynecol 1991;165:934-938.

188 Romero R, Salafia CM, Athanassiadis AP, Hanaoka S, Mazor M, Sepulveda W, Bracken MB: The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology. Am J Obstet Gynecol 1992;166:1382-1388.

189 Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C: Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society 2003;6:435-448.

190 Redline RW: Placental inflammation. Semin Neonatol 2004;9:265-274.

191 Redline RW: Infections and other inflammatory conditions. Semin Diagn Pathol 2007;24:5-13.

192 Menon R, Taylor RN, Fortunato SJ: Chorioamnionitis--a complex pathophysiologic syndrome. Placenta 2010;31:113-120.

193 Redline RW: Inflammatory response in acute chorioamnionitis. Semin Fetal Neonatal Med 2012;17:20-25.

194 Martinelli P, Sarno L, Maruotti GM, Paludetto R: Chorioamnionitis and prematurity: a critical review. J Matern Fetal Neonatal Med 2012;25 Suppl 4:29-31.

195 Torricelli M, Voltolini C, Toti P, Vellucci FL, Conti N, Cannoni A, Moncini I, Occhini R, Severi FM, Petraglia F: Histologic chorioamnionitis: different histologic features at different gestational ages. J Matern Fetal Neonatal Med 2014;27:910-913.

196 Kim SM, Romero R, Park JW, Oh KJ, Jun JK, Yoon BH: The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis in preterm gestation. J Matern Fetal Neonatal Med 2015;28:1500-1509.

197 Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM: Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol 2015;213:S29-S52.

198 Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, Ghezzi F, Berry SM, Qureshi F, Jacques SM, Kim JC, Kadar N, Romero R: Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. J Matern Fetal Neonatal Med 2002;11:18-25.

199 Kim CJ, Yoon BH, Park SS, Kim MH, Chi JG: Acute funisitis of preterm but not term placentas is associated with severe fetal inflammatory response. Hum Pathol 2001;32:623-629.

200 Kim EN, Kim CJ, Park JW, Yoon BH: Acute funisitis is associated with distinct changes in fetal hematologic profile. J Matern Fetal Neonatal Med 2015;28:588-593.

201 Lee J, Oh KJ, Park CW, Park JS, Jun JK, Yoon BH: The presence of funisitis is associated with a decreased risk for the development of neonatal respiratory distress syndrome. Placenta 2011;32:235-240.

202 Lee J, Romero R, Kim SM, Chaemsaithong P, Park CW, Park JS, Jun JK, Yoon BH: A new anti-microbial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM. J Matern Fetal Neonatal Med 2016;29:707-720.

203 Mi Lee S, Romero R, Lee KA, Jin Yang H, Joon Oh K, Park CW, Yoon BH: The frequency and risk factors of funisitis and histologic chorioamnionitis in pregnant women at term who delivered after the spontaneous onset of labor. J Matern Fetal Neonatal Med 2011;24:37-42.

204 Yoon BH, Romero R, Shim JY, Shim SS, Kim CJ, Jun JK: C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. J Matern Fetal Neonatal Med 2003;14:85-90.

205 Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM: Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol 2015;213:S29-52.

206 Park CW, Park JS, Moon KC, Jun JK, Yoon BH: Preterm labor and preterm premature rupture of membranes have a different pattern in the involved compartments of acute histologoic chorioamnionitis and/or funisitis: Patho-physiologic implication related to different clinical manifestations. Pathol Int 2016;66:325-332.

207 Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L: A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. Am J Reprod Immunol 2014;71:330-358.

208 Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L, Kim YM: Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. J Matern Fetal Neonatal Med 2014:1-17.

209 Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z, Ahmed AI, Shaman M, Lannaman K, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L, Kim YM: Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2015;28:1394-1409.

210 Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsaithong P, Martinez A, Gotsch F, Dong Z, Ahmed AI, Shaman M, Lannaman K, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L, Kim YM: Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. J Perinat Med 2015;43:19-36.

211 Musilova I, Kutova R, Pliskova L, Stepan M, Menon R, Jacobsson B, Kacerovsky M: Intraamniotic Inflammation in Women with Preterm Prelabor Rupture of Membranes. PLoS One 2015;10:e0133929.

212 Hecht JL, Fichorova RN, Tang VF, Allred EN, McElrath TF, Leviton A: Relationship Between Neonatal Blood Protein Concentrations and Placenta Histologic Characteristics in Extremely Low GA Newborns. Pediatr Res 2011;69:68-73.

213 Committee opinion no 611: method for estimating due date. Obstet Gynecol 2014;124:863-866.

214 Madan I, Romero R, Kusanovic JP, Mittal P, Chaiworapongsa T, Dong Z, Mazaki-Tovi S, Vaisbuch E, Alpay Savasan Z, Yeo L, Kim CJ, Hassan SS: The frequency and clinical significance of intra-amniotic infection and/or inflammation in women with placenta previa and vaginal bleeding: an unexpected observation. J Perinat Med 2010;38:275-279.

215 DiGiulio DB, Gervasi M, Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Seok KS, Gomez R, Mittal P, Gotsch F, Chaiworapongsa T, Oyarzun E, Kim CJ, Relman DA: Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods. J Perinat Med 2010;38:503-513.

216 Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, Kusanovic JP, Kim CJ, Hassan SS: Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. J Matern Fetal Neonatal Med 2011;24:1444-1455.

217 Park KH, Kim SN, Oh KJ, Lee SY, Jeong EH, Ryu A: Noninvasive prediction of intra-amniotic infection and/or inflammation in preterm premature rupture of membranes. Reproductive sciences 2012;19:658-665.

218 Redline RW: Inflammatory responses in the placenta and umbilical cord. Semin Fetal Neonatal Med 2006;11:296-301.

219 Redline RW: Classification of placental lesions. Am J Obstet Gynecol 2015;213:S21-28.

220 Kim JS, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, Kim CJ: Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. Histopathology 2008;52:457-464.

221 Redline RW: Villitis of unknown etiology: noninfectious chronic villitis in the placenta. Hum Pathol 2007;38:1439-1446.

222 Khong TY, Bendon RW, Qureshi F, Redline RW, Gould S, Stallmach T, Lipsett J, Staples A: Chronic deciduitis in the placental basal plate: definition and interobserver reliability. Hum Pathol 2000;31:292-295.

223 Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, Hobbins JC: Infection in the pathogenesis of preterm labor. Semin Perinatol 1988;12:262-279.

224 Romero R, Mazor M: Infection and preterm labor. Clin Obstet Gynecol 1988;31:553-584.

225 Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, Sabo V, Athanassiadis AP, Hobbins JC: Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol 1989;161:817-824.

226 Gomez R, Romero R, Edwin SS, David C: Pathogenesis of preterm labor and preterm premature rupture of membranes associated with intraamniotic infection. Infect Dis Clin North Am 1997;11:135-176.

227 Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM: The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol 2001;15 Suppl 2:41-56.

228 Romero R, Espinoza J, Chaiworapongsa T, Kalache K: Infection and prematurity and the role of preventive strategies. Semin Neonatol 2002;7:259-274.

229 Leigh J, Garite TJ: Amniocentesis and the management of premature labor. Obstet Gynecol 1986;67:500-506.

230 Romero R, Avila C, Brekus CA, Morotti R: The role of systemic and intrauterine infection in preterm parturition. Annals of the New York Academy of Sciences 1991;622:355-375.

231 Gauthier DW, Meyer WJ, Bieniarz A: Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes. Am J Obstet Gynecol 1991;165:1105-1110.

232 Coultrip LL, Grossman JH: Evaluation of rapid diagnostic tests in the detection of microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1992;167:1231-1242.

233 Watts DH, Krohn MA, Hillier SL, Eschenbach DA: The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol 1992;79:351-357.

234 Coultrip LL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH: The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1994;171:901-911.

235 Goncalves LF, Chaiworapongsa T, Romero R: Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 2002;8:3-13.

236 Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S: The role of inflammation and infection in preterm birth. Semin Reprod Med 2007;25:21-39.

237 Romero R, Quintero R, Oyarzun E, Wu YK, Sabo V, Mazor M, Hobbins JC: Intraamniotic infection and the onset of labor in preterm premature rupture of the membranes. Am J Obstet Gynecol 1988;159:661-666.

238 Romero R, Ghidini A, Mazor M, Behnke E: Microbial invasion of the amniotic cavity in premature rupture of membranes. Clin Obstet Gynecol 1991;34:769-778.

239 Romero R, Gonzalez R, Sepulveda W, Brandt F, Ramirez M, Sorokin Y, Mazor M, Treadwell MC, Cotton DB: Infection and labor. VIII. Microbial invasion of the amniotic cavity in patients with suspected cervical incompetence: prevalence and clinical significance. Am J Obstet Gynecol 1992;167:1086-1091.

240 Romero R, Avila C, Sepulveda W, et al: The role of systemic and intrauterine infection in preterm labor. In *Preterm Birth: Causes, Prevention, and Management*, A Fuchs, F Fuchs, P Stubblefield (eds). New York, McGraw-Hill Inc., 1993, p 97.

241 Matzinger P: The danger model: a renewed sense of self. Science 2002;296:301-305.

242 Oppenheim JJ, Yang D: Alarmins: chemotactic activators of immune responses. Curr Opin Immunol 2005;17:359-365.

243 Harris HE, Raucci A: Alarmin(g) news about danger: workshop on innate danger signals and HMGB1. EMBO Rep 2006;7:774-778.

244 Bianchi ME: DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1-5.

245 Romero R, Espinoza J, Hassan S, Gotsch F, Kusanovic JP, Avila C, Erez O, Edwin S, Schmidt AM: Soluble receptor for advanced glycation end products (sRAGE) and endogenous secretory RAGE (esRAGE) in amniotic fluid: modulation by infection and inflammation. J Perinat Med 2008;36:388-398.

246 Gotsch F, Romero R, Chaiworapongsa T, Erez O, Vaisbuch E, Espinoza J, Kusanovic JP, Mittal P, Mazaki-Tovi S, Kim CJ, Kim JS, Edwin S, Nhan-Chang CL, Hamill N, Friel L, Than NG, Mazor M, Yoon BH, Hassan SS: Evidence of the involvement of caspase-1 under physiologic and pathologic cellular stress during human pregnancy: a link between the inflammasome and parturition. J Matern Fetal Neonatal Med 2008;21:605-616.

247 Chaiworapongsa T, Erez O, Kusanovic JP, Vaisbuch E, Mazaki-Tovi S, Gotsch F, Than NG, Mittal P, Kim YM, Camacho N, Edwin S, Gomez R, Hassan SS, Romero R: Amniotic fluid heat shock protein 70 concentration in histologic chorioamnionitis, term and preterm parturition. J Matern Fetal Neonatal Med 2008;21:449-461.

248 Bianchi ME, Manfredi AA: Immunology. Dangers in and out. Science 2009;323:1683-1684.

249 Piccinini AM, Midwood KS: DAMPening inflammation by modulating TLR signalling. Mediators Inflamm 2010;2010.

250 Chen GY, Nunez G: Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010;10:826-837.

251 Nunez G: Intracellular sensors of microbes and danger. Immunol Rev 2011;243:5-8.

252 Romero R, Chaiworapongsa T, Savasan ZA, Hussein Y, Dong Z, Kusanovic JP, Kim CJ, Hassan SS: Clinical chorioamnionitis is characterized by changes in the expression of the alarmin HMGB1 and one of its receptors, sRAGE. J Matern Fetal Neonatal Med 2012;25:558-567.

253 Bredeson S, DeFord J, Yin H, Papaconstantinou J, Saade G, Menon R: Abstract No.710: Acetylated HMGB1 in human amniotic fluid as an important factor in premature preterm rupture of the membranes. Am J Obstet Gynecol 2014;2010:S348.

254 Ahmed AI, Chaemsaithong P, Chaiworapongsa T, Zhong D, Shaman M, Lannaman K, Yeo L, Hassan S, Yoon BH, Romero R: Abstract No. 599: A receptor for danger signals, advanced glycation and products (RAGE) in fetal inflammation and clinical chorioamnionitis. Am J Obstet Gynecol 2015;212:S298.

255 Behnia F, Taylor BD, Woodson M, Kacerovsky M, Hawkins H, Fortunato SJ, Saade GR, Menon R: Chorioamniotic membrane senescence: a signal for parturition? Am J Obstet Gynecol 2015;213:359 e351-359 e316.

256 Behnia F, Saade G, Micheal V, Kacerovsky M, Dutta E, Polettini J, Huaizhi Y, Campisi J, Menon R: Abstract No. 98: Term fetal membranes and senescence associated secretory phenotype (SASP)-like gene expression: a signal for parturition? Am J Obstet Gynecol 2015;212:S66.

257 Polettini J, Dutta E, Kechichian T, Tamayo E, Bytautiene E, Boldogh I, Saade G, Menon R: Abstract No.73: Activation of p38MAPK and senescence in fetal membranes induced by telomere overhang sequence: a novel mechanism for preterm birth. Am J Obstet Gynecol 2015;212:S51.

258 Dutta E, Kacerovsky M, Behnia F, Kechichian T, Saade G, Menon R: Abstract No.152: Development of DNA damage foci, loss of lamin B and activation of pp38MAPK: classic signs of senescence in human amniochorion. Am J Obstet Gynecol 2015;212:S92.

259 Montenegro D, Romero R, Pineles BL, Tarca AL, Madsen-Bouterse SA, Hassan S, Kusanovic JP, Draghici S, Espinoza J, Kim CJ: Differential expression of the inflammasome components in the fetal inflammatory response syndrome. Reproductive Sciences 2007;14:59A-60A.

260 Pineles BL, Romero R, Montenegro D, Tarca AL, Than NG, Hassan S, Gotsch F, Draghici S, Espinoza J, Kim CJ: The inflammasome in human parturition Reproductive Sciences 2007;14 59A.

261 Abrahams VM: The role of the Nod-like receptor family in trophoblast innate immune responses. J Reprod Immunol 2011;88:112-117.

262 Lappas M: Caspase-1 activation is increased with human labour in foetal membranes and myometrium and mediates infection-induced interleukin-1beta secretion. Am J Reprod Immunol 2014;71:189-201.

263 Romero R, Gomez-Lopez N, Xu Y, Plazyo O, Chaemsaithong P, Chaiworapongsa T, Than NG, Chiang PJ, Dong Z, Xu Z, Tarca AL, Hassan S: A role of inflammasome in spontaneous labor at term. Abstract presented at 12th World Congress of Perinatal Medicine, 3rd-6th November, 2015, Madrid, Spain 2015.

264 Gomez-Lopez N, Romero R, Plazyo O, Panaitescu B, Furcron AE, Miller D, Roumayah T, Flom E, Hassan SS: Intra-Amniotic Administration of HMGB1 Induces Spontaneous Preterm Labor and Birth. Am J Reprod Immunol 2016;75:3-7.

265 Plazyo O, Romero R, Unkel R, Balancio A, Mial TN, Xu Y, Dong Z, Hassan SS, Gomez-Lopez N: HMGB1 Induces an Inflammatory Response in the Chorioamniotic Membranes That Is Partially Mediated by the Inflammasome. Biol Reprod 2016;95:130.

266 Romero R, Gomez R, Galasso M, Munoz H, Acosta L, Yoon BH, Svinarich D, Cotton DB: Macrophage inflammatory protein-1 alpha in term and preterm parturition: effect of microbial invasion of the amniotic cavity. Am J Reprod Immunol 1994;32:108-113.

267 Dudley DJ, Hunter C, Mitchell MD, Varner MW: Elevations of amniotic fluid macrophage inflammatory protein-1 alpha concentrations in women during term and preterm labor. Obstet Gynecol 1996;87:94-98.

268 Kusanovic JP, Romero R, Chaiworapongsa T, Mittal P, Mazaki-Tovi S, Vaisbuch E, Erez O, Gotsch F, Than NG, Edwin SS, Pacora P, Jodicke C, Yeo L, Hassan SS: Amniotic fluid sTREM-1 in normal pregnancy, spontaneous parturition at term and preterm, and intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med 2010;23:34-47.

269 Wei SQ, Fraser W, Luo ZC: Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. Obstet Gynecol 2010;116:393-401.

270 Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J: Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. BJOG 2011;118:1042-1054.

271 Romero R, Grivel JC, Tarca AL, Chaemsaithong P, Xu Z, Fitzgerald W, Hassan SS, Chaiworapongsa T, Margolis L: Evidence of Perturbations of the Cytokine Network in Preterm Labor. Am J Obstet Gynecol 2015.

272 Rotondi M, Rosati A, Buonamano A, Lasagni L, Lazzeri E, Pradella F, Fossombroni V, Cirami C, Liotta F, La Villa G, Serio M, Bertoni E, Salvadori M, Romagnani P: High pretransplant serum levels of CXCL10/IP-10 are related to increased risk of renal allograft failure. Am J Transplant 2004;4:1466-1474.

273 Hoffman SA, Wang L, Shah CV, Ahya VN, Pochettino A, Olthoff K, Shaked A, Wille K, Lama VN, Milstone A, Ware LB, Orens J, Weinacker A, Demissie E, Bellamy S, Kawut SM, Hancock WW, Christie JD: Plasma cytokines and chemokines in primary graft dysfunction post-lung transplantation. Am J Transplant 2009;9:389-396.

274 Matz M, Beyer J, Wunsch D, Mashreghi MF, Seiler M, Pratschke J, Babel N, Volk HD, Reinke P, Kotsch K: Early post-transplant urinary IP-10 expression after kidney transplantation is predictive of short- and long-term graft function. Kidney Int 2006;69:1683-1690.

275 Suthanthiran M, Schwartz JE, Ding R, Abecassis M, Dadhania D, Samstein B, Knechtle SJ, Friedewald J, Becker YT, Sharma VK, Williams NM, Chang CS, Hoang C, Muthukumar T, August P, Keslar KS, Fairchild RL, Hricik DE, Heeger PS, Han L, Liu J, Riggs M, Ikle DN, Bridges ND, Shaked A: Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. N Engl J Med 2013;369:20-31.

276 Agostini C, Calabrese F, Rea F, Facco M, Tosoni A, Loy M, Binotto G, Valente M, Trentin L, Semenzato G: Cxcr3 and its ligand CXCL10 are expressed by inflammatory cells infiltrating lung allografts and mediate chemotaxis of T cells at sites of rejection. Am J Pathol 2001;158:1703-1711.

277 Melter M, Exeni A, Reinders ME, Fang JC, McMahon G, Ganz P, Hancock WW, Briscoe DM: Expression of the chemokine receptor CXCR3 and its ligand IP-10 during human cardiac allograft rejection. Circulation 2001;104:2558-2564.

278 Panzer U, Reinking RR, Steinmetz OM, Zahner G, Sudbeck U, Fehr S, Pfalzer B, Schneider A, Thaiss F, Mack M, Conrad S, Huland H, Helmchen U, Stahl RA: CXCR3 and CCR5 positive T-cell recruitment in acute human renal allograft rejection. Transplantation 2004;78:1341-1350.

279 Segerer S, Cui Y, Eitner F, Goodpaster T, Hudkins KL, Mack M, Cartron JP, Colin Y, Schlondorff D, Alpers CE: Expression of chemokines and chemokine receptors during human renal transplant rejection. Am J Kidney Dis 2001;37:518-531.

280 Tatapudi RR, Muthukumar T, Dadhania D, Ding R, Li B, Sharma VK, Lozada-Pastorio E, Seetharamu N, Hartono C, Serur D, Seshan SV, Kapur S, Hancock WW, Suthanthiran M: Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine. Kidney Int 2004;65:2390-2397.

281 Lazzeri E, Rotondi M, Mazzinghi B, Lasagni L, Buonamano A, Rosati A, Pradella F, Fossombroni V, La Villa G, Gacci M, Bertoni E, Serio M, Salvadori M, Romagnani P: High CXCL10 expression in rejected kidneys and predictive role of pretransplant serum CXCL10 for acute rejection and chronic allograft nephropathy. Transplantation 2005;79:1215-1220.

282 Segerer S, Bohmig GA, Exner M, Kerjaschki D, Regele H, Schlondorff D: Role of CXCR3 in cellular but not humoral renal allograft rejection. Transpl Int 2005;18:676-680.

283 Schaub S, Nickerson P, Rush D, Mayr M, Hess C, Golian M, Stefura W, Hayglass K: Urinary CXCL9 and CXCL10 levels correlate with the extent of subclinical tubulitis. Am J Transplant 2009;9:1347-1353.

284 Lo DJ, Weaver TA, Kleiner DE, Mannon RB, Jacobson LM, Becker BN, Swanson SJ, Hale DA, Kirk AD: Chemokines and their receptors in human renal allotransplantation. Transplantation 2011;91:70-77.

285 Fahmy NM, Yamani MH, Starling RC, Ratliff NB, Young JB, McCarthy PM, Feng J, Novick AC, Fairchild RL: Chemokine and chemokine receptor gene expression indicates acute rejection of human cardiac transplants. Transplantation 2003;75:72-78.

286 Fahmy NM, Yamani MH, Starling RC, Ratliff NB, Young JB, McCarthy PM, Feng J, Novick AC, Fairchild RL: Chemokine and receptor-gene expression during early and late acute rejection episodes in human cardiac allografts. Transplantation 2003;75:2044-2047.

287 Crescioli C, Buonamano A, Scolletta S, Sottili M, Francalanci M, Giomarelli P, Biagioli B, Lisi G, Pradella F, Serio M, Romagnani P, Maccherini M: Predictive role of pretransplant serum CXCL10 for cardiac acute rejection. Transplantation 2009;87:249-255.

288 Zhao DX, Hu Y, Miller GG, Luster AD, Mitchell RN, Libby P: Differential expression of the IFN-gamma-inducible CXCR3-binding chemokines, IFN-inducible protein 10, monokine induced by IFN, and IFN-inducible T cell alpha chemoattractant in human cardiac allografts: association with cardiac allograft vasculopathy and acute rejection. J Immunol 2002;169:1556-1560.

289 Burns WR, Wang Y, Tang PC, Ranjbaran H, Iakimov A, Kim J, Cuffy M, Bai Y, Pober JS, Tellides G: Recruitment of CXCR3+ and CCR5+ T cells and production of interferon-gamma-inducible chemokines in rejecting human arteries. Am J Transplant 2005;5:1226-1236.

290 Shahzad K, Cadeiras M, Memon S, Zeeberg B, Klingler T, Sinha A, Tabak EG, Unniachan S, Deng MC: Gene Expression Signatures of Peripheral Blood Mononuclear Cells during the Early Post-Transplant Period in Patients Developing Cardiac Allograft Vasculopathy. J Transplant 2010;2010:719696.

291 Ogge G, Romero R, Lee DC, Gotsch F, Than NG, Lee J, Chaiworapongsa T, Dong Z, Mittal P, Hassan SS, Kim CJ: Chronic chorioamnionitis displays distinct alterations of the amniotic fluid proteome. J Pathol 2011;223:553-565.

292 Alok A, Mukhopadhyay D, Karande AA: Glycodelin A, an immunomodulatory protein in the endometrium, inhibits proliferation and induces apoptosis in monocytic cells. Int J Biochem Cell Biol 2009;41:1138-1147.

293 Cuenca AG, Wynn JL, Kelly-Scumpia KM, Scumpia PO, Vila L, Delano MJ, Mathews CE, Wallet SM, Reeves WH, Behrns KE, Nacionales DC, Efron PA, Kunkel SL, Moldawer LL: Critical role for CXC ligand 10/CXC receptor 3 signaling in the murine neonatal response to sepsis. Infect Immun 2011;79:2746-2754.

294 Groom JR, Luster AD: CXCR3 ligands: redundant, collaborative and antagonistic functions. Immunol Cell Biol 2011;89:207-215.

295 Chan T, Gu F: Early diagnosis of sepsis using serum biomarkers. Expert Rev Mol Diagn 2011;11:487-496.

296 Liu M, Guo S, Hibbert JM, Jain V, Singh N, Wilson NO, Stiles JK: CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev 2011;22:121-130.

297 Herzig DS, Luan L, Bohannon JK, Toliver-Kinsky TE, Guo Y, Sherwood ER: The role of CXCL10 in the pathogenesis of experimental septic shock. Crit Care 2014;18:R113.

298 Herzig DS, Driver BR, Fang G, Toliver-Kinsky TE, Shute EN, Sherwood ER: Regulation of lymphocyte trafficking by CXC chemokine receptor 3 during septic shock. Am J Respir Crit Care Med 2012;185:291-300.

299 Herzig DS, Guo Y, Fang G, Toliver-Kinsky TE, Sherwood ER: Therapeutic efficacy of CXCR3 blockade in an experimental model of severe sepsis. Crit Care 2012;16:R168.

300 Ng PC, Li K, Chui KM, Leung TF, Wong RP, Chu WC, Wong E, Fok TF: IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants. Pediatr Res 2007;61:93-98.

301 Punyadeera C, Schneider EM, Schaffer D, Hsu HY, Joos TO, Kriebel F, Weiss M, Verhaegh WF: A biomarker panel to discriminate between systemic inflammatory response syndrome and sepsis and sepsis severity. J Emerg Trauma Shock 2010;3:26-35.

302 Chen HL, Hung CH, Tseng HI, Yang RC: Plasma IP-10 as a predictor of serious bacterial infection in infants less than 4 months of age. J Trop Pediatr 2011;57:145-151.

Table 1: Descriptive characteristics of the study population

Descriptive Characteristics	Median (IQR) or % (n)
Maternal age (years)	24.0 (20.3-30.0)
Body mass index (kg/m^2)	25.0 (21.0-30.9)
Nulliparity (%)	33.9% (57/168)
Gestational age at amniocentesis (weeks)	30.2 (26.6-32.3)
African-American Race (%)	88.1% (148/168)
Amniotic fluid white blood cell (cells/mm^3)	3.5 (0-39.3)
Amniotic fluid glucose (mg/dL)	23.0 (10.0-30.0)
Positive amniotic fluid culture (%)	20.2% (34/168)
Amniocentesis-to-delivery interval (days)	4 (1-26.8)
Preterm delivery (%)	83.3% (140/168)
Gestational age at delivery (weeks)	32.1 (27.7-34.9)
Birthweight (grams)	1,822.5 (1,086.3-2,472.5)
Acute histologic chorioamnionitis (%)	48.8% (82/168)
Chronic chorioamnionitis (%)	26.8% (45/168)
Placental lesions associated with maternal anti-fetal rejection*	41.1% (69/168)

Data presented as median (IQR) or % (n); IQR= interquartile range

*Placental lesions associated with maternal anti-fetal rejection: chronic chorioamnionitis, villitis of unknown etiology and chronic deciduitis with plasma cells

Table 2: Clinical characteristics and placental lesions according to amniotic fluid interleukin-6 and CXCL10 concentrations

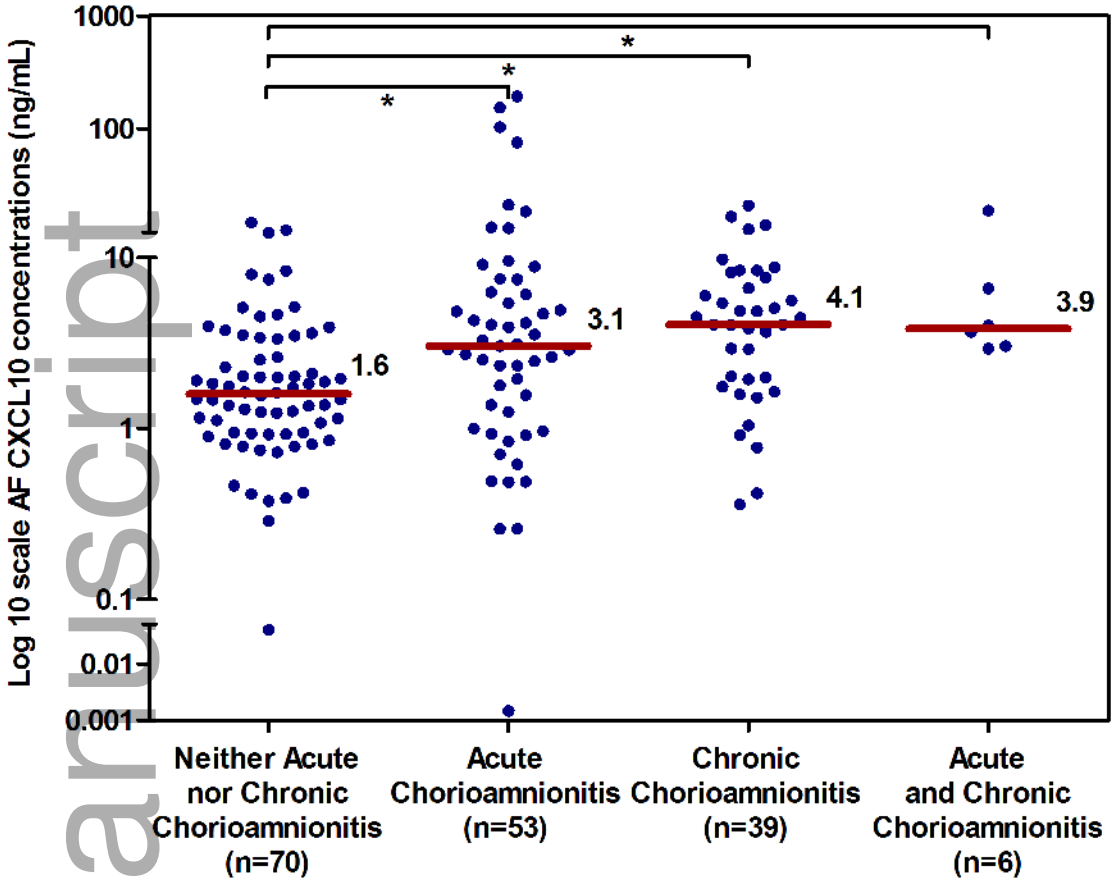
Outcomes	Normal AF IL-6 and CXCL10 (n=53)	Isolated increase of AF IL-6 (n=26)	Isolated increase of CXCL10 (n=30)	Increase of both AF IL-6 and CXCL10 (n=59)
GA at amniocentesis (weeks)	31.3 (28.4-32.7)	30.6 (26.7-32.4)*	31.4 (29.4-32.2)#	26.9 (23.6-30.7)
GA at delivery (weeks)	34.9 (32.0-38.1)	31.0 (27.1-33.0)*	34.1 (31.8-36.5)#	27.9 (24.3-32.1)
Preterm delivery (83.3%; n=140/168)	64.2% (34/53)	93.3% (24/26)*	76.7% (23/30)#	100% (59/59)
Spontaneous preterm delivery within 48 hours of amniocentesis (36.3%; n=61/168)	20.8% (11/53)	61.5% (16/26)	20.0% (6/30)#	47.5% (28/59)
Spontaneous preterm delivery before 34 weeks of gestation (50.6%; n=85/168)	30.2% (16/53)	65.4% (17/26)	26.7% (8/30)#	74.6% (44/59)
Birthweight (grams)	2,485 (1,900-2,941)	1,557 (1,008-2,119)*	2,210 (1,701-2,743)#	1,155 (600-1,700)
Placental pathology				
No acute/chronic chorioamnionitis (41.7%; n=70/168)	67.9% (36/53)	50% (13/26)*	36.7% (11/30)#	16.9% (10/59)
Acute chorioamnionitis ≥stage 2 (31.5%; n=53/168)	13.2% (7/53)	42.3% (11/26)	13.3% (4/30)#	52.5% (31/59)
Chronic chorioamnionitis (23.2%; n=39/168)	18.9% (10/53)	7.7% (2/26)	46.7% (14/30)#	22.0% (13/59)
Acute (≥stage 2) and chronic chorioamnionitis (3.6%; n=6/168)	0% (0/53)	0% (0/26)	3.3% (1/30)	8.5% (5/59)
Acute funisitis (33.3%; n=56/168)	17% (9/53)	34.6% (9/26)	20% (6/30)#	54.2% (32/59)

Data presented as % (n) or median (interquartile); AF: amniotic fluid; CXCL: C-X-C motif chemokine; GA: gestational age; IL: interleukin; acute chorioamnionitis: the presence of acute chorioamnionitis ≥stage 2 in the absence of chronic chorioamnionitis; chronic chorioamnionitis: the presence of chronic chorioamnionitis in the absence of acute chorioamnionitis ≥stage 2

Normal AF IL-6 and CXCL10 concentrations: IL-6 <2.6 ng/mL and CXCL10 <2.2 ng/mL; Isolated increase of AF IL-6 concentrations: IL-6 ≥2.6 ng/mL; Isolated increase of AF CXCL10 concentrations: CXCL10 ≥2.2 ng/mL; Increase of both AF IL-6 and CXCL10 concentrations: IL-6 ≥2.6 ng/mL and CXCL10 ≥2.2 ng/mL

[*] $p<0.05$ for the comparison between group of isolated increase of amniotic fluid IL-6 concentration and group of increase of both amniotic fluid IL-6 and CXCL10 concentrations

[#] $p<0.05$ for the comparison between group of isolated increase of amniotic fluid CXCL10 concentration and group of increase of both amniotic fluid IL-6 and CXCL10 concentrations



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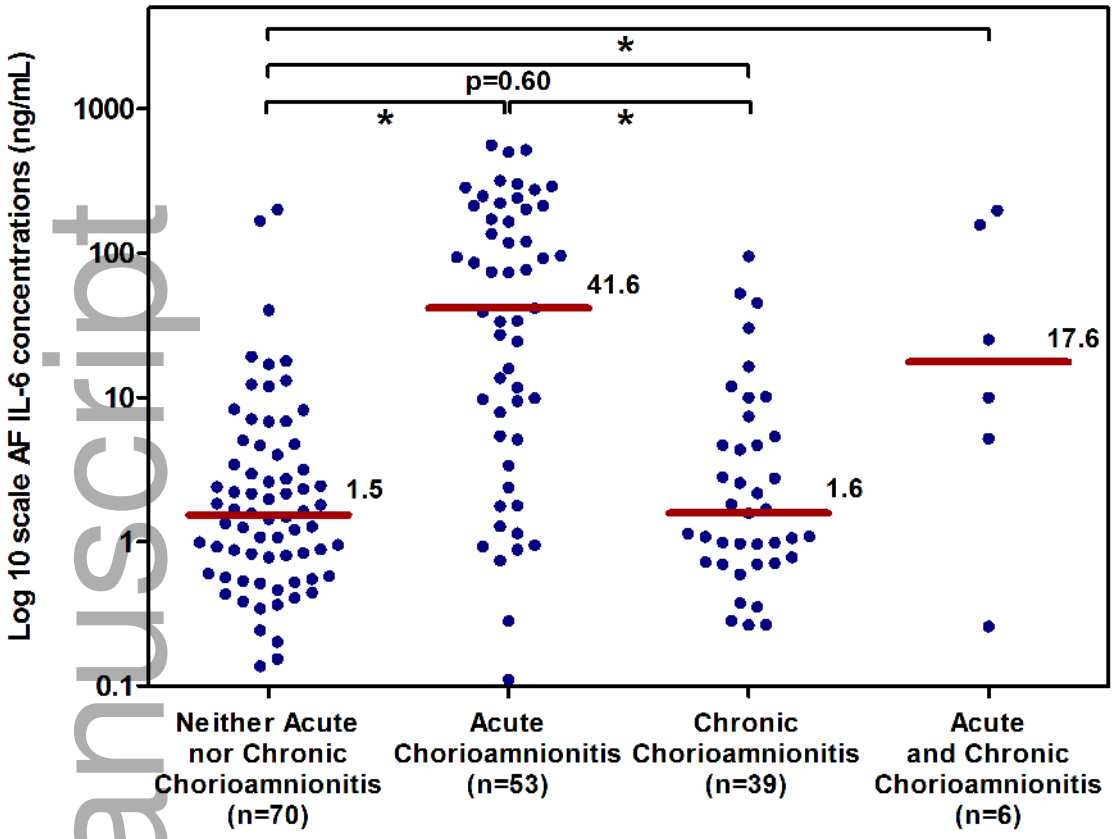

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			Chronic chorioamnionitis (n=39)	Acute chorioamnionitis (n=53)
	Normal AF IL-6 and CXCL10	32% (53/168)	Reference	Reference
	Isolated increase of AF IL-6	15% (26/168)	0.5 (0.1-2.8)	4.2 (1.3-13.7)
	Isolated increase of CXCL10	18% (30/168)	4.8 (1.7-14)	2.1 (0.5-8.9)
	Increased AF IL-6 and CXCL10	35% (59/168)	3.8 (1.3-11.6)	9.6 (3.1-30.1)

Data presented as odds ratio (95% confidence interval)

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			Placental lesions associated with maternal anti-fetal rejection (n=54)	Acute chorioamnionitis (n=44)
	Normal AF IL-6 and CXCL10	32% (53/168)	Reference	Reference
	Isolated increase of AF IL-6	15% (26/168)	0.4 (0.1-1.7)	2.6 (0.7-9.2)
	Isolated increase of CXCL10	18% (30/168)	3.7 (1.3-10.4)	1.6 (0.3-8.3)
	Increased AF IL-6 and CXCL10	35% (59/168)	4.3 (1.4-13.4)	10.9 (3.1-39)

Data presented as odds ratio (95% confidence interval)

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