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European Journal of Obstetrics and Gynecology 272 (2022) 24–29

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.journals.elsevier.com/european-journal-of-obstetrics-and-gynecology-and-reproductive-biology

Calprotectin levels in amniotic fluid in relation to intra-amniotic inflammation and infection in women with preterm labor with intact membranes: A retrospective cohort study

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ARTICLE INFO

Keywords:
Intra-amniotic inflammation
Intra-amniotic infection
IL-6
Calprotectin
Spontaneous preterm labor with intact membranes

ABSTRACT

Objective: To evaluate the concentrations of calprotectin in amniotic fluid with respect to intra-amniotic inflammation and infection and to assess the presence or absence of bacteria in the amnio-chorionic niche with respect to presence or absence of intra-amniotic inflammation.

Study design: Seventy-nine women with singleton pregnancies and preterm labor with intact membranes (PTL) were included in the study. Amniotic fluid was collected at the time of admission by amniocentesis and calprotectin levels were analyzed from frozen/thawed samples using ELISA. Interleukin (IL)-6 concentration was measured by point-of-care test. Samples from amniotic fluid and the amnio-chorionic niche (space between amniotic and chorionic membranes) were microbiologically analyzed. Microbial invasion of the amniotic cavity (MIAC) was diagnosed based on a positive PCR result for Ureaplasma species, Mycoplasma hominis, 16S rRNA or positive culture. Intra-amniotic inflammation (IAI) was defined as amniotic fluid point-of-care IL-6 concentration $\geq$ 745 pg/mL. The cohort of included women was divided into 4 subgroups based on the presence or absence of IAI/MIAC; i) intra-amniotic infection, ii) sterile IAI, iii) intra-amniotic colonization and iv) neither MIAC nor IAI.

Results: Women with intra-amniotic infection had a significantly higher intra-amniotic calprotectin concentration (median; 101.6 μg/mL) compared with women with sterile IAI (median; 9.2 μg/mL), women with intra-amniotic colonization (median; 2.6 μg/mL) and women with neither MIAC nor IAI (median 4.6 μg/mL) ($p = 0.001$). Moreover, significantly higher amniotic fluid calprotectin concentration was seen in women who delivered within 7 days ($p = 0.003$). A significant negative correlation was found between amniotic fluid calprotectin and gestational age at delivery (rho = 0.32, $p = 0.003$). Relatively more bacteria in the amnio-chorionic niche were found in the sterile IAI group compared with the other groups.

Conclusions: Calprotectin concentrations in amniotic fluid were significantly higher in the intra-amniotic infection group compared with the other groups. Moreover, the bacterial presence in the amnio-chorionic niche was higher in IAI group.

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https://doi.org/10.1016/j.ejogrb.2022.03.006
Received 23 January 2022; Accepted 1 March 2022
Available online 4 March 2022

Introduction

Preterm delivery (PTD; <37 gestational weeks) is the leading cause of death in children under the age of five [1–3]. It is also associated with a substantial health burden in terms of perinatal and long-term morbidity [3,4]. The World Health Organization (WHO) estimates a yearly incidence of 15 million preterm born children [1–3]. In some low- and middle-income countries, the rate of PTD is around 12%, compared to 9% in higher income countries [5,6]. In Scandinavia, PTD rates have been stable, around 5–7%, for the past three decades [3,7].

Preterm labor with intact membranes (PTL) accounts for 40–45% of all PTD and is defined as regular contractions together with cervical changes [8]. A complex network of different overlapping pathophysiological pathways, including molecular and genetic factors, contributes to the occurrence of PTL [9–11]. Intra-amniotic inflammation (IAI), defined as presence of intra-amniotic inflammatory mediators in amniotic fluid, is accompanied in most PTL cases [12]. This inflammation may be triggered by microbial invasion of the amniotic cavity (MIAC), called intra-amniotic infection or other non-microbial immunologically mediated processes, defined as sterile IAI. Sterile IAI is determined by elevated concentrations of amniotic fluid interleukin (IL)-6 in the absence of MIAC [13–15]. The mechanism of sterile IAI is still not completely understood but damage and infection of the fetal membranes and placenta are believed to be among the inducing factors [13,14,16]. During infection and inflammation leukocytes invade amniotic cavity, neutrophils and macrophages being the most frequent. Their amount differs depending on the phenotype of inflammation and they are more abundant in infection compared to sterile IAI [17].

Endogenous molecules called alarmins are released from leukocytes, triggering inflammatory responses through pattern recognition receptors (PRR) [13,14,16]. Calprotectin is one of the proteins, secreted from cytosol of immunocompetent cells. It is widely used in the follow-up of inflammatory processes [18–24] and has even previously been associated with both IAI and spontaneous PTD [25]. The number of immune cells differs among sterile IAI and intra-amniotic infection [17,26–29] but there is no evidence regarding concentrations of calprotectin in these phenotypes of IAI. Therefore, the aim of this study was to evaluate amniotic fluid calprotectin concentrations with respect to the presence and absence of sterile IAI and intra-amniotic infection. The second aim was to assess the presence of microorganisms and/or their nucleic acids in the amnio-chorionic niche with respect to the presence and absence of sterile IAI and intra-amniotic infection.

Materials and methods

In this retrospective cohort study, women that were admitted to the Department of Obstetrics and Gynecology, Sahlgrenska University Hospital/Östra, Gothenburg, Sweden, between 2008 and 2017 were approached to participate in the study. Inclusion criteria were women ≥ 18 years of age with a viable singleton pregnancy, presenting with PTL at 22^{+0} and 33^{+6} weeks of gestation. PTL was defined as regular uterine contractions at admission in combination with one of the following criteria: cervical dilatation ≥ 2 cm and/or cervical length < 25 mm (as documented by digital examination and transvaginal sonography at enrollment or at sampling). Exclusion criteria were women with uterine abnormalities, significant vaginal bleeding, severe preeclampsia, severe rheumatic disease, contractions as a result of urinary tract infection, kidney stones or pelvic inflammatory disease, genetic disease or chromosomal abnormality (including translocation, deletion and duplication), blood disease (Hepatitis B, C or HIV), active infectious disease (malaria or tuberculosis), oocyte donation, treatment with high dose of anticoagulant medications, cervical cerclage and women who were unable to provide an informed consent due to language barriers. Cases of fetal distress, imminent delivery and fetal abnormalities were also excluded.

Sample collection and processing

Women participating in the study underwent amniocentesis and amniotic fluid was aspirated under sterile conditions through an ultrasound guided transabdominal amniocentesis. After amniocentesis, approximately 3.5 mL of amniotic fluid was used for the detection of MIAC and assessment of IL-6 levels for the detection of IAI. The remaining amniotic fluid was further processed, where samples were aliquoted and stored either as non-centrifuged, or as centrifuged for 15 min at 2000 g at 4 °C to remove cells and debris. In the latter cases, supernatant was aspirated and further divided into aliquots while pellets were left in the original polypropylene tubes. All aliquots were stored at −80 °C until analysis. After delivery, the chorionic and amniotic membrane were separated approximately 10 cm from the initial rupture of the membranes and samples were collected using Charcoal swab and nylon swabs (E-swab 480CE, Copan, Italy).

Analysis of amniotic fluid and amnio-chorionic niche

A total of 3 mL of fresh non-centrifuged amniotic fluid was sent to the microbiology laboratory at Sahlgrenska University Hospital for aerobic and anaerobic culture, Ureaplasma species and Mycoplasma hominis analysis with polymerase chain reaction (PCR) and for pan-bacterial PCR (16S rRNA). The same analyses were performed on the swab samples from the space between the membranes and the placental amnio-chorionic niche. The detailed analytical microbiological procedures are presented in Supplement S1.

Amniotic fluid IL-6 assessment

A total of 100 μL of fresh, non-centrifuged amniotic fluid was used for individual assessment of IL-6 concentration with lateral flow immunoassay Milenia QuickLine IL-6 using the Milenia PicoScan System and POCScan systems (Milenia Biotec, GmbH, Giessen, Germany). The detection range for IL-6 was 50–10 000 pg/mL.

Amniotic fluid calprotectin assessment

Calprotectin (S100A8/S100A9 heterodimer) concentrations in amniotic fluid were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Human S100A8/100A9 Heterodimer, R&D systems, Minneapolis, Minnesota) on stored and thawed samples, according to the manufacturer's instructions. ELISA kits were chosen with a working range to include the most likely concentration ranges in amniotic fluid calprotectin. Amniotic fluid samples were analyzed in duplicate, within 24 h of thawing. The majority of the calprotectin concentrations were detectable in a dilution of 1:500 but 15 samples had to be re-analyzed in duplicates in dilutions of 1:5000 up to 1:40000. The limit of detection for amniotic fluid calprotectin concentrations were 0.97–39 ng/mL. The absorbance values were read at 450 nm using a microplate reader. Intra-assay variation was < 5%, inter-assay variation was < 6%.

Clinical management of PTL

Antenatal steroids (betamethasone, 12 mg intramuscularly in 2 doses with 24 h interval when possible) were individually administered from 23^{+0} to 33^{+6} weeks of gestation (or earlier after consultation with neonatologist). Tocolytic therapy was individually administered according to clinical routine. Women with suspected clinical chorioamnionitis, diagnosed by a combination of maternal fever and tachycardia, uterine tenderness and/or fetal tachycardia, were treated with antenatal antibiotics but not prompt delivered if asymptomatic after the treatment. Presence of IAI and MIAC were reported to the clinicians and served as a basis for the continued individual clinical management.

Clinical definitions

IAI was defined as amniotic fluid IL-6 concentration ≥ 745 pg/mL,

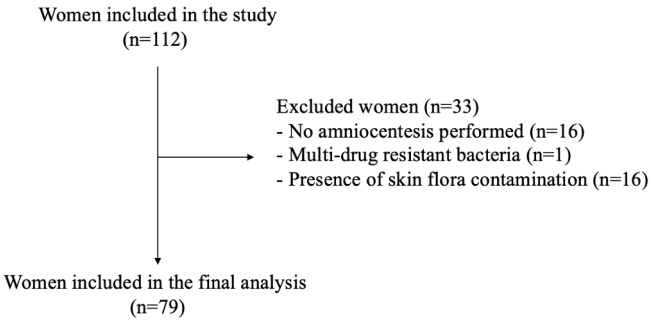

Fig. 1. Flowchart of the study.

measured by point of care (POC) test based on the data by Chaemsaithong et al [30]. MIAC was defined as positive PCR for Ureaplasma species, Mycoplasma hominis, and/or positive 16S rRNA and/or positive amniotic fluid aerobic or anaerobic culture. A positive amniotic fluid 16S rRNA for skin flora but negative culture was considered contamination. Intra-amniotic infection was defined as presence of both MIAC and IAI. Sterile IAI was defined as IAI without MIAC. Intra-amniotic colonization was defined as presence of MIAC without IAI. Microbial invasion of the membranes was defined as a positive PCR for Ureaplasma species and M. hominis and/or growth of any bacteria from the amnio-chorionic niche.

Ethics statement

The study was approved by the local ethics committee at the University of Gothenburg (Dnr 476–05, Dnr 690–11 T 704–16). Informed consent was obtained from all participants before inclusion.

Statistical analysis

Normality of data was assessed with Shapiro-Wilk test and a mainly skewed distribution was observed. Continuous variables were analyzed using Kruskal Wallis H test or Mann-Whitney *U* test. Pearson chi-square test was used for categorical variables. Spearman's partial correlation was used to adjust all results for gestational age at sampling, nulliparity and administration of prenatal antibiotics, corticosteroids and tocolysis, since those were the variables that were significantly different among groups. Spearman's correlation was used to test associations between calprotectin and IL-6 concentrations, gestational age at sampling and gestational age at delivery. Mann-Whitney *U* test was used for comparison between calprotectin concentration and delivery within 7 days. Two-sided statistical tests were used and a *p*-value of < 0.05 was considered to be statistically significant. All statistical analyses were computed using SPSS 25.0 for Mac (IBM SPSS Statistics, Armonk, NY:

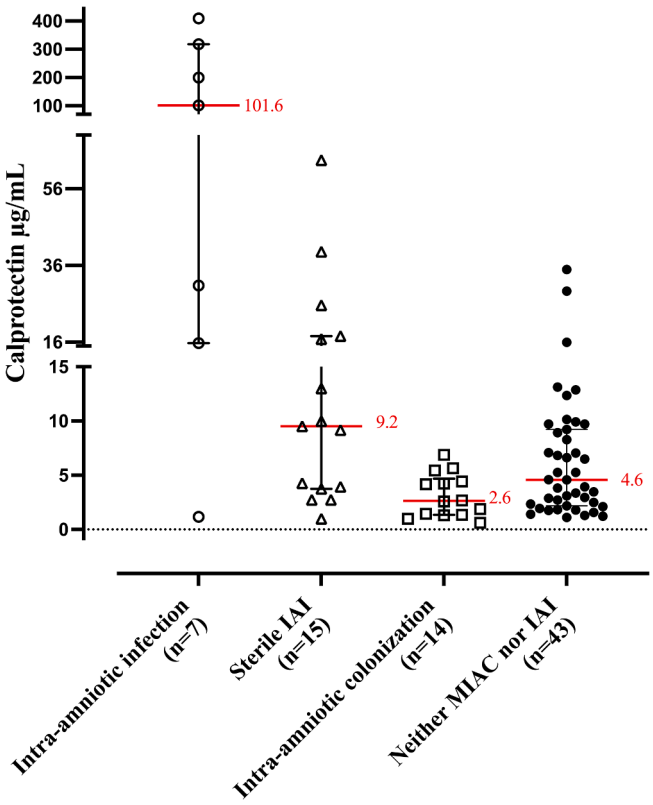

Fig. 2. Amniotic fluid calprotectin concentrations in women with PTL among the four subgroups.

Table 1
Demographics and clinical characteristics in women with spontaneous preterm labor (n = 79) among the four sub-groups.

	Intra-amniotic infection (n = 7)	Sterile IAI (n = 15)	Intra-amniotic colonization (n = 14)	Neither MIAC nor IAI (n = 43)	*p-value*
Maternal age (years)	26 (±9)	31 (±10)	31 (±6)	29 (±7)	0.109
Maternal BMI	25 (±7)	25 (±12)	24 (±9)	24 (±6)	0.737
Nulliparity	6 (86%)	11 (73%)	7 (50%)	16 (37%)	**0.024**
Smoking	0 (0%)	2 (13%)	1 (7%)	6 (14%)	0.614
Previous preterm delivery	0 (0%)	1 (7%)	2 (14%)	6 (14%)	0.642
Cervical length at sampling (mm)	0 (±8)	0 (±12)	16 (±16)	17 (±14)	**<0.001**
Gestational age at sampling (days)	163 (±40)	182 (±53)	208 (±38)	214 (±30)	**0.005**
Tocolysis before sampling	4 (57%)	13 (87%)	6 (43%)	28 (65%)	0.100
Tocolysis after sampling	4 (57%)	12 (80%)	5 (36%)	28 (65%)	0.093
Antenatal corticosteroid administration before sampling	7 (100%)	15 (100%)	13 (93%)	40 (93%)	0.653
Antenatal corticosteroid administration after sampling	4 (57%)	3 (20%)	4 (29%)	16 (37%)	0.340
Antenatal antibiotics (≤7 days from sampling)	5 (71%)	7 (47%)	3 (21%)	6 (14%)	**0.003**
Antenatal antibiotics after sampling	5 (71%)	11 (73%)	9 (64%)	23 (53%)	0.502
Gestational age at delivery (days)	173 (±39)	196 (±56)	239 (±41)	260 (±32)	**<0.001**
Delivery ≤ 7 days from sampling	6 (86%)	9 (60%)	2 (14%)	2 (5%)	**<0.001**
Delivery < 37^{+0} weeks of gestation	7 (100%)	14 (93%)	9 (64%)	19 (44%)	**0.001**
Latency (days from sampling to delivery)	1 (±3)	3 (±13)	48 (±54)	45 (±39)	**<0.001**
Calprotectin (µg/mL)	101.6 (±302.2)	9.2 (±13.9)	2.6 (±3.3)	4.6 (±7.0)	**0.001**
IL-6 (pg/mL)	10,000 (±0)	10,000 (±8304)	181 (±285)	100 (±167)	**<0.001**

Continuous variables were analyzed using Kruskal Wallis H test and presented as median (±IQR). Categorical variables were analyzed using Pearson chi-square test and presented as n (%). Statistically significant results (p-value) are marked in bold. IAI: Intra-amniotic inflammation; MIAC: Microbial invasion of the amniotic cavity.

Table 2
Comparisons of amniotic fluid calprotectin concentrations among the four subgroups of women with spontaneous preterm labor based on the presence of IAI and/or MIAC.

	Intra-amniotic infection Calprotectin: 101.6 (±302.2) μg/mL	Sterile IAI Calprotectin: 9.2 (±13.9) μg/mL	Intra-amniotic colonization Calprotectin: 2.6 (±3.3) μg/mL	Neither MIAC nor IAI Calprotectin: 4.6 (±7.0) μg/mL
Intra-amniotic infection Calprotectin: 101.6 (±302.2) μg/mL	–	***p* = 0.026; *p1* = 0.007; *p2* = 0.001**	***p* = 0.006; *p1* = 0.019; *p2* = 0.045**	***p* = 0.003; *p1* < 0.001; *p2* < 0.001**
Sterile IAI Calprotectin: 9.2 (±13.9) μg/mL		–	***p* = 0.005; *p1* = 0.017**; *p2* = 0.077	***p* = 0.042; *p1* = 0.007; *p2* = 0.008**
Intra-amniotic colonization Calprotectin: 2.6 (±3.3) μg/mL			–	***p* = 0.032**; *p1* = 0.097; *p2* = 0.059
Neither MIAC nor IAI Calprotectin: 4.6 (±7.0) μg/mL				–

Differences were assessed using Mann-Whitney *U* test (*p*-value). Data was adjusted using Spearman partial correlation for gestational age at sampling (*p*1-value) and gestational age at sampling, tocolysis, antibiotics and corticosteroids administered prior to sampling (*p*2-value). Statistically significant results are marked in bold. Calprotectin levels are presented in μg/mL as median (±IQR). IAI: Intra-amniotic inflammation; MIAC: Microbial invasion of the amniotic cavity.

IBM Crop.) and Prism 9.0.2 for Mac (GraphPad Software, LLC).

Results

Characteristics of the study population

From 2008 to 2017, 112 women with PTL were enrolled. Amniocentesis could not be performed on 16 patients due to oligohydramnios and were therefore excluded from further analysis. Another woman was excluded retrospectively due to the presence of multi-drug resistant bacteria due to laboratory routines and samples had to be removed from the freezer. Sixteen additional women were excluded from analysis as the test results showed polymicrobial growth of bacteria of skin flora, identified by 16S rRNA PCR but not any other method. This led to the changing of the antiseptic routines during amniocentesis. The remaining women (n = 79) were included in the analysis (Fig. 1) (Fig. 1 Flowchart of the study). These were divided into 4 subgroups based on the presence or absence of IAI/MIAC as follows; i) intra-amniotic infection (n = 7; 9%), ii) sterile IAI (n = 15; 19%), iii) intra-amniotic colonization (n = 14; 18%) and iv) neither MIAC nor IAI (n = 43; 54%). Demographic and clinical data for each subgroup are presented in Table 1.

Table 3
Microorganisms identified with both culture, 16S rRNA and PCR in the amniotic fluid of women with PTL in intra-amniotic infection and colonization. Ranking of microorganisms is presented by incidence.

Intra-amniotic infection	Intra-amniotic colonization
Campylobacter ureolyticus (x 2)	*Micrococcus* species (x 4)
Fusobacterium species (x 2)	*Ureaplasma* species (x 2)
Ureaplasma species	*Propionibacterium acnes* (x 2)
Prevotella ioeschii	*Moraxella* species (x 2)
Staphylococcus aureus	*Enterobacteriacae*
Aerococcus urinae	*Coagulase negative staphylococcus*
Sneathia sanguinegens	*Enterococcus* species
	Corynebacterium species
	Mycoplasma hominis

Amniotic fluid calprotectin concentrations

Amniotic fluid calprotectin concentrations were significantly different among the four subgroups (intra-amniotic infection: 101.6 ± 302.2 μg/mL; sterile IAI 9.2 ± 13.9 μg/mL; intra-amniotic colonization 2.6 ± 3.3 μg/mL; neither MIAC nor IAI 4.6 ± 7.0 μg/mL; $p = 0.001$; Table 1, Fig. 2) (Fig. 2 Amniotic fluid calprotectin concentrations in women with PTL among the four subgroups). Amniotic fluid calprotectin concentrations in women with intra-amniotic infection were higher compared to all other three subgroups in both crude and adjusted analyses (Table 2). Differences were seen when the sterile IAI group was compared to other groups; however, no difference was found when compared to intra-amniotic colonization, after adjustment for gestational age at sampling, prenatal antibiotics, tocolysis and corticosteroids prior to sampling. Moreover, no differences were observed when comparing the intra-amniotic colonization and neither MIAC nor IAI groups after adjustment. Higher amniotic fluid calprotectin concentrations were seen among women who delivered within 7 days from sampling (9.5 ± 35.7 μg/mL vs 4.1 ± 7.1 μg/mL; $p = 0.003$). A positive correlation was identified between amniotic fluid calprotectin and IL-6 concentrations (rho = 0.25, $p = 0.02$). However, a negative correlation was seen between amniotic fluid calprotectin concentrations and gestational age at delivery (rho = -0.32, $p = 0.003$). No correlation was found between amniotic fluid calprotectin concentrations and gestational age at sampling (rho = -0.08, $p = 0.48$).

Microbial invasion in the amniotic fluid and amnio-chorionic niche in the subgroups of women with PTL

Microbial species were identified both in amniotic fluid (in all women) and from the amnio-chorionic niche of the fetal membranes in a selection of the women (n = 43). Microbial species identified in the amniotic fluid are shown in Table 3, while microbial species identified in the amnio-chorionic niche of the fetal membranes are presented in Table 4. A lot of the samples from amnio-chorionic niche were not taken in the intra-amniotic colonization and neither MIAC nor IAI group because women delivered at term and were lost in the follow-up process.

Discussion

The main findings of this study were that; i) amniotic fluid calprotectin concentrations were elevated in the presence of both sterile IAI and intra-amniotic infection in pregnancies complicated with PTL, ii) amniotic fluid calprotectin concentrations were significantly higher in intra-amniotic infection compared to sterile IAI and iii) calprotectin concentrations were significantly higher in amniotic fluid of women delivered within 7 days from amniotic fluid sampling compared to delivery after 7 days.

A previous study on intra-amniotic calprotectin has shown that intra-amniotic concentration of calprotectin increases normally with gestational age and both MIAC and IAI have been shown to be associated with

Table 4
Microorganisms identified with aerobic/anaerobic cultures, PCR or 16S rRNA in the amnio-chorionic niche of women with spontaneous preterm labor who delivered vaginally among the four subgroups.

	Intra-amniotic infection (n = 7)	Sterile IAI (n = 11)	Intra-amniotic colonization (n = 6)	Neither MIAC nor IAI (n = 19)
Bacteria found in amnio-chorionic niche	*Coagulase negative staphylococcus (CoNS)* *Mycoplasma hominis*	*Ureaplasma* species *(x3)* *Mycoplasma* species *Gardenella vaginalis* *Kocuria* species *Escherichia coli*	*Acinetobacter* species *Staphylococcus aureus*	*Ureaplasma* species *(x2)* *Diphteroid rods* *Streptococcus* species *Exiguobacterium* species *Streptococcus agalactiae (GBS)*
Percentage of positive findings	29%	45%	30%	32%

IAI: Intra-amniotic inflammation; MIAC: Microbial invasion of the amniotic cavity.

increased concentrations of amniotic fluid calprotectin amniotic fluid calprotectin. Calprotectin concentrations were significantly higher in the presence of intra-amniotic infection [25]. However, in our study, a negative correlation was found between amniotic fluid calprotectin concentrations and gestational age at delivery, indicating that higher amniotic fluid calprotectin concentrations were present at delivery in earlier gestational ages. The results of our study confirm the previous findings about the association with increased levels of amniotic fluid calprotectin in the case of MIAC and IAI [25]. Furthermore, we have shown that intra-amniotic infection and not colonization is related to higher concentrations of intra-amniotic calprotectin. It seems that MIAC itself does not significantly affect calprotectin concentrations. Moreover, our study has shown that amniotic fluid calprotectin concentrations were significantly higher in intra-amniotic infection compared to sterile IAI, indicating that the inflammatory events in amniotic fluid are more intense when caused by microbial agents and not only by alarmins. It has been hypothesized that sterile IAI could be caused by inflammatory mediators originating from the space between the fetal membranes [13,14,16]. Bacteria that reside in the amnio-chorionic niche and the subsequent maternal inflammatory response, triggered by this microbial presence, could be the factor that cause inflammation without any bacteria found in the amniotic fluid.

A strength with this study was that the presence of MIAC was assessed with a combination of microbial cultures and specific PCR (Ureaplasma species, Mycoplasma hominis, and evaluation of the 16S RNA gene) limiting the possibility of falsely including bacterial species that are normally part of the skin flora. A new routine was introduced to the clinical ward after finding out that a lot of our early samples were contaminated with skin flora. An important limitation of the study is the relatively small cohorts of respective subgroups of women with PTL which decrease the generalizability of the study conclusions. Furthermore, only women who understood the Swedish language were enrolled in the study, which limits the ethnical variability in the cohort. It is well known that spontaneous PTD rates vary between ethnicities, and this may have an effect on the generalization of the study results [31]. Finally, the microbiological analytical methods changed remarkably during the duration of the study. The initial microbiological analyses were based on a manual extraction method and conventional PCR, whereas automatic extraction methods and real-time PCR were used from 2018, which has improved the sensitivity of the method.

Conclusions

Calprotectin concentrations in amniotic fluid were elevated in both sterile IAI and intra-amniotic infection, where concentrations were higher in cases of intra-amniotic infection. Relatively more bacteria in the amnio-chorionic niche were found in the sterile IAI group compared with the other groups.

Funding

The study was financed by grants from the Swedish state under the agreement between the Swedish government and Region Västra Götaland, the ALF-agreement(ALFGBG-11522, ALFGBG-136431, ALFGBG-426411, ALFGBG-507701, ALFGBG-717501).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejogrb.2022.03.006.

References

[1] Platt MJ. Outcomes in preterm infants. Public Health 2014;128(5):399–403.
[2] Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health 2019;7(1):e37–46.
[3] Jacobsson B, Pettersson K, Modzelewska D, Abrahamsson T, Bergman L, Håkansson S. Preterm delivery: an overview on epidemiology, pathophysiology and consequences for the individual and the society. Lakartidningen 2019;116.
[4] Morken NH, Källen K, Jacobsson B. Outcomes of preterm children according to type of delivery onset: a nationwide population-based study. Paediatr Perinat Epidemiol 2007;21(5):458–64.
[5] Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet (London, England) 2016;388(10063):3027–35.
[6] Purisch SE, Gyamfi-Bannerman C. Epidemiology of preterm birth. Semin Perinatol 2017;41(7):387–91.
[7] Morken NH, Vogel I, Kallen K, Skjaerven R, Langhoff-Roos J, Kesmodel US, et al. Reference population for international comparisons and time trend surveillance of preterm delivery proportions in three countries. BMC Womens Health 2008;8:16.
[8] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371(9606):75–84.
[9] Zhang G, Feenstra B, Bacelis J, Liu X, Muglia LM, Juodakis J, et al. Genetic Associations with Gestational Duration and Spontaneous Preterm Birth. N Engl J Med 2017;377(12):1156–67.
[10] Cobo T, Kacerovsky M, Jacobsson B. Risk factors for spontaneous preterm delivery. International Journal of Gynecology & Obstetrics 2020;150(1):17–23.
[11] Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014;345(6198):760–5.
[12] 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(6):719–24.
[13] Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. American journal of reproductive immunology (New York, NY : 1989). 2014;72(5):458-74.
[14] Musilova I, Kutova R, Pliskova L, Stepan M, Menon R, Jacobsson B, et al. Intraamniotic Inflammation in Women with Preterm Prelabor Rupture of Membranes. PLoS ONE 2015;10(7):e0133929.
[15] Kacerovsky M, Musilova I, Stepan M, Andrys C, Drahosova M, Jacobsson B. Detection of intraamniotic inflammation in fresh and processed amniotic fluid samples with the interleukin-6 point of care test. Am J Obstet Gynecol 2015;213(3):435–6.
[16] Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, et al. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. J Matern Fetal Neona 2011;24(12):1444–55.
[17] Martinez-Varea A, Romero R, Xu Y, Miller D, Ahmed AI, Chaemsaithong P, et al. Clinical chorioamnionitis at term VII: the amniotic fluid cellular immune response. J Perinat Med 2017;45(5):523–38.
[18] Shabani F, Farasat A, Mahdavi M, Gheibi N. Calprotectin (S100A8/S100A9): a key protein between inflammation and cancer. Inflamm Res 2018;67(10):801–12.
[19] Park SY, Kim WJ. A Study of Fecal Calprotectin in Obese Children and Adults. J Obes Metab Syndr 2018;27(4):233–7.
[20] Ometto F, Friso L, Astorri D, Botsios C, Raffeiner B, Punzi L, et al. Calprotectin in rheumatic diseases. Exp Biol Med (Maywood) 2017;242(8):859–73.

[21] Mortensen OH, Nielsen AR, Erikstrup C, Plomgaard P, Fischer CP, Krogh-Madsen R, et al. Calprotectin–a novel marker of obesity. PLoS ONE 2009;4(10):e7419.
[22] Manceau H, Chicha-Cattoir V, Puy H, Peoc'h K. Fecal calprotectin in inflammatory bowel diseases: update and perspectives. Clin Chem Lab Med (CCLM) 2017:474.
[23] Lee HJ, Savelieff MG, Kang J, Brophy MB, Nakashige TG, Lee SJC, et al. Calprotectin influences the aggregation of metal-free and metal-bound amyloid-β by direct interaction. Metallomics 2018;10(8):1116–27.
[24] D'Angelo F, Felley C, Frossard JL. Calprotectin in Daily Practice: Where Do We Stand in 2017? Digestion 2017;95(4):293–301.
[25] Espinoza J, Chaiworapongsa T, Romero R, Edwin S, Rathnasabapathy C, Gomez R, et al. Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. The Journal of Maternal-Fetal & Neonatal Medicine 2003;13(1):2–21.
[26] Gomez-Lopez N, Romero R, Xu Y, Miller D, Arenas-Hernandez M, Garcia-Flores V, et al. Fetal T Cell Activation in the Amniotic Cavity during Preterm Labor: A Potential Mechanism for a Subset of Idiopathic Preterm Birth. J Immunol 2019;203(7):1793–807.
[27] Gomez-Lopez N, Romero R, Galaz J, Xu Y, Panaitescu B, Slutsky R, et al. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. American journal of reproductive immunology (New York. NY 1989;2019;82(5):e13171.
[28] Galaz J, Romero R, Xu Y, Miller D, Slutsky R, Levenson D, et al. Cellular immune responses in amniotic fluid of women with preterm clinical chorioamnionitis. Inflamm Res 2020;69(2):203–16.
[29] Galaz J, Romero R, Slutsky R, Xu Y, Motomura K, Para R, et al. Cellular immune responses in amniotic fluid of women with preterm prelabor rupture of membranes. J Perinat Med 2020;48(3):222–33.
[30] Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, et al. A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. J Matern Fetal Neonatal Med 2016;29(3):349–59.
[31] Wilms FF, Vis JY, Oudijk MA, Kwee A, Porath MM, Scheepers HC, et al. The impact of fetal gender and ethnicity on the risk of spontaneous preterm delivery in women with symptoms of preterm labor. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet 2016;29(21):3563–9.