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Clinical chorioamnionitis at term IV: the maternal plasma cytokine profile

Abstract

Introduction: Fever is a major criterion for clinical chorioamnionitis; yet, many patients with intrapartum fever do not have demonstrable intra-amniotic infection. Some cytokines, such as interleukin (IL)-1, IL-6, interferon-gamma (IFN- γ), and tumor necrosis factor alpha (TNF- α), can induce a fever. The objective of this study was to determine whether maternal plasma concentrations of cytokines could be of value in the identification of patients with the diagnosis of clinical chorioamnionitis at term who have microbial-associated intra-amniotic inflammation.

Methods: A retrospective cross-sectional study was conducted, including patients with clinical chorioamnionitis at term (n=41; cases) and women in spontaneous labor at term without clinical chorioamnionitis (n=77; controls). Women with clinical chorioamnionitis were classified into three groups according to the results of amniotic fluid culture, broad-range polymerase chain reaction coupled with electrospray ionization mass spectrometry (PCR/ESI-MS), and amniotic fluid IL-6 concentration:

1) no intra-amniotic inflammation; 2) intra-amniotic inflammation without detectable microorganisms; or 3) microbial-associated intra-amniotic inflammation. The maternal plasma concentrations of 29 cytokines were determined with sensitive and specific V-PLEX immunoassays. Nonparametric statistical methods were used for analysis, adjusting for a false discovery rate of 5%.

Results: 1) The maternal plasma concentrations of pyrogenic cytokines (IL-1 β , IL-2, IL-6, IFN- γ , and TNF- α) were significantly higher in patients with clinical chorioamnionitis at term than in those with spontaneous term labor without clinical chorioamnionitis; 2) the maternal plasma concentrations of cytokines were not significantly different among the three subgroups of patients with clinical chorioamnionitis (intra-amniotic inflammation with and without detectable bacteria and those without intra-amniotic inflammation); and 3) among women with the diagnosis of clinical chorioamnionitis, but without evidence of intra-amniotic inflammation, the maternal plasma concentrations of pyrogenic cytokines were significantly higher than in patients with spontaneous labor at term. These observations suggest that a fever can be

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mediated by increased circulating concentrations of these cytokines, despite the absence of a local intra-amniotic inflammatory response.

Conclusions: 1) The maternal plasma concentrations of pyrogenic cytokines (e.g. IL-1 β , IL-2, IL-6, IFN- γ , and TNF- α) are higher in patients with intra-partum fever and the diagnosis of clinical chorioamnionitis at term than in those in spontaneous labor at term without a fever; and 2) maternal plasma cytokine concentrations have limited value in the identification of patients with bacteria in the amniotic cavity. Accurate assessment of the presence of intra-amniotic infection requires amniotic fluid analysis.

Keywords: Acute histologic chorioamnionitis; amniotic fluid; biomarkers; chemokines; fever; funisitis; interferon-gamma (IFN- γ); interleukin-1 β (IL-1 β); interleukin-2 (IL-2); interleukin-6 (IL-6); microbial-associated intra-amniotic inflammation; pyrogens; tumor necrosis factor- α (TNF- α); V-PLEX immunoassays.

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Introduction

Clinical chorioamnionitis is characterized by maternal fever accompanied by at least two of the following signs: maternal or fetal tachycardia, maternal leukocytosis, uterine tenderness, or foul-smelling amniotic fluid [1–13]. This pregnancy complication is associated with adverse maternal [14–19], fetal, and neonatal/infant outcomes [20–40]. Most of the signs of clinical chorioamnionitis (except for fetal tachycardia and foul-smelling amniotic fluid) are thought to reflect a maternal inflammatory response to microbial invasion of the amniotic cavity (MIAC) [1, 7]. Fever, the cardinal sign of clinical chorioamnionitis, is mediated by the effect of pyrogenic cytokines [41–43], which include interleukin (IL)-1 [43–51], IL-2 [52–54], IL-6 [55–59], interferon-gamma (IFN- γ) [60, 61], and tumor necrosis factor-alpha (TNF- α) [54, 62–65]. Some of these cytokines have primary pyrogenic effects (i.e., IL-1 [43–51]), or TNF- α [54, 62–65], while others induce fever indirectly (i.e., IL-2 [52–54]). Fever can be generated independently of IL-1 and TNF- α by the engagement of Toll-like receptors [66–70]. These different pathways converge in the production of cyclooxygenase-2 [69–73] and prostaglandin E₂ [74–80], which bind to prostanoid receptors in the hypothalamus [43, 70]. Thermosensitive neurons in

the thermoregulatory center then raise the hypothalamic thermostatic set point [69, 81, 82].

Many cases of intra-partum fever are not related to infection [9, 12, 83–91]. We recently reported that only 54% of women with clinical chorioamnionitis (all had fever) at term have microorganisms isolated from the amniotic cavity; 24% had intra-amniotic inflammation without demonstrable bacteria and 22% had no intra-amniotic inflammation [9]. Epidural analgesia can cause maternal hyperthermia in the absence of intra-amniotic infection [83, 86, 92–107]. Therefore, when patients have a fever, the accurate diagnosis of infection is challenging, but important. We recently reported that the signs used in the diagnosis of clinical chorioamnionitis do not accurately identify patients with proven intra-amniotic infection [4].

The objective of this study was to determine if the maternal plasma concentration of cytokines could distinguish patients with clinical chorioamnionitis with and without proven intra-amniotic infection.

Materials and methods

Study population

A retrospective cross-sectional case-control 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 (NICHD/NIH/DHHS). The inclusion criteria were: 1) singleton gestation; 2) gestational age of ≥ 37 weeks; and 3) the absence of fetal malformations.

Cases consisted of women with clinical chorioamnionitis at term ($n=41$). These women had also been included in a prior study that provided a detailed description of sample collection, microbiological studies, and determination of amniotic fluid IL-6 concentrations using a sensitive and specific enzyme-linked immunosorbent assay [9]. Controls were women with spontaneous term labor without clinical and histological chorioamnionitis ($n=77$; gestational age of 37–42 weeks).

All patients provided written informed consent, and the use of biological specimens as well as clinical and ultrasound data for research purposes was approved by the Institutional Review Boards of NICHD, Wayne State University, and S tero del R o Hospital, Santiago, Chile.

Clinical definitions

MIAC was defined according to the results of amniotic fluid culture and broad-range polymerase chain reaction coupled with electrospray ionization mass spectrometry (PCR/ESI-MS), (Ibis® Technology, Athogen, Carlsbad, CA, USA) [108–111]. Intra-amniotic inflammation was diagnosed when the amniotic fluid IL-6 concentration was ≥ 2.6 ng/mL [10, 112–125]. Based on the results of amniotic fluid cultures, PCR/ESI-MS, and amniotic fluid concentrations of IL-6, patients with

clinical chorioamnionitis at term were classified as having: 1) no intra-amniotic inflammation; 2) intra-amniotic inflammation without detectable bacteria (an elevated amniotic fluid IL-6 concentration without evidence of bacteria using cultivation and molecular methods); or 3) microbial-associated intra-amniotic inflammation (combination of MIAC and intra-amniotic inflammation). Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature $>37.8^{\circ}\text{C}$) accompanied by two or more of the following criteria: 1) maternal tachycardia (heart rate >100 beats/min); 2) uterine tenderness; 3) foul-smelling amniotic fluid; 4) fetal tachycardia (heart rate >160 beats/min); and 5) maternal leukocytosis (leukocyte count $>15,000$ cells/mm³) [3, 4, 7, 8, 126, 127].

Controls were women with spontaneous labor without clinical or histological chorioamnionitis, who delivered a normal term (≥ 37 weeks) infant whose birthweight was appropriate for the gestational age (10th–90th percentile) [128, 129].

Acute histologic chorioamnionitis was diagnosed based on the presence of inflammatory cells in the chorionic plate and/or chorioamniotic membranes [130–139]. Acute funisitis was diagnosed based on the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly, using criteria previously described [130, 140–145]. Acute inflammatory lesions of the placenta or placental lesions consistent with amniotic fluid infection were defined by the presence of acute histologic chorioamnionitis and/or acute funisitis [130].

Sample collection and cytokines/chemokines immunoassays

Maternal blood samples were collected at admission during labor in both cases and controls. These samples were placed into tubes containing EDTA, then centrifuged for 10 min at 4°C and stored at -70°C . Laboratory personnel were blinded to clinical diagnosis. The maternal plasma concentrations of the following 29 cytokines/chemokines were determined with sensitive and specific V-PLEX immunoassays (Meso Scale Discovery, Gaithersburg, MD, USA) [Pro-inflammatory cytokines: IFN- γ , IL-1 α , IL-1 β , IL-2, IL-6, IL-7, IL-12p70, IL-12/IL-23p40, IL-15, IL-16, IL-17 α , TNF- α , TNF- β , vascular endothelial growth factor (VEGF), granulocyte macrophage colony-stimulating factor (GM-CSF); anti-inflammatory cytokines: IL-4, IL-5, IL-10, IL-13; and chemokines: IL-8, thymus and activation-regulated chemokine (TARC), eotaxin, eotaxin-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1 α , MIP-1 β , monocyte chemoattractant protein (MCP)-1, MCP-4, C-X-C motif chemokine 10 (CXCL-10) or interferon gamma-induced protein 10 (IP-10)]. Briefly, 50 μL of a maternal

blood sample or calibrator were dispensed into separate wells of the plates and incubated for 2 h with vigorous shaking at room temperature. The samples and calibrators were discarded, and the plates were washed three times with phosphate-buffered saline and 0.05% Tween-20, followed by an addition of 25 μL of the 1 \times detection antibody solution into each well. Plates were then incubated for 2 h with vigorous shaking at room temperature. The detection antibody was removed, and the plates were washed three times. One hundred and fifty microliters of 2 \times Read Buffer T were added to each well, and the signals were read by the SECTOR® Imager 2400 (Meso Scale Discovery). Standard curves were generated, and the assay values of the samples were interpolated from the curves. The assay characteristics are described in the Supplementary Table. The coefficient of variation was $\leq 14.6\%$ for 20 of the 29 analytes. For samples with concentrations below the limits of detection, missing values were replaced with 99% of the lowest detectable concentration.

Statistical analysis

For demographics data analysis: the Kolmogorov-Smirnov test was used to test whether the distribution of continuous variables was normal. Chi-square and Fisher's exact tests were used for comparisons of proportions. Kruskal-Wallis and the Mann-Whitney *U*-tests were used to compare median concentrations of analytes between and among groups. Statistical analysis of demographics data was performed using SPSS 19 (IBM Corp, Armonk, NY, USA). A *P*-value < 0.05 was considered statistically significant.

Comparison of analyte concentrations determined by multiplex assay was restricted to the analytes that were detected in a number of samples larger than one-half of the size of the smallest group. Statistical analysis was performed using the Wilcoxon rank-sum test and R statistical environment [146]. Nominal *P*-values were adjusted using the Benjamini and Hochberg method [147], controlling the false discovery rate at 5%.

Results

Characteristics of the study population

Clinical characteristics of the study population are displayed in Table 1. Patients with clinical chorioamnionitis

Table 1: Clinical characteristics of the study population.

	Term in labor (n=77)	Clinical chorioamnionitis at term (n=41)	P-value
Maternal age (years)	25 (19.5–30)	23.7 (21.6–24.6)	0.007
Body mass index (kg/m ²)	23.9 (21.3–26.1)	23.7 (21.6–24.6)	0.53
Amniotic fluid glucose (mg/dL)	N/A	9 (9–9)	N/A
Amniotic fluid white blood cell count (cell/mm ³)	N/A	58 (5–695)	N/A
Gestational age at amniocentesis (weeks)	N/A	39.6 (38.9–40.7)	N/A
Gestational age at delivery (weeks)	39.6 (38.9–40.7)	40 (39.1–40.7)	0.33
Birthweight (g)	3450 (3200–3665)	3550 (3200–3780)	0.39

Data presented as median (interquartile range). N/A=not applicable.

had a significantly lower median maternal age than those with spontaneous term labor without clinical chorioamnionitis ($P=0.007$). Otherwise, there were no significant differences in the clinical characteristics between these two groups. All patients with clinical chorioamnionitis received epidural analgesia.

When classified according to the presence or absence of both intra-amniotic inflammation and microorganisms (by amniotic fluid culture and PCR/ESI-MS), 58.5% (24/41) of the cases had microbial-associated intra-amniotic inflammation, 22% (9/41) had intra-amniotic inflammation without demonstrable bacteria, and 19.5% (8/41) had no evidence of intra-amniotic inflammation. The microorganisms identified in the amniotic fluid were previously reported [9]. A placental histopathology report was not available for one patient with clinical chorioamnionitis. Acute inflammatory lesions of the placenta were found in 62.5% (25/40) of patients with clinical chorioamnionitis. Among cases with intra-amniotic inflammation with and without demonstrable microorganisms, 79.2% (19/24) and 62.5% (5/8) had acute inflammatory lesions of the placenta, respectively. Only 12.5% (1/8) of cases without intra-amniotic inflammation had acute inflammatory lesions of the placenta.

Maternal plasma cytokine and chemokine concentrations in clinical chorioamnionitis at term and spontaneous term labor without clinical chorioamnionitis

Clinical chorioamnionitis vs. spontaneous labor at term without clinical chorioamnionitis

The maternal plasma cytokine and chemokine concentrations of women with spontaneous labor at term and those with clinical chorioamnionitis at term are described in Table 2. Women with clinical chorioamnionitis had significantly higher median maternal plasma concentrations of 10/19 inflammation-related cytokines than controls (after correcting for false discovery). The fold difference in median maternal plasma concentrations of IL-2, IL-6, IL-1 β , IL-17 α , IFN- γ , TNF- β , TNF- α , IL-15, IL-12/IL-23p40, IL-10, and IL-5 ranged from 1.17 to 13.13. IL-2 had the greatest fold change of 13.13 (Table 2). Importantly, the maternal plasma concentrations of several pyrogenic cytokines (IL-6, IL-2, TNF- α , and IL-1 β) were higher in women with clinical chorioamnionitis (all with a fever) than in those with spontaneous labor at term without clinical chorioamnionitis.

Patients with clinical chorioamnionitis at term had significantly higher median maternal plasma concentrations of three of the ten chemokines (MCP-1, MIP-1 α , and IL-8) than controls, with a fold difference in the median of approximately 1.2 for the three of them. Eotaxin, MCP-4, and eotaxin-3 were significantly lower in women with clinical chorioamnionitis than in controls, and fold differences in the median were 0.83, 0.70, and 0.24, respectively (Table 2).

Maternal plasma cytokine concentrations in patients with clinical chorioamnionitis according to the presence of inflammation and bacteria in the amniotic fluid

In this study, six of the ten pro-inflammatory cytokines (IL-6, IL-2, IFN- γ , TNF- α , IL-17 α , and IL-15) had significantly higher concentrations in cases without intra-amniotic inflammation than in controls, and fold differences in the median ranged from 1.82 to 18.69 (Table 3). IL-2 and IFN- γ had fold changes >15 when comparing cases without intra-amniotic inflammation to women in spontaneous labor at term (Figures 1 and 2). IL-6 was the only pro-inflammatory cytokine that was significantly higher in cases with intra-amniotic inflammation without demonstrable microorganisms than in those with spontaneous labor at term (Figure 3).

Patients with clinical chorioamnionitis and microbial-associated intra-amniotic inflammation had significantly higher median maternal plasma concentrations of IL-2, IL-6, and IL-15 than those with spontaneous labor at term, with fold changes of 13.13, 6.16, and 1.26, respectively (Table 3). IFN- γ had a fold change >15 , and the difference was detected only in the subgroup of clinical chorioamnionitis without intra-amniotic inflammation, but not in those with intra-amniotic inflammation, when compared to controls. In contrast, maternal IL-6 concentrations were significantly higher in all subgroups with clinical chorioamnionitis than in the control group (i.e., clinical chorioamnionitis without intra-amniotic inflammation: fold change 2.66, $P=0.008$; clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria: fold change 3.85, $P=0.009$; clinical chorioamnionitis with microbial-associated intra-amniotic inflammation: fold change 6.16, $P=0.0000025$; Table 3; Figure 3).

Among the four anti-inflammatory cytokines, the median maternal plasma IL-10 concentration was four times greater in women with chorioamnionitis without intra-amniotic inflammation than in controls (Table 3). However, the difference did not reach statistical significance in cases with intra-amniotic inflammation with or

Table 2: Concentrations of maternal plasma cytokines and chemokines in term in labor vs. clinical chorioamnionitis at term.

Analytes (pg/mL)	Term in labor median (IQR) (n=77)	Clinical chorioamnionitis at term median (IQR) (n=41)	Fold change	Adjusted P-value
Pro-inflammatory cytokines				
IL-2	0.01 (0.01–0.12)	0.13 (0.06–0.22)	13.13	0.001
IL-6	2.53 (1.64–4.08)	10.94 (5.36–22.08)	4.32	<0.0001
IL-1 β	0.12 (0.01–0.31)	0.21 (0.11–0.34)	1.75	0.03
IL-17 α	0.55 (0.34–0.71)	0.8 (0.63–1.32)	1.45	0.002
TNF- β	0.07 (0.02–0.11)	0.1 (0.05–0.15)	1.43	0.04
TNF- α	2.06 (1.62–2.48)	2.84 (1.9–3.89)	1.38	0.008
IL-15	2.05 (1.71–2.42)	2.66 (2.25–3.67)	1.30	<0.0001
IL-12/IL-23p40	76.89 (62.43–98.69)	89.81 (70.86–112.47)	1.17	0.046
IFN- γ	5.24 (3.26–8.34)	6.15 (3.66–19.81)	1.17	0.09
IL-1 α	2.61 (1.65–4.27)	2.59 (1.94–3.52)	0.99	0.78
IL-12p70	0.07 (0.01–0.13)	0.06 (0.01–0.19)	0.86	0.24
GM-CSF	0.02 (0.02–0.09)	0.02 (0.02–0.12)	1.00	0.88
IL-16	158.99 (129.21–224.54)	173.57 (127.9–220.27)	1.10	0.93
IL-7	3.51 (2.04–6.51)	3.41 (2.15–7.64)	0.97	0.88
VEGF	14.36 (10.67–23.71)	13.48 (9.41–17.25)	0.94	0.24
Anti-inflammatory cytokines				
IL-10	0.41 (0.25–0.86)	2.04 (0.32–5)	4.97	0.0006
IL-5	0.36 (0.21–0.57)	0.48 (0.27–0.83)	1.33	0.04
IL-13	0.65 (0.28–1.01)	0.65 (0.28–1.04)	1.00	0.88
IL-4	0.03 (0.01–0.03)	0.03 (0.01–0.04)	1.00	0.97
Chemokines				
MCP-1	57.38 (51.48–70.38)	73.61 (53.78–104.7)	1.28	0.03
MIP-1 α	10.41 (7.35–12.25)	13.27 (9.29–17.36)	1.27	0.02
IL-8	3.0 (1.9–5.09)	3.67 (3.29–7.47)	1.22	0.03
Eotaxin	56.8 (43.72–77.74)	46.93 (31.29–56.09)	0.83	0.008
MCP-4	43.79 (29.91–73.38)	30.48 (24.06–43.03)	0.70	0.02
Eotaxin-3	19.36 (14.89–25.59)	4.67 (0.64–13.1)	0.24	<0.0001
MDC	520.65 (443.02–644.53)	515.51 (403.33–637.29)	0.99	0.76
CXCL-10 or IP-10	238.32 (172.2–327.48)	242.38 (179.13–485.75)	1.02	0.37
TARC	34.99 (18.74–52.3)	35.59 (22.81–52.87)	1.02	0.78
MIP-1 β	59.25 (45.93–78.56)	59.33 (41.53–80.4)	1.001	0.93

IQR=interquartile range, IL=interleukin, TNF=tumor necrosis factor, IFN- γ =interferon gamma, GM-CSF=granulocyte macrophage colony-stimulating factor, VEGF=vascular endothelial growth factor, MCP=monocyte chemoattractant protein, MIP=macrophage inflammatory protein, MDC=macrophage-derived chemokine, CXCL-10=C-X-C motif chemokine 10, IP-10=interferon gamma-induced protein 10, TARC=thymus and activation-regulated chemokine. The units of all analytes are pg/mL.

without detectable microorganisms, when each one was compared to controls (Table 3).

Patients with clinical chorioamnionitis without intra-amniotic inflammation had significantly higher maternal plasma CXCL-10 (or IP-10) concentrations than controls (fold change=2.11; P=0.02) (Table 3). Eotaxin-3 is the only chemokine that had a significantly lower concentration in cases of intra-amniotic inflammation without demonstrable microorganisms than in controls (fold change=0.24; P=0.008). The concentrations of MCP-4 and eotaxin-3 were significantly lower in cases with microbial-associated intra-amniotic inflammation than in controls (eotaxin-3: fold change=0.20, P=0.003; MCP-4: fold change=0.59, P=0.003; Table 3). Overall, no differences could be detected in the maternal plasma concentrations

of cytokines among the subgroups of patients with clinical chorioamnionitis at term (Table 3).

The supplementary material contains the scatter-plots of maternal plasma concentrations of cytokines/chemokines among the different groups (Supplementary Figures 1–3).

Discussion

Principal findings of this study

1) The maternal plasma concentrations of pyrogenic cytokines (IL-1 β , IL-2, IL-6, IFN- γ , and TNF- α) were

Table 3: Concentrations of maternal plasma cytokines and chemokines in the subgroups of clinical chorioamnionitis and term in labor.

Analytes (pg/mL)	Term in labor (controls) (n=77)	Clinical chorioamnionitis at term (n=41)														
		Without intra-amniotic inflammation (n=8)					With intra-amniotic inflammation without demonstrable bacteria (n=9)					With microbial-associated intra-amniotic inflammation (n=24)				
		Median (IQR)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to term in labor)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to without intra-amniotic inflammation)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to intra-amniotic inflammation)	Adjusted P-value (compared to with intra-amniotic inflammation without demonstrable bacteria)	
Pro-inflammatory cytokines																
IL-6	2.53 (1.64–4.08)	6.72 (5.05–12.91)	2.66	0.008		9.75 (6.14–22.92)	3.85	0.009	0.78		15.58 (5.54–22.34)	6.16	<0.0001	0.70	0.92	
IL-2	0.01 (0.01–0.12)	0.18 (0.14–0.24)	18.69	0.008		0.12 (0.05–0.14)	12.12	0.27	0.74		0.13 (0.02–0.21)	13.13	0.04	0.40	0.92	
IFN-γ	5.24 (3.26–8.34)	88.73 (13.78–135.99)	16.93	0.008		6.15 (3.83–20.57)	1.17	0.45	0.74		5.04 (3.31–7.92)	0.96	0.82	0.08	0.87	
TNF-α	2.06 (1.62–2.48)	4.46 (3.2–5.79)	2.17	0.04		2.98 (2.2–3.48)	1.45	0.05	0.74		2.42 (1.55–3.3)	1.17	0.26	0.26	0.87	
IL-17α	0.55 (0.34–0.71)	1.32 (0.71–3.87)	2.40	0.04		0.83 (0.68–1.32)	1.51	0.05	0.74		0.74 (0.48–1.07)	1.35	0.09	0.40	0.87	
IL-15	2.05 (1.71–2.42)	3.73 (2.93–4.51)	1.82	0.008		2.75 (2.25–3.33)	1.34	0.07	0.74		2.58 (2.23–3.18)	1.26	0.002	0.40	0.97	
TNF-β	0.07 (0.02–0.11)	0.1 (0.07–0.11)	1.43	0.51		0.13 (0.08–0.15)	1.86	0.27	0.74		0.1 (0.05–0.15)	1.43	0.11	0.84	0.92	
IL-12/IL-23p40	76.89 (62.43–98.69)	112.2 (87.39–147.02)	1.46	0.07		96.63 (58.76–128.84)	1.26	0.30	0.74		83.45 (70.45–101.04)	1.09	0.37	0.31	0.87	
IL-16	158.99 (129.21–224.54)	118.8 (89.15–155.07)	0.75	0.13		133.63 (128.67–191.54)	0.84	0.53	0.74		187.65 (159.96–229.7)	1.18	0.31	0.26	0.87	
IL-1α	2.61 (1.65–4.27)	2.65 (1.81–3.68)	1.02	0.91		2.7 (2.25–5.08)	1.03	0.53	0.74		2.57 (2.07–3.19)	0.98	0.82	1.00	0.87	
GM-CSF	0.02 (0.02–0.09)	0.02 (0.02–0.13)	1.00	0.83		0.02 (0.02–0.17)	1.00	0.53	0.88		0.02 (0.02–0.06)	1.00	0.88	0.90	0.87	
IL-7	3.51 (2.04–6.51)	7.79 (3.83–9.14)	2.22	0.15		4.58 (3.68–7.64)	1.3	0.27	0.74		2.72 (1.55–5.07)	0.78	0.27	0.26	0.46	
IL-1β	0.12 (0.01–0.31)	0.21 (0.1–0.4)	1.79	0.32		0.31 (0.12–0.35)	2.58	0.27	0.74		0.21 (0.11–0.28)	1.71	0.11	1.00	0.87	
IL-12p70	0.07 (0.01–0.13)	0.13 (0.04–0.24)	1.93	0.27		0.13 (0.04–0.21)	1.86	0.15	0.96		0.05 (0.01–0.19)	0.64	0.93	0.30	0.87	
VEGF	14.36 (10.67–23.71)	13.98 (10.43–17.51)	0.97	0.61		16.8 (12.11–26.54)	1.17	0.98	0.74		12.21 (9.25–15.12)	0.85	0.19	0.84	0.87	
Anti-inflammatory cytokines																
IL-10	0.41 (0.25–0.86)	1.9 (1.3–5.42)	4.63	0.02		3.19 (0.92–3.8)	7.78	0.05	0.96		1.75 (0.3–5.49)	4.27	0.05	0.70	0.95	
IL-5	0.36 (0.21–0.57)	0.28 (0.2–0.47)	0.79	0.83		0.38 (0.27–0.54)	1.06	0.61	0.74		0.59 (0.4–0.91)	1.64	0.01	0.26	0.87	
IL-4	0.03 (0.01–0.03)	0.02 (0.01–0.02)	0.67	0.36		0.02 (0.01–0.03)	0.67	0.81	0.74		0.03 (0.01–0.04)	1.00	0.61	0.40	0.87	
IL-13	0.65 (0.28–1.01)	0.28 (0.28–0.41)	0.43	0.07		0.28 (0.28–0.78)	0.43	0.45	0.74		0.93 (0.28–1.37)	1.42	0.27	0.19	0.87	

(Table 3 Continued)

Analytes (pg/mL)	Term in labor (controls) (n=77)		Clinical chorioamnionitis at term (n=41)										
	Without intra-amniotic inflammation (n=8)			With intra-amniotic inflammation without demonstrable bacteria (n=9)			With microbial-associated intra-amniotic inflammation (n=24)						
	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to term in labor)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to intra-amniotic inflammation without demonstrable bacteria)
Chemokines													
CXCL-10 or IP-10	238.32 (172.2–327.48)	503.96 (284.8–998.46)	0.02	242.6 (179.13–557.89)	1.02	0.46	0.74	209.99 (169.03–296.8)	0.88	0.70	0.08		0.87
Eotaxin-3	19.36 (14.89–25.59)	10.12 (0.64–22.92)	0.22	4.67 (0.64–9.54)	0.24	0.008	0.74	3.93 (0.64–12.97)	0.20	0.0003	0.84		0.92
MCP-4	43.79 (29.91–73.38)	33.11 (25.28–52.48)	0.50	38.85 (34.99–46.92)	0.89	0.98	0.74	26.05 (22.15–37.84)	0.59	0.003	0.40		0.41
MCP-1	57.38 (51.48–70.38)	94.93 (65.69–130.46)	0.07	73.01 (55.47–91.16)	1.27	0.27	0.74	69.27 (52.17–99.48)	1.21	0.26	0.47		0.92
MIP-1β	59.25 (45.93–78.56)	68.62 (52.61–165.9)	0.29	57.28 (44.39–69.42)	0.97	0.81	0.74	60.51 (38.55–80.72)	1.02	0.82	0.40		0.92
MIP-1α	10.41 (7.35–12.25)	20.64 (9.81–25.13)	0.10	11.84 (8.95–13.77)	1.14	0.41	0.74	12.63 (9.4–15.2)	1.21	0.11	0.40		0.92
IL-8	3 (1.9–5.09)	4 (2.77–7.97)	0.44	3.58 (3.43–4.71)	1.19	0.27	0.96	3.61 (3.14–8.6)	1.20	0.09	1.00		0.92
TARC	34.99 (18.74–52.3)	26.89 (21.74–34.94)	0.50	42.57 (20.45–73.51)	1.22	0.53	0.74	36.75 (24.22–55.2)	1.05	0.61	0.40		0.92
MDC	520.65 (443.02–644.53)	526.94 (471.77–590.9)	0.73	463.75 (421.72–732.89)	0.89	0.98	0.78	512.05 (396.98–639.64)	0.98	0.68	1.00		0.87
Eotaxin	56.8 (43.72–77.74)	47.95 (41.02–58.23)	0.27	40.04 (31.29–54.04)	0.70	0.10	0.74	46.68 (32.87–57.96)	0.82	0.08	1.00		0.92

IQR=interquartile range, IL=interleukin, TNF=tumor necrosis factor, IFN-γ=interferon gamma, GM-CSF=granulocyte macrophage colony-stimulating factor, VEGF=vascular endothelial growth factor, MCP=monocyte chemoattractant protein, MIP=macrophage inflammatory protein, MDC=macrophage-derived chemokine, CXCL-10=C-X-C motif chemokine 10, IP-10=interferon gamma-induced protein 10, TARC=thymus and activation-regulated chemokine. The units of all analytes are pg/mL.

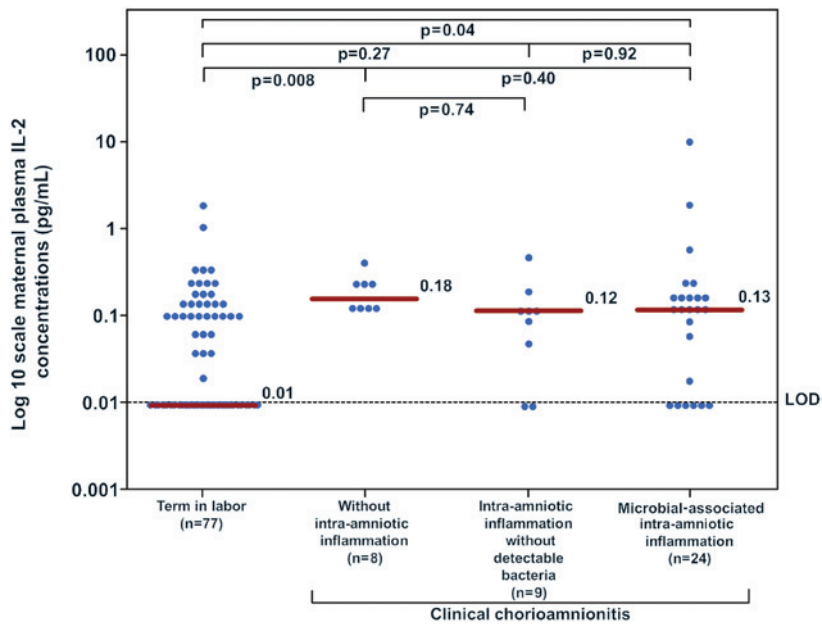


Figure 1: The maternal plasma concentrations of interleukin (IL)-2 in patients with spontaneous term labor without clinical chorioamnionitis ($n=77$, controls), clinical chorioamnionitis without intra-amniotic inflammation ($n=8$), clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria ($n=9$), and clinical chorioamnionitis with microbial-associated intra-amniotic inflammation ($n=24$). The median (interquartile range) maternal plasma concentrations of IL-2 are 0.01 (0.01–0.12) pg/mL (term in labor), 0.18 (0.14–0.24) pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 0.12 (0.05–0.14) pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria), and 0.13 (0.02–0.21) pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). LOD=limit of detection.

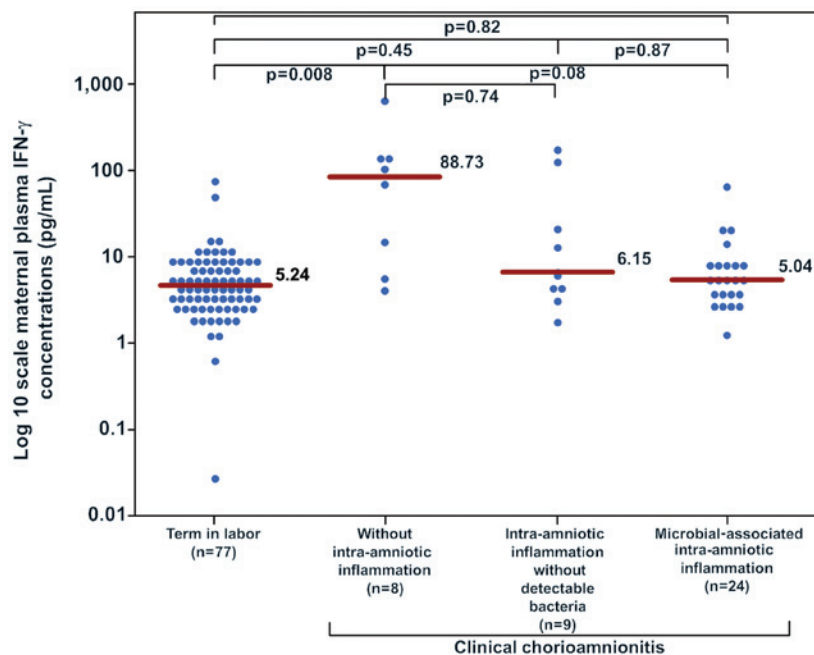


Figure 2: The maternal plasma concentrations of interferon (IFN)- γ in patients with spontaneous term labor without clinical chorioamnionitis ($n=77$, controls), clinical chorioamnionitis without intra-amniotic inflammation ($n=8$), clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria ($n=9$), and clinical chorioamnionitis with microbial-associated intra-amniotic inflammation ($n=24$). The median (interquartile range) maternal plasma concentrations of IFN- γ are 5.24 (3.26–8.34) pg/mL (term in labor), 88.73 (13.78–135.99) pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 6.15 (3.83–20.57) pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria), and 5.04 (3.31–7.92) pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation).

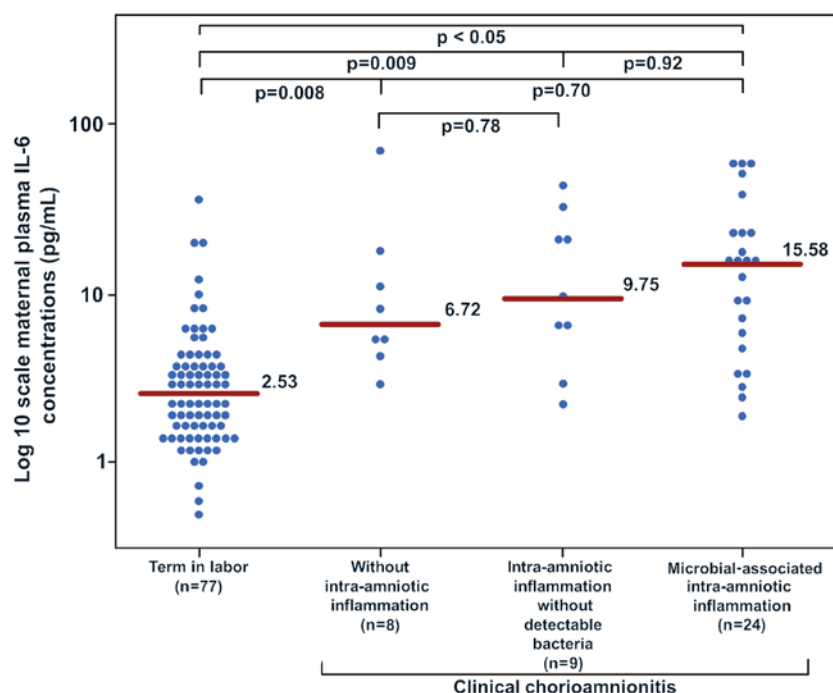


Figure 3: The maternal plasma concentrations of interleukin (IL)-6 in patients with spontaneous term labor without clinical chorioamnionitis ($n=77$, controls), clinical chorioamnionitis without intra-amniotic inflammation ($n=8$), clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria ($n=9$), and clinical chorioamnionitis with microbial-associated intra-amniotic inflammation ($n=24$). The median (interquartile range) maternal plasma concentrations of IL-6 are 2.53 (1.64–4.08) pg/mL (term in labor), 6.72 (5.05–12.91) pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 9.75 (6.14–22.92) pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria), and 15.58 (5.54–22.34) pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation).

significantly higher in patients with clinical chorioamnionitis at term than in those with spontaneous term labor without clinical chorioamnionitis; 2) the maternal plasma concentrations of cytokines were not significantly different among the three subgroups of patients with clinical chorioamnionitis (intra-amniotic inflammation with and without detectable bacteria, and those without intra-amniotic inflammation); and 3) among women with the diagnosis of clinical chorioamnionitis but without evidence of intra-amniotic inflammation, the maternal plasma concentrations of pyrogenic cytokines were significantly higher than in women with spontaneous labor at term. These observations suggest that the intra-amniotic inflammatory response observed in some patients with clinical chorioamnionitis is not reflected in the maternal circulation.

Maternal plasma cytokines in patients with clinical chorioamnionitis

Intra-amniotic inflammation and bacteria are frequently present in patients with clinical chorioamnionitis at term [1, 3, 7, 9, 12] and other complications of pregnancy, such

as preterm labor [13, 116, 119, 148–164], preterm prelabor rupture of the membranes (PPROM) [109, 117, 121, 135, 165–176], cervical insufficiency [177–179], and in patients with an asymptomatic short cervix [118, 180–185]. Microorganisms and their products can initiate an inflammatory response by the engagement of pattern recognition receptors [163, 172, 186–200] and the production of cytokines [112, 122, 151, 201–224], chemokines [213, 215, 216, 219, 223, 225–244], prostaglandins [245–261], and proteases [142, 145, 262–277]. If microorganisms in the amniotic cavity reach the fetus, this can result in a fetal systemic inflammatory response [278, 279], which is associated with multi-organ involvement [141, 278, 280–289] and preterm delivery [290].

We report herein, for the first time, a systematic study of the maternal cytokine profile using multiplex immunoassays in patients with clinical chorioamnionitis at term stratified according to the status of the amniotic cavity (presence/absence of inflammation and bacteria). The results showed that clinical chorioamnionitis at term is associated with higher maternal plasma pro- and anti-inflammatory cytokines, as well as some chemokines, than in spontaneous labor at term without a fever. The greatest fold change was observed in the maternal plasma

concentration of IL-2, followed by IL-6 (13.13 vs. 4.32, respectively). Other cytokines and chemokines had fold changes of a lesser magnitude (<5).

IL-2 is a lymphocyte growth factor involved in the differentiation of CD4⁺ and CD8⁺ cells, as well as immune tolerance [291–296], and it has been implicated in the pathogenesis of sepsis [297–300]. Therapeutic administration of IL-2 to patients with cancer can lead to a sepsis-like syndrome [301–303]. *In vitro* and *in vivo* studies have shown that IL-2 up-regulates the production of IL-1 β and TNF- α in peripheral blood mononuclear cells [52, 304], which are also involved in the pathogenesis of sepsis [305, 306]. Moreover, IL-2 administration is associated with the activation of complement [307–311], neutrophils [311, 312], and the coagulation system [313]. Circulating IL-2 receptor concentrations can also predict the likelihood of sepsis in both newborns [314] and adults [315–317].

Maternal inflammatory response in clinical chorioamnionitis without intra-amniotic inflammation

We have previously reported that a subset of patients with clinical chorioamnionitis at term did not have intra-amniotic inflammation [9], and that their amniotic fluid inflammatory response was similar to that of women with spontaneous labor at term without fever or complications [12]. In the current study, patients with clinical chorioamnionitis without intra-amniotic inflammation had higher concentrations of several maternal plasma cytokines (e.g., IL-2, IFN- γ , IL-6, TNF- α , IL-17 α , and IL-15) than patients with spontaneous term labor. Of interest, the maternal plasma IL-2 and IFN- γ concentrations had a fold change >15 in patients with clinical chorioamnionitis without intra-amniotic inflammation when compared to controls. Of note, IFN- γ was significantly higher only in patients with clinical chorioamnionitis without intra-amniotic inflammation, but not in those with intra-amniotic inflammation either with or without demonstrable bacteria when each was compared to controls (spontaneous labor at term without a fever).

IFN- γ is a potent pro-inflammatory cytokine with antiviral properties [318, 319]. The administration of IFN- γ in patients with cancer can induce a fever [320, 321]. However, the pyrogenic effect appears to be due to the induction of IL-1 and TNF- α [60, 322, 323]. The administration of IFN- γ to human subjects initiates fever 3–4 h after injection [53]. Therefore, the fever of some patients with the diagnosis of clinical chorioamnionitis may be mediated by IFN- γ or other pyrogenic cytokines.

Maternal plasma cytokines in patients with intra-amniotic inflammation with or without bacteria in the amniotic cavity

We observed a dramatic elevation in the concentration of maternal plasma IL-6 (fold change >5) in patients with bacteria in the amniotic cavity. This is not unexpected, as this cytokine is a major mediator of the host response to infection and tissue damage [324–328]. Bacterial products are known to up-regulate the expression of IL-6 [329, 330] and so are other inflammatory cytokines elevated in cases of intra-amniotic infection [329, 330]. Despite the large magnitude of the elevation in maternal plasma IL-6, we are not persuaded that this information can be used to distinguish between patients with and without infection in the amniotic cavity. This is in keeping with the observations of others [331, 332]. The most likely explanation for this is that IL-6 is a mediator of the acute phase response, and therefore, its concentration is elevated in the presence of an inflammatory insult unrelated to infection [87, 333, 334]. For example, the maternal plasma concentration of IL-6 is elevated in patients with spontaneous labor at term [335–344] without proven infection, which was assessed by amniotic fluid analysis.

In contrast to maternal plasma, amniotic fluid IL-6 concentrations are of value in the identification of the patient at risk for preterm delivery [112, 116–119, 122, 123, 169, 211, 212] or neonatal complications [112, 116, 119, 122, 123, 214, 345, 346], and are superior to amniotic fluid white blood cell counts, glucose concentrations, or Gram stains [331, 347–353]. Recent studies suggest that amniotic fluid IL-6 analysis is equivalent to other techniques, such as proteomic analysis of amniotic fluid [353], and rapid assays are now available which provide results within 20 min [120, 124, 125, 354–356]. These assays allow IL-6 to be used as a point-of-care test [120, 124, 125, 354–356].

Conclusion

We demonstrate, for the first time, that patients with clinical chorioamnionitis at term have higher maternal plasma concentrations of pyrogenic cytokines identified thus far (IL-1 β , IL-2, IL-6, IFN- γ , and TNF- α) than patients in spontaneous labor at term without a fever. However, among patients with clinical chorioamnionitis, the absolute concentration of cytokines cannot be used to identify those who have bacteria in the amniotic fluid. This suggests that amniotic fluid assessment is required to identify intra-amniotic inflammation. Recently, a transcervical amniotic

fluid collector was described that showed promising results in the analysis of the amniotic fluid of patients with preterm PROM [357]. This device may be of value to a subset of patients who will benefit from anti-inflammation or antibiotic treatment.

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