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CELLULAR IMMUNE RESPONSES IN AMNIOTIC FLUID OF WOMEN WITH A SONOGRAPHIC SHORT CERVIX

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Abstract

Background: A sonographic short cervix is one of the strongest predictors of a preterm delivery. However, the cellular immune composition of amniotic fluid in women with a short cervix has not yet been described. Herein, we determined cellular and soluble immune responses in amniotic fluid from pregnant women with a mid-trimester asymptomatic short cervix.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

Methods: Amniotic fluid samples (n=77) were collected from asymptomatic women with a cervical length between 15–25 mm (n=36, short cervix) or < 15 mm (n=41, severely short cervix) diagnosed by ultrasound. Flow cytometry and multiplex measurement of cytokines/chemokines were performed.

Results: 1) the cellular immune composition of amniotic fluid was not different between women with a severely short cervix (< 15 mm) and those with a short cervix 15–25 mm; 2) amniotic fluid concentrations of multiple cytokines/chemokines were higher in women with a severely short cervix (< 15 mm) than in those with a short cervix 15–25 mm; 3) the cellular immune composition of amniotic fluid was not different between women with a severely short cervix (< 15 mm) who ultimately underwent preterm delivery compared to those who delivered at term; and 4) amniotic fluid concentrations of IL-2, but not other immune mediators, were increased in women with a severely short cervix (< 15 mm) who ultimately delivered preterm compared to those who delivered at term.

Conclusion: Women with a severely short cervix (< 15 mm) have increased concentrations of pro-inflammatory mediators in the amniotic cavity; yet, this is not translated to changes in the cellular immune response.

Keywords

Amniotic fluid; cytokines; chemokines; immune cells; intra-amniotic inflammation; preterm

INTRODUCTION

Preterm birth is one of the great obstetrical syndromes (1–4) and a primary cause of perinatal morbidity and mortality worldwide (5, 6). Among the known risk factors that predispose to preterm birth, a sonographic short cervix is one of the strongest predictors (2, 7–23). Indeed, an asymptomatic woman with a mid-trimester cervical length of < 15 mm has over a 50% chance of undergoing spontaneous preterm birth at 32 weeks or less (13, 24). Similarly, 50% of women diagnosed with a short cervix at 16–22 weeks will deliver at 32 weeks or less (25). Therefore, the shorter the sonographic cervical length, the higher is the risk of spontaneous preterm labor and birth (2, 7–19, 23).

A short cervix can be considered a syndrome caused by multiple pathological processes (1, 17, 26, 27), including prior cervical surgery (28–35), congenital disorders (e.g. cervical hypoplasia after diethylstilbestrol exposure) (36–40), genetic syndromes (e.g. Ehlers-Danlos syndrome) (41, 42), progesterone deficiency (17, 26, 27), vaginal microbiome composition (43–45) and, more importantly, intra-amniotic inflammation and/or infection (19, 24, 25, 46–51) - the well-known etiology for preterm birth (1, 2, 18, 52–62). Recently, we used a combination of cultivation and molecular microbiological techniques to establish the nature of the inflammatory process in the amniotic cavity of women with a sonographic short cervix (19). Most women with a short cervix do not have detectable microorganisms or intra-amniotic inflammation [elevated concentrations of interleukin-6 > 2.6 ng/mL (63)] (19). Yet, a fraction of these women have intra-amniotic inflammation in the absence of detectable microorganisms (19), a clinical condition that is referred to as sterile intra-amniotic inflammation (19, 61, 64–71). Importantly, sterile intra-amniotic inflammation is more

frequently observed than proven intra-amniotic infection in women with a short cervix (19, 25). Hence, the clinical condition of a short cervix can occur in the presence or absence of intra-amniotic inflammation/infection and, therefore, may involve diverse immune responses in the amniotic cavity.

The cellular immune responses in the amniotic cavity have been recently characterized during normal pregnancy (72) and in complications such as clinical chorioamnionitis at term (73), preterm labor with intact membranes (74), preterm clinical chorioamnionitis (75), and preterm prelabor rupture of membranes (PPROM) (76). However, the cellular immune composition of the amniotic fluid in women with a short cervix has not yet been described. Increased amniotic fluid concentrations of several cytokines and other soluble factors (e.g. complement) have been linked to a short cervix and/or cervical insufficiency (25, 49, 51, 77, 78). Indeed, the cytokine network of women with a short cervix who delivered preterm includes elevated concentrations of pro-inflammatory cytokines such as MIP-1 α , MCP-1, and IL-6 (25). Hence, in the current study we determined both the cellular immune composition and cytokine/chemokine concentrations in amniotic fluid from women with a mid-trimester asymptomatic short cervix.

METHODS

Study design and population:

This cross-sectional study included patients who underwent amniocentesis due to clinical indications and amniotic fluid samples were immediately processed for immunophenotyping by flow cytometry. The collection of samples was approved by the Institutional Review Boards of the Detroit Medical Center, Wayne State University, and the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (Detroit, MI, USA). All women provided written informed consent prior to the collection of amniotic fluid.

This study included 77 amniotic fluid samples collected from asymptomatic women with a short cervix diagnosed by ultrasound (see clinical definitions and amniotic fluid sample collection below). Women were divided into two groups: those with a cervical length 15–25 mm (n=36) (hereafter referred to as a short cervix) and those with ≤ 15 mm (n=41) (hereafter referred to as a severely short cervix) (Table 1). For all patients, the amniocentesis was performed at the time of diagnosis. The demographic and clinical characteristics of the study population are shown in Table 1. Patients with multiple pregnancies, preterm prelabor rupture of membranes (pPROM) or spontaneous preterm labor occurring prior to diagnosis with a short cervix, fetal malformations, or genetic disorders were excluded from this study. In addition, women with positive amniotic fluid bacterial cultures were also excluded.

Clinical definitions

Gestational age was determined by the date of the last menstrual period and confirmed by ultrasound examination. The gestational age derived from sonographic fetal biometry was used if the estimation was inconsistent with menstrual dating. Spontaneous preterm labor

was diagnosed by the presence of regular uterine contractions (at least two contractions every 10 minutes) associated with cervical changes in patients <37 weeks of gestation. Intra-amniotic inflammation was diagnosed when the interleukin-6 (IL-6) amniotic fluid concentration was ≥ 2.6 ng/mL (63, 79).

Placental histopathological examination

Placentas were examined histologically by perinatal pathologists blinded to clinical diagnoses and obstetrical outcomes according to standardized Perinatology Research Branch protocols (80, 81). Briefly, three to nine sections of the placenta were examined, and at least one full-thickness section was taken from the center of the placenta; others were taken randomly from the placental disc. Acute inflammatory lesions of the placenta (maternal inflammatory response and fetal inflammatory response) were diagnosed according to established criteria including staging and grading (80, 82). The proportions of patients whose placentas presented acute maternal and/or fetal inflammatory responses are displayed in Table 1.

Amniotic fluid sample collection

Amniotic fluid samples were collected to detect intra-amniotic inflammation and/or infection in patients with a short cervix. Samples of amniotic fluid were transported to the laboratory in a sterile, capped syringe and immunophenotyping by flow cytometry was performed immediately. The rest of the sample was centrifuged at $1300 \times g$ for 10 minutes at 4°C , and the supernatant was stored at -80°C until use. Additionally, an aliquot of amniotic fluid was transported to the clinical laboratory for culture of aerobic/anaerobic bacteria and genital mycoplasmas. The clinical laboratory also determined the amniotic fluid white blood cell (WBC) count (83), performed a Gram stain (84), and determined the amniotic fluid glucose concentration (85).

Determination of IL-6 concentration in amniotic fluid

Amniotic fluid concentrations of IL-6 were determined as previously established (63) using a sensitive and specific enzyme immunoassay obtained from R&D Systems (Minneapolis, MN, USA). The IL-6 concentrations were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation for IL-6 were 8.7% and 4.6%, respectively. The sensitivity of the IL-6 assay was 0.09 pg/mL.

Multiplex determination of inflammatory-related proteins

Amniotic fluid samples were assessed using sensitive and specific V-PLEX immunoassays (Meso Scale Discovery, Gaithersburg, MD, USA) to measure amniotic fluid concentrations of several immune mediators: the Pro-inflammatory Panel 1 kit [Cat# K15049D (IL-1 β , TNF α , IL-8, IL-12p70, IFN γ , IL-2, IL-4, IL-10, IL-13)], and Chemokine Panel 1 kit [Cat# K15047D; MCP-1 (CCL2), MIP-1 α (CCL3), MIP-1 β (CCL4), MCP-4 (CCL13), MDC (CCL22), IP-10 (CXCL10), TARC (CCL17), Eotaxin (CCL11), Eotaxin-3 (CCL26)], according to the manufacturer's instructions. The plate signals were read using the QuickPlex SQ 120 (Meso Scale Discovery). Standard curves were generated and the assay values of the samples were interpolated from the curves. The detection limits of the assays

were 0.05 pg/mL (IL-1 β), 0.04 pg/mL (TNF- α), 0.07 pg/mL (IL-8), 0.11 pg/mL (IL-12p70), 0.37 pg/mL (IFN- γ), 0.09 pg/mL (IL-2), 0.02 pg/mL (IL-4), 0.04 pg/mL (IL-10), 0.24 pg/mL (IL-13), 0.09 pg/mL (MCP-1), 3.02 pg/mL (MIP-1 α), 0.17 pg/mL (MIP-1 β), 1.69 pg/mL (MCP-4), 1.22 pg/mL (MDC), 0.37 pg/mL (IP-10), 0.22 pg/mL (TARC), 3.26 pg/mL (Eotaxin), 1.77 pg/mL (Eotaxin-3).

Immunophenotyping by flow cytometry

Amniotic fluid samples (0.5–1 mL) were centrifuged at $300 \times g$ for 5 minutes at room temperature. The resulting amniotic fluid pellet was resuspended in 1 mL of 1X phosphate-buffered saline (PBS) (Life Technologies, Grand Island, NY, USA) and stained with BD Horizon Fixable Viability Stain 510 dye (BD Biosciences, San Jose, CA, USA). Cells were washed in 1X PBS and incubated with 20 μ L of human FcR blocking reagent (Miltenyi Biotec, San Diego, CA, USA) in 80 μ L of stain buffer (BD Biosciences) for 10 minutes at 4°C. Next, cells were incubated with extracellular fluorochrome-conjugated anti-human monoclonal antibodies for 30 minutes at 4°C in the dark (Supplementary Table 1). Stained cells were then washed with 1X PBS, resuspended in 0.5 mL of stain buffer, and acquired using the BD LSR II or LSRFortessa Flow Cytometer (BD Bioscience) and BD FACSDiva 6.0 software (BD Bioscience). The analysis was performed and the figures were generated using the FlowJo version 10 software (FlowJo, Ashland, OR, USA). The absolute number of cells was determined using CountBright absolute counting beads (Molecular Probes, Eugene, OR, USA).

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). For patient demographics, the Mann-Whitney *U*-test was used to compare continuous variables and the Fisher's exact test was used for nominal variables. The Mann-Whitney *U*-test was performed to compare the number of amniotic fluid immune cells and cytokine concentrations between the two study groups. A *p*-value <0.05 was considered statistically significant for all tests.

RESULTS

Characteristics of the study population

The clinical and demographic characteristics of the study population are shown in Table 1. A total of 77 amniotic fluid samples were collected from asymptomatic women with a short cervix diagnosed using ultrasound. Thirty-six women had a cervical length 15–25 mm (hereafter referred to as a short cervix) and 41 had a cervical length ≤ 15 mm (hereafter referred to as a severely short cervix). Women with a severely short cervix had significantly higher amniotic fluid concentrations of IL-6 and consequently a higher rate of intra-amniotic inflammation than those with a short cervix (Table 1). In addition, women with a cervical length ≤ 15 mm delivered at an earlier gestational age and their newborns had a lower birthweight than those women who had a cervical length 15–25 mm (Table 1). No differences in maternal age, body mass index, race, gestational age at amniocentesis, amniotic fluid glucose concentrations, or WBC counts were found between the study groups. No differences in the treatment received by women with a short cervix were found

between the study groups (Table 1). Patients with a cervical length ≤ 15 mm had a greater prevalence of severe maternal inflammatory responses in the placenta (i.e. stage 3) than those with a cervical length 15–25 mm (Table 1). Yet, no differences were observed in acute fetal inflammatory responses between the study groups (Table 1).

Amniotic fluid cellular and soluble immune responses in women with a short cervix

First, we determined the cellular immune composition of amniotic fluid using multi-color flow cytometry. Viable immune cell populations were identified using previously reported gating strategies (72–76). The following immune cell types are reported: total leukocytes (CD45+ cells), neutrophils (CD45+CD15+CD14– cells), monocytes/macrophages (CD45+CD15–CD14+ cells), B cells (CD45+CD15–CD14–CD3–CD19+ cells), and T cells (CD45+CD15–CD14–CD3+CD19– cells). T cells were further identified as CD4+ T cells (CD3+CD4+CD8– cells) or CD8+ T cells (CD3+CD4–CD8+ cells).

No differences were found in the overall number of amniotic fluid leukocytes in women with a cervical length ≤ 15 mm compared to those with a cervical length 15–25 mm (Figure 1A). Similarly, no differences were found in the numbers of amniotic fluid innate immune cells (neutrophils and monocytes/macrophages) between the study groups (Figure 1B&C). Lastly, the numbers of adaptive immune cells (T cells and B cells) in amniotic fluid were not different between the study groups (Figure 1D–G). These results show that women with a severely short cervix (≤ 15 mm) do not have a different cellular immune composition than those with a short cervix (15–25 mm).

Given this negative result, we next evaluated whether the concentrations of cytokines/chemokines in amniotic fluid were different between women with a short cervix and those with a severely short cervix. Sixty-three amniotic fluid samples were available to evaluate inflammatory mediator concentrations. Amniotic fluid concentrations of IL-1 β (Figure 2A), TNF- α (Figure 2B), IL-8 (Figure 2C), IL-12p70 (Figure 2D), IL-2 (Figure 2F), IL-4 (Figure 2G), IL-10 (Figure 2H), IL-13 (Figure 2I), MCP-1 (Figure 2J), MIP-1 α (Figure 2K), MIP-1 β (Figure 2L), MCP-4 (Figure 2M), and eotaxin (Figure 2Q) were higher in women with a cervical length ≤ 15 mm than in those with a cervical length 15–25 mm. No differences were found in the amniotic fluid concentrations of IFN- γ (Figure 2E), MDC (Figure 2N), IP-10 (Figure 2O), TARC (Figure 2P) and eotaxin-3 (Figure 2R) between the study groups. These findings demonstrate that a severely short cervix (≤ 15 mm) is accompanied by elevated concentrations of pro- and anti-inflammatory mediators in the amniotic cavity compared to those with a short cervix (15–25 mm).

Amniotic fluid soluble and cellular immune responses in women with a severely short cervix (≤ 15 mm) who delivered preterm or at term

Thus far, we showed that women with a severely short cervix (≤ 15 mm) had elevated concentrations of pro-inflammatory mediators in amniotic fluid. Hence, we focused on this patient group and determined whether women who delivered preterm could be differentiated from those who delivered at term based on amniotic fluid cellular immune composition and/or soluble immune mediator concentrations. Interestingly, the amniotic fluid cellular immune composition was not different between women with a severely short cervix who

ultimately delivered preterm compared to those who delivered at term (Figure 3A–G). Among the 18 amniotic fluid cytokines/chemokines evaluated, only IL-2 concentrations were significantly elevated in women with a severely short cervix who ultimately delivered preterm compared to those who delivered at term (Figure 4F). No other differences were found in the amniotic fluid cytokine/chemokine concentrations between women with a severely short cervix who ultimately delivered preterm and those who delivered at term (Figure 4A–E, G–R). Taken together, these results show that the cellular and soluble immune responses in the amniotic cavity are not altered in women with a severely short cervix who are at high risk of preterm birth compared to those who will deliver at term.

DISCUSSION

The principal findings of the current study are: 1) the cellular immune composition of amniotic fluid was not different between women with a severely short cervix (< 15 mm) and those with a short cervix 15–25 mm; 2) amniotic fluid concentrations of multiple cytokines/chemokines were higher in women with a severely short cervix (< 15 mm) than in those with a short cervix 15–25 mm; 3) the cellular immune composition of amniotic fluid was not different between women with a severely short cervix (< 15 mm) who ultimately underwent preterm delivery compared to those who delivered at term; and 4) amniotic fluid concentrations of IL-2, but not other immune mediators, were increased in women with a severely short cervix (< 15 mm) who ultimately delivered preterm compared to those who delivered at term. Collectively, these findings show that women with a severely short cervix (< 15 mm) have increased concentrations of immune mediators in the amniotic cavity compared to those with a short cervix 15–25 mm; yet, this is not translated to changes in the cellular immune composition.

Herein, we found that women with a severely short cervix (< 15 mm) had higher amniotic fluid concentrations of multiple soluble mediators than those with a short cervix 15–25 mm. This finding is consistent with previous reports focused on the cytokine responses in amniotic fluid of women with a short cervix (25, 49, 51, 77, 78). Yet, this elevated cytokine profile in the amniotic fluid was not accompanied by a concomitant increase in the cellular immune composition. This result has two potential explanations: 1) some women with a short cervix have elevated concentrations of IL-6 (some of these cases may be due to sterile intra-amniotic inflammation) and low white blood cell counts (19); or 2) some women with a short cervix have increased concentrations of specific inflammatory mediators (independent of increased IL-6 or related inflammatory mediators) and low white blood cell counts (86). These two scenarios occur in the absence of culturable microorganisms in amniotic fluid because we excluded cases with positive microbiological cultures; therefore, immune cells are increased when viable microbes invade the amniotic cavity. For example, an enhanced cellular immune response is commonly observed in cases of microbial invasion of the amniotic cavity accompanied by intra-amniotic inflammation (i.e. intra-amniotic infection) (73–76). This intra-amniotic inflammatory response is characterized by the massive invasion of maternal and/or fetal immune cells such as neutrophils and monocytes/macrophages (87, 88), which enter the amniotic fluid to facilitate host defense against invaders. Neutrophils in the amniotic cavity possess an arsenal of weapons for combating microorganisms including the formation of neutrophil extracellular traps (NETS) (89),

phagocytosis (90), production of reactive oxygen species (91), and by degranulation, which leads to the release of anti-microbial molecules such as elastase (92–94), alpha-defensins (94–97), lactoferrin (98), pentraxin-3 (99), myeloperoxidase (94, 100, 101), cathelicidin (94, 100) and cathepsin G (94, 102). Monocytes/macrophages in the amniotic cavity participate in host responses by releasing massive amounts of pro-inflammatory mediators such as IL-1 β (73, 75). Moreover, macrophage extracellular traps (METs) have also been detected in the placental tissues upon infection with *Streptococcus agalactiae* (103). Our unpublished data also suggest that monocytes/macrophages orchestrate inflammasome activation in the amniotic cavity of women with intra-amniotic infection. In conclusion, increased cellular immune responses in the amniotic cavity are largely governed by the presence of viable microorganisms, and in the specific context of a sonographic short cervix inflammation is characterized by high concentrations of cytokines/chemokines without an exacerbated cellular immune response.

Interestingly, women with a severely short cervix (< 15 mm) who ultimately delivered preterm had elevated amniotic fluid concentrations of IL-2 compared to those who delivered at term. Interleukin-2 is a cytokine released upon T-cell activation (104) and has been previously shown to be increased in the amniotic cavity of women with a short cervix (25). This cytokine is also increased in amniotic fluid of women with intra-amniotic inflammation and/or infection who delivered preterm (86, 105, 106). Recently, we have also shown that fetal T cells found in amniotic fluid can express IL-2 upon stimulation (86). In addition, amniotic fluid concentrations of IL-2 tend to increase in women with idiopathic preterm labor and birth compared to those who had an episode of preterm labor but delivered at term (86). Taken together, these data suggest that IL-2, and potentially T-cell activation processes, are involved in the pathophysiology of preterm labor and birth in women with a severely short cervix.

Despite the absence of an augmented cellular immune response, women with a severely short cervix had an elevated pro-inflammatory cytokine/chemokine profile in the amniotic cavity. The most likely source of these cytokines is the fetal tissues. For example, it is likely that in women with a severely short cervix (< 15 mm) the chorioamniotic membranes are under greater tension than those of women with a short cervix 15–25 mm. This increased tensile force on the chorioamniotic membranes results in the release of several inflammatory mediators into the amniotic cavity (107). Indeed, it has been proposed that the cervical zone of the chorioamniotic membranes displays a unique inflammatory profile, which is different from that of the middle and peri-placental zones (108–115). However, whether the chorioamniotic membranes from women with a severely short cervix (who are at higher risk of preterm delivery) display a different cytokine profile than those from women with a short cervix 15–25 mm requires further investigation.

It is worth mentioning this descriptive cross-sectional study does not allow us to determine causal links between intra-amniotic inflammation and a sonographic short cervix. It would be interesting to investigate whether inflammatory stimuli (e.g. bacteria) can induce cervical shortening or whether a short cervix leads to intra-amniotic inflammation. It is also likely that a short cervix is not accompanied by intra-amniotic inflammation, highlighting the syndromic nature of this clinical entity.

A limitation of this study is that we could not include gestational age-matched controls with a normal cervical length (>25 mm). This is due to the fact that cervical length measurement is not routinely performed in women who underwent genetic amniocentesis. However, the aim of this study was to compare cellular and soluble immune responses among women diagnosed with a sonographic short cervix.

CONCLUSION

In the current study, we report that the cellular immune composition of amniotic fluid was not different between women with a severely short cervix (< 15 mm) and those with a short cervix 15–25 mm. Yet, amniotic fluid concentrations of multiple cytokines/chemokines were higher in women with a severely short cervix (< 15 mm) than in those with a short cervix 15–25 mm. Interestingly, amniotic fluid concentrations of IL-2, but not other immune mediators, were increased in women with a severely short cervix (< 15 mm) who ultimately delivered preterm compared to those who delivered at term. These findings provide evidence that women with a severely short cervix (< 15 mm) have increased concentrations of pro-inflammatory mediators in the amniotic cavity; yet, this is not translated to changes in the cellular immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cellular immune composition of amniotic fluid.

Numbers of (A) total leukocytes (CD45+ cells/mL), (B) neutrophils (CD15+ cells/mL), (C) monocytes/macrophages (CD14+ cells/mL), (D) B cells (CD19+ cells/mL), (E) total T cells (cells/mL), (F) CD4+ T cells (cells/mL) and (G) CD8+ T cells (cells/mL) in amniotic fluid from women with a short cervix with a cervical length 15–25 mm or ≤15 mm. N = 36–41 per group. Midlines = median, boxes = interquartile ranges, and whiskers = minimum/maximum ranges.

Figure 2. Immune mediators in amniotic fluid.

Amniotic fluid concentrations of cytokines [(A) IL-1 β (pg/mL), (B) TNF- α (pg/mL), (C) IL-8 (pg/mL), (D) IL-12p70 (pg/mL), (E) IFN- γ (pg/mL), (F) IL-2 (pg/mL), (G) IL-4 (pg/mL), (H) IL-10 (pg/mL), (I) IL-13 (pg/mL)] and chemokines [(J) MCP-1 (pg/mL), (K) MIP-1 α (pg/mL), (L) MIP-1 β (pg/mL), (M) MCP-4 (pg/mL), (N) MDC (pg/mL), (O) IP-10 (pg/mL), (P) TARC (pg/mL), (Q) eotaxin (pg/mL), and (R) eotaxin-3 (pg/mL)] in women with a short cervix with a cervical length 15–25 mm or ≤ 15 mm. N = 29–34 per group. Midlines = median, boxes = interquartile ranges, and whiskers = minimum/maximum ranges.

Figure 3. Cellular immune composition of amniotic fluid from women with a severely short cervix.

Numbers of (A) total leukocytes (CD45+ cells/mL), (B) neutrophils (CD15+ cells/mL), (C) monocytes/macrophages (CD14+ cells/mL), (D) B cells (CD19+ cells/mL), (E) total T cells (cells/mL), (F) CD4+ T cells (cells/mL) and (G) CD8+ T cells (cells/mL) in amniotic fluid from women with a cervical length ≤ 15 mm who delivered preterm or at term. N = 14–27 per group. Midlines = median, boxes = interquartile ranges, and whiskers = minimum/maximum ranges.

Figure 4. Immune mediators in amniotic fluid of women with a severely short cervix.

Amniotic fluid concentrations of cytokines [(A) IL-1 β (pg/mL), (B) TNF- α (pg/mL), (C) IL-8 (pg/mL), (D) IL-12p70 (pg/mL), (E) IFN- γ (pg/mL), (F) IL-2 (pg/mL), (G) IL-4 (pg/mL), (H) IL-10 (pg/mL), (I) IL-13 (pg/mL)] and chemokines [(J) MCP-1 (pg/mL), (K) MIP-1 α (pg/mL), (L) MIP-1 β (pg/mL), (M) MCP-4 (pg/mL), (N) MDC (pg/mL), (O) IP-10 (pg/mL), (P) TARC (pg/mL), (Q) eotaxin (pg/mL), and (R) eotaxin-3 (pg/mL)] in women with a cervical length ≤ 15 mm who delivered preterm or at term. N = 13–21 per group. Midlines = median, boxes = interquartile ranges, and whiskers = minimum/maximum ranges.

Table 1.

Clinical and demographic characteristics of women with an asymptomatic short cervix.

	>15 mm (n=36)	15 mm (n=41)	p-value
Maternal age (years; median [IQR]) ^a	25 (21.8–30)	26 (22–31)	0.4
Body mass index (kg/m ² ; median [IQR]) ^a	27 (23.3–31.8) ^c	31.8 (27–34.9)	0.09
Primiparity ^b	11.1% (4/36)	9.8% (4/41)	1.0
Race/ethnicity ^b			0.4
African-American	88.9% (32/36)	82.9% (34/41)	
White	2.8% (1/36)	9.8% (4/41)	
Other	8.3% (3/36)	7.3% (3/41)	
Gestational age at amniocentesis (weeks; median [IQR]) ^a	20.9 (20–24.3)	22.1 (20.7–23.1)	0.5
Sonographic cervical length at diagnosis (millimeters; median [IQR]) ^a	21 (18–22.3)	6 (0–12)	<0.001
IL-6 (ng/mL; median [IQR]) ^a	0.3 (0.2–0.7)	0.7 (0.2–3.5)	0.02
Amniotic fluid glucose (mg/dl; median [IQR]) ^a	29 (24–37) ^c	35.5 (24.3–41) ^c	0.3
Amniotic fluid WBC (cells/mm ³ ; median [IQR]) ^a	1 (0–4) ^e	2 (0–5.5) ^d	0.8
Intra-amniotic inflammation ^b	2.8% (1/36)	29.3% (12/41)	0.002
Treatment ^b			
Cerclage	11.1% (4/36)	14.6% (6/41)	0.7
Progesterone	27.8% (10/36)	29.3% (12/41)	1.0
Cerclage and progesterone	41.7% (15/36)	34.1% (14/41)	0.6
No treatment	19.4% (7/36)	22% (9/41)	1.0
Gestational age at delivery (weeks; median [IQR]) ^a	38.4 (36.1–39.4)	35.9 (25.1–37.4)	<0.001
Cesarean section ^b	16.7% (6/36)	39% (16/41)	0.04
Birthweight (grams; median [IQR]) ^a	2935 (2425–3300)	2475 (740–3185)	0.04
Acute maternal inflammatory response ^b			
Stage 1 (Early acute subchorionitis or chorionitis)	17.2% (5/29) ^g	10.8% (4/37) ^f	0.4
Stage 2 (Acute chorioamnionitis)	17.2% (5/29) ^g	24.3% (9/37) ^f	0.55
Stage 3 (Necrotizing chorioamnionitis)	3.4% (1/29) ^g	24.3% (9/37) ^f	0.03
Acute fetal inflammatory response ^b			
Stage 1 (Chorionic vasculitis or umbilical phlebitis)	27.6% (8/29) ^g	18.9% (7/37) ^f	0.55
Stage 2 (Umbilical arteritis)	10.3% (3/29) ^g	21.6% (8/37) ^f	0.3
Stage 3 (Necrotizing funisitis)	0% (0/29) ^g	10.8% (4/37) ^f	0.1

Data are given as median (interquartile range, IQR) and percentage (n/N).

^aMann-Whitney U test,

^bFisher's exact test,

^cOne missing data,

^dTwo missing data,

^eThree missing data,

^fFour missing data,

^gSeven missing data.

Abbreviations: WBC, white blood cells.

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