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ORIGINAL RESEARCH ARTICLE

Second-trimester amniotic fluid proteins changes in subsequent spontaneous preterm birth

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Abstract

Introduction: The global sequence of the pathogenesis of preterm labor remains unclear. This study aimed to compare amniotic fluid concentrations of extracellular matrix-related proteins (procollagen, osteopontin and IL-33), and of cytokines (IL-19, IL-6, IL-20, TNFα, TGFβ, and IL-1β) in asymptomatic women with and without subsequent spontaneous preterm delivery.

Material and methods: We used amniotic fluid samples of singleton pregnancy, collected by amniocentesis between 16 and 20 weeks' gestation, without stigmata of infection (i.e., all amniotic fluid samples were tested with broad-range 16S rDNA PCR to distinguish samples with evidence of past bacterial infection from sterile ones), during a randomized, double-blind, placebo-controlled trial to perform a nested case–control laboratory study. Cases were women with a spontaneous delivery before 37 weeks of gestation (preterm group). Controls were women who gave birth at or after 39 weeks (full term group). Amniotic fluid concentrations of the extracellular matrix-related proteins and cytokines measured by immunoassays were compared for two study groups. ClinicalTrials.gov: NCT00718705.

Results: Between July 2008 and July 2011, in 12 maternal-fetal medicine centers in France, 166 women with available PCR-negative amniotic fluid samples were retained for the analysis. Concentrations of procollagen, osteopontin, IL-19, IL-6, IL-20, IL-33, TNFα, TGFβ, and IL-1β were compared between the 37 who gave birth preterm and the 129 women with full-term delivery. Amniotic fluid levels of procollagen, osteopontin, IL-19, IL-33, and TNFα were significantly higher in the preterm than the full-term group. IL-6, IL-20, TGFβ, and IL-1β levels did not differ between the groups.

Conclusions: In amniotic fluid 16S rDNA PCR negative samples obtained during second-trimester amniocentesis, extracellular matrix-related protein concentrations (procollagen, osteopontin and IL-33), together with IL-19 and TNFα, were observed higher at this time in cases of later spontaneous preterm birth.

Abbreviations: AF, amniotic fluid; PPROM, preterm premature rupture of the membranes; SPTB, spontaneous preterm birth.

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KEYWORDS
amniotic fluid, IL-33, *Mycoplasma hominis*, osteopontin, preterm birth, procollagen, second-trimester pregnancy, *Ureaplasma urealiticum*

1 | INTRODUCTION

The cause of spontaneous preterm birth (SPTB) most often remains unknown, unless an intrauterine infection has been clearly identified.[1,2] The onset of spontaneous preterm labor, with or without infection, is concomitant to an intrauterine burst of inflammation that releases proinflammatory cytokines at the maternal-fetal interface.[3,4] At term, on the other hand, the spontaneous onset of labor accompanies inflammation that appears to result from an accumulation of earlier molecular events leading to the loss of immune tolerance at the maternal-fetal interface and to tissue senescence.[3,5] It is not yet known if spontaneous preterm delivery shares the same pathogenetic pathways as either late preterm delivery or parturition (at term). We hypothesized that preterm (i.e., as early as the second trimester) modifications in gestational tissues related to extracellular matrix remodeling may play a role in the subsequent onset of labor or be dysregulated in SPTB without any evidence of past infection.

High concentrations of various proinflammatory cytokines (such as IL-6, and TNFα) have been found in mid-second trimester amniotic fluid samples from asymptomatic women with a subsequent preterm birth.[6,7] Only one of those studies, however, mentioned amniotic fluid bacterial cultures, and more than half of them were positive.[7] It is not yet known whether the dysregulation of amniotic fluid proteins precedes or results from the clinical context. No study has investigated early remodeling and extracellular matrix changes in the second-trimester amniotic fluid of asymptomatic women without amniotic fluid bacterial colonization or infection. Prior evidence suggest a role for the procollagen alteration in the mechanisms of preterm labor notably in Ehlers-Danlos syndrome,[8] a role of osteopontin in attachment of the conceptus and placentation in animal models[9,10] and a protective role of IL-33, a mucosal alarmin, that signals tissue damage following stress or infection.[11] In addition to these three extracellular matrix-related proteins, we selected inflammatory/immunomodulatory cytokines (IL-19, IL-6, IL-20, IL-33, TNFα, TGFβ, and IL-1β) to investigate whether variations in these intra-amniotic concentrations during the second trimester of an asymptomatic pregnancy are associated with SPTB.

Key message

In amniotic fluid obtained during second-trimester amniocentesis, extracellular matrix-related protein concentrations (procollagen, osteopontin and IL-33) together with IL-19 and TNFα were higher in women who later had preterm delivery, especially those with late preterm delivery.

2 | MATERIAL AND METHODS

2.1 | Setting and study population

This study is an ancillary case–control laboratory analysis of amniotic fluid samples collected during the PREMYC trial, the design of which is detailed in the original report.[12] Briefly, it was a double-blind, placebo-controlled, randomized clinical trial, assessing the benefit of treatment by josamycin on the risk of SPTB in asymptomatic women with a singleton pregnancy and PCR+ for *Mycoplasma genitalium*, *M. hominis*, and *Ureaplasma* spp.

The PREMYC trial included asymptomatic women with singleton pregnancies from 12 hospitals in France who underwent a second trimester amniocentesis from 16 to 20 weeks of gestation to screen for Down syndrome between July 2008 and July 2011. Women were excluded if they were younger than 18 years, had a known allergy to macrolides or lactose, had a fetus with a known major structural or chromosomal abnormality, or did not speak and understand French. Amniotic fluid samples were obtained by transabdominal amniocentesis under ultrasound guidance. The amniotic fluid samples were centrifuged at 1200 *g* for 10 minutes at 4°C, and supernatants were collected. Aliquots of those samples were stored at −80°C until further processing. All amniotic fluid samples were tested for *M. hominis*, *M. genitalium*, *U. urealyticum* and *U. parvum* with specific real-time PCR and with broad-range 16 S rDNA PCR to distinguish samples with evidence of past bacterial infection from sterile ones.[12] This technique allows to detect presence of bacteria and identify bacterial species in microbial communities with amplicon-based sequencing.

2.2 | Study groups

Among the 1043 women included in the PREMYC study, 951 of the 980 (97.0%) with complete PCR data had negative PCR results for all tests and were eligible for this analysis. Women with spontaneous deliveries before 37 weeks, with or without preterm premature rupture of the membranes (PPROM), comprised the "preterm group." The control or "full-term" group contained the four women with negative PCR and a full-term birth (≥39 weeks) recruited just after each case to compare the preterm births with full-term (between 39 weeks and 42 weeks) rather than simply term births (>37 weeks) that may be associated with more obstetrical complications. We also

stratified women with early preterm birth (<34 weeks' gestation) and late preterm birth ($34^{0/7}$–$36^{6/7}$ weeks) and compared them with the control group.

2.3 | Clinical data

For each woman, the data potentially related to preterm birth were collected, including general characteristics (age, body mass index, country of birth, isolated women, professional activity during pregnancy, use of tobacco during pregnancy); medical and surgical history (eg preexisting diabetes and hypertension, previous conization); obstetrical history (eg previous curettage, previous fetal loss and preterm delivery). Data of the pregnancy course (prophylactic cerclage, gestational hypertension, bleeding during pregnancy, gestational age at birth).

2.4 | Measurement of cytokine concentrations

After completion of the the PREMYC study, procollagen, osteopontin, IL-19, IL-6, IL-20, IL-33, TNFα, TGFβ, and IL-1β were assayed in serum samples by ELISA (R&D Systems, Inc.), according to the manufacturer's instructions. The range of determination was 7.5–3500 pg/mL.[13] Concentrations below 7.5 pg/mL were undetectable and considered to be 0 pg/mL for statistical analysis. Each sample was tested in duplicate and the mean value calculated. The intra- and interassay coefficients of variation of the ELISA kits were <10%. When more than half the assay samples were null, results are reported as the number of positive tests. A test was considered positive when the assay result was different from 0 ng/mL.

2.5 | Statistical analyses

All data were collected in a computerized database and analyzed with Stata 13.0 (StataCorp). Data are presented as means ± SD or medians (interquartile ranges), as appropriate. Because the distributions of the amniotic fluid procollagen, osteopontin, IL-19, and IL-6 concentrations were non-Gaussian, statistical comparisons between the two groups were performed with the Mann–Whitney U test. Pearson's χ^2 or Fisher's exact test, as appropriate, were used for the categorical variables (for IL-20, IL-33, TNFα, TGFβ, and IL-1β). Each variable was studied independently, and no multitest procedures were used. Statistical significance was defined by a p-value <0.05.

2.6 | Ethics statement

The trial was approved by the national data protection authority (Commission Nationale de l'Informatique et des Libertés, CNIL) and by the committee for the protection of people participating in biomedical research (Comité de Protection des Personnes [CPP], CPP Poissy Saint Germain, no. 07051) on July 21, 2008. It was registered at ClinicalTrials.gov (no. NCT00718705). The study was performed in accordance with the Declaration of Helsinki and Good Scientific Practice guidelines. All patient records were anonymized and de-identified before the analyses. All participants gave written informed consent before enrolment.

3 | RESULTS

Among the 1016 women with PCR tests in the PREMYC study between July 2008 and July 2011, 980 had clinical data for the primary outcome (delivery before 37 weeks), and 951 women had negative PCR results for *M. genitalium*, *M. hominis*, *Ureaplasma* spp. and 16 S rDNA. Among those with negative PCR results, 37 gave birth spontaneously before 37 weeks' gestation (with or without PPROM) and comprise the preterm group. The full-term group was formed with only 129 control women of the 148 expected, because of inadequate amniotic fluid volume for the others when their frozen samples were thawed. The selection process of these women is detailed in the flow chart (Figure 1).

The women's baseline characteristics are presented in Table 1. The preterm and full-term groups were similar for demographic and most obstetric characteristics, except known risk factors of PTB (previous preterm delivery, bleeding during each trimester, and cerclage), all of which were more frequent in the preterm group (Table 1).

Mean gestational age at amniocentesis was 17 weeks 3 days in both groups ($p = 0.99$). Amniotic fluid protein levels were measured in the 166 women studied. Preterm labor and PPROM for either early or late preterm births were analyzed together for reasons of power in the early and combined preterm delivery groups, after verifying the absence of any major difference between them for the proteins of interest (Table S1). Amniotic fluid levels of procollagen, osteopontin, and IL-19 were significantly higher in the preterm than the full-term group, whereas IL-6 was not (Figure 2A). Positive tests for IL-33 and TNFα were significantly more frequent in the preterm than the full-term group (Figure 2B). Table S2 summarizes the numeric data.

When the 37 women in the preterm group were stratified into early (birth <34 weeks, $n = 12$) and late (34–$36^{6/7}$ weeks, $n = 25$) SPTB groups. Procollagen, osteopontin, and IL-19 levels were significantly lower in the early than in the late preterm group ($p < 0.01$). They were significantly higher in the late preterm than in the full-term group ($p < 0.001$). Osteopontin was significantly lower in the early preterm than in the full-term group ($p = 0.03$), but no such difference was observed for procollagen or IL-19 (Table 2).

Procollagen and osteopontin tends to be lower in the two women with PPROM before 22 weeks' gestation than the 13 women with PPROM after 22 weeks (procollagen, 1.6 ng/mL ± 2.9 vs 51.9 ng/mL ± 32.5; and osteopontin, 127.6 ng/mL ± 28.9 vs 245 ng/mL ± 85.9).

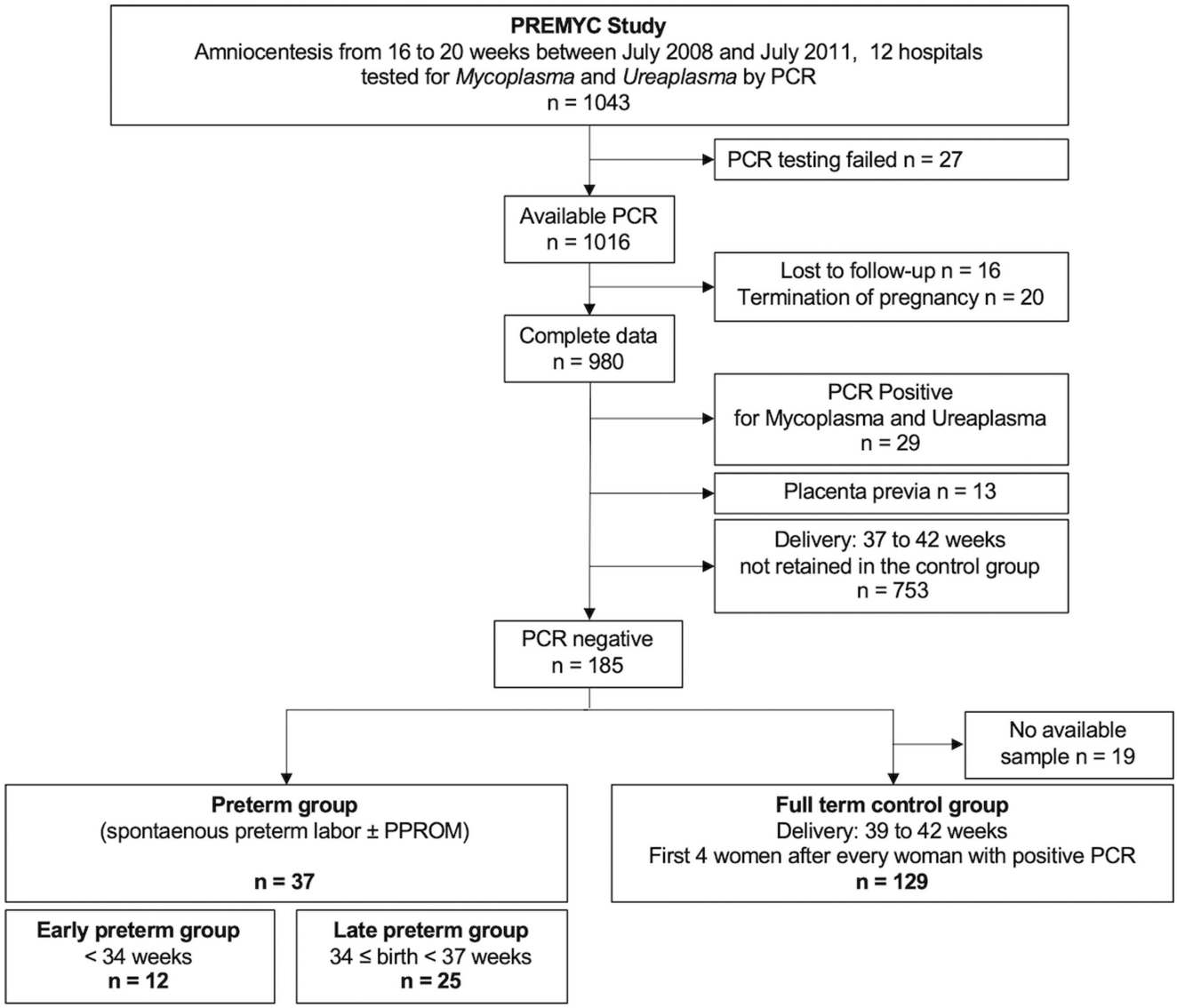

FIGURE 1 Flow chart.

4 | DISCUSSION

This study showed significantly higher levels of the extracellular matrix-related proteins procollagen and osteopontin and of cytokine IL-33 as well as of IL-19 and TNFα in the second-trimester amniotic fluid of women with SPTB. These proteins were higher in women with late preterm births than with both early preterm and full-term births.

The primary strength of this study is its prospective design in a population of women asymptomatic during the second trimester. All the samples included had negative PCR results for *M. genitalium*, *M. hominis*, and *Ureaplasma* spp. as well as for 16 S rDNA to confirm the absence of past bacterial colonization, evidence unavailable in previous studies.[6,7] We choose to compare preterm births with full-term (between 39 weeks and 42 weeks) rather than simply term births (>37 weeks) to increase contrast between the groups. Our study nonetheless has several limitations, due notably to the small number of patients in the preterm group (n = 37) and thus insufficient power to ensure the significance of some comparisons. The rates of previous preterm deliveries, bleeding during each of the first, second, and third trimesters, and cerclage were also higher in the preterm group, but the protein profiles in these women did not differ from those observed in the control women (data not shown). In addition, the cervical lengths of the patients at second trimester were not considered in this study although extracellular matrix remodeling is a key process in the cervical remodeling observed in patients with a short cervix. The risk of SPTB in asymptomatic women with a sonographic short cervix increases as cervical length decreases.[14] Previous reports have demonstrated that a shorter cervix is associated with a more severe inflammatory response in the amniotic

TABLE 1 Baseline characteristics and obstetric history of participants, $n = 166$.

Factor studied	Preterm group ($n = 37$)	Full-term group ($n = 129$)	p-value
Maternal age, med (IQR)	37.0 (34.0–40.0)	36.0 (32.0–39.0)	0.28
Body mass index, med (IQR)	21.2 (19.7–23.5)	21.1 (19.3–24.0)	0.97
Country of birth			
France	18 (48.7)	86 (66.7)	0.08
North Africa	6 (16.2)	14 (10.9)	
Sub-Saharan Africa	5 (13.5)	7 (5.4)	
Other	8 (21.6)	19 (14.7)	
Isolated woman	1 (2.7)	7 (5.4)	0.40
No professional activity during pregnancy	29 (78.4)	116 (89.9)	0.25
Use of tobacco during pregnancy	4 (10.8)	20 (15.5)	0.37
Nulliparity	14 (37.8)	37 (28.7)	0.29
Preexisting diabetes	0 (0)	2 (1.6)	0.76
Preexisting hypertension	1 (2.7)	0 (0)	0.06
Previous conization	0 (0)	2 (1.6)	0.46
Previous curettage	10 (27.0)	20 (15.5)	0.08
Previous preterm delivery between $22^{0/7}$ and $36^{6/7}$ weeks' gestation	4 (10.8)	6 (4.7)	<0.01
Previous fetal loss between $14^{0/7}$ and $21^{6/7}$ weeks' gestation	2 (5.4)	0 (0)	
Prophylactic cerclage	4 (10.8)	0(0)	<0.01
Gestational hypertension	1 (2.7)	0	0.05
Bleeding during the first trimester	4 (10.8)	2(1.6)	<0.01
Bleeding during the second trimester	6 (16.2)	0 (0)	<0.01
Bleeding during the third trimester	5 (13.5)	0 (0)	<0.01
Gestational age at birth med (IQR)	$34^{6/7}$ ($33^{4/7}$–$36^{2/7}$)	$40^{2/7}$ ($39^{4/7}$–$40^{5/7}$)	-
Preterm birth <$34^{0/7}$ weeks' gestation	12 (32.4)	0	-
Late preterm birth $34^{0/7}$–$36^{6/7}$ weeks' gestation	25 (73.5)	0	-

Note: Data are presented as *n* (%) unless otherwise stated.

cavity.[15] Further analysis are necessary to analyze proteins concentration in the second-trimester amniotic fluid of women according to cervical length. In addition, amniotic fluid protein levels may significantly fluctuate during the antenatal period, and it is not certain that the presented differences persist (or even increase) in the third trimester and contribute to preterm birth. Studies regarding these proteins in the third trimester AF samples are needed to verify a correlation with preterm birth, sustaining possible persistent amniotic fluid differences among preterm birth and term deliveries from second trimester to delivery. Finally, despite considered the amniotic fluid samples as sterile at the time of the amniocentesis thanks to negative 16 S PCR, we cannot exclude a later infection involved in the process of preterm birth.

The molecular structure of collagen and its interaction with adhesive proteins and proteoglycans guarantee the cohesive and extensible properties of the fetal membranes.[16] Increased production of procollagen during the second trimester in women who will deliver preterm may be part of a gradual compensatory mechanism to strengthen the fetal membrane structure to counteract the degradation of the amnion layer and its activation of prostaglandin production.[17]

The concentration of osteopontin increased during the second trimester among the women with preterm births in our study. It is an extracellular matrix protein that upregulates expression of interferon-γ and promotes uterine contractility in maternal and fetal immune cell-related preterm labor.[1819] Osteopontin also drives fibrogenesis in the liver,[20] while IL-33 triggers fibrotic processes.[21–23] Fibrosis is thought to be a consequence of tissue aggression and damage,[24] and IL-33 is constitutively expressed in normal human tissues as an alarmin,[13] that is, released as a "danger" signal to the immune system, notably activating TH2 immune cells after cell damage occurs.[25,26] Variations in amniotic fluid concentrations of osteopontin and IL-33 during the second trimester may play a role in regulating maternal-fetal tolerance.

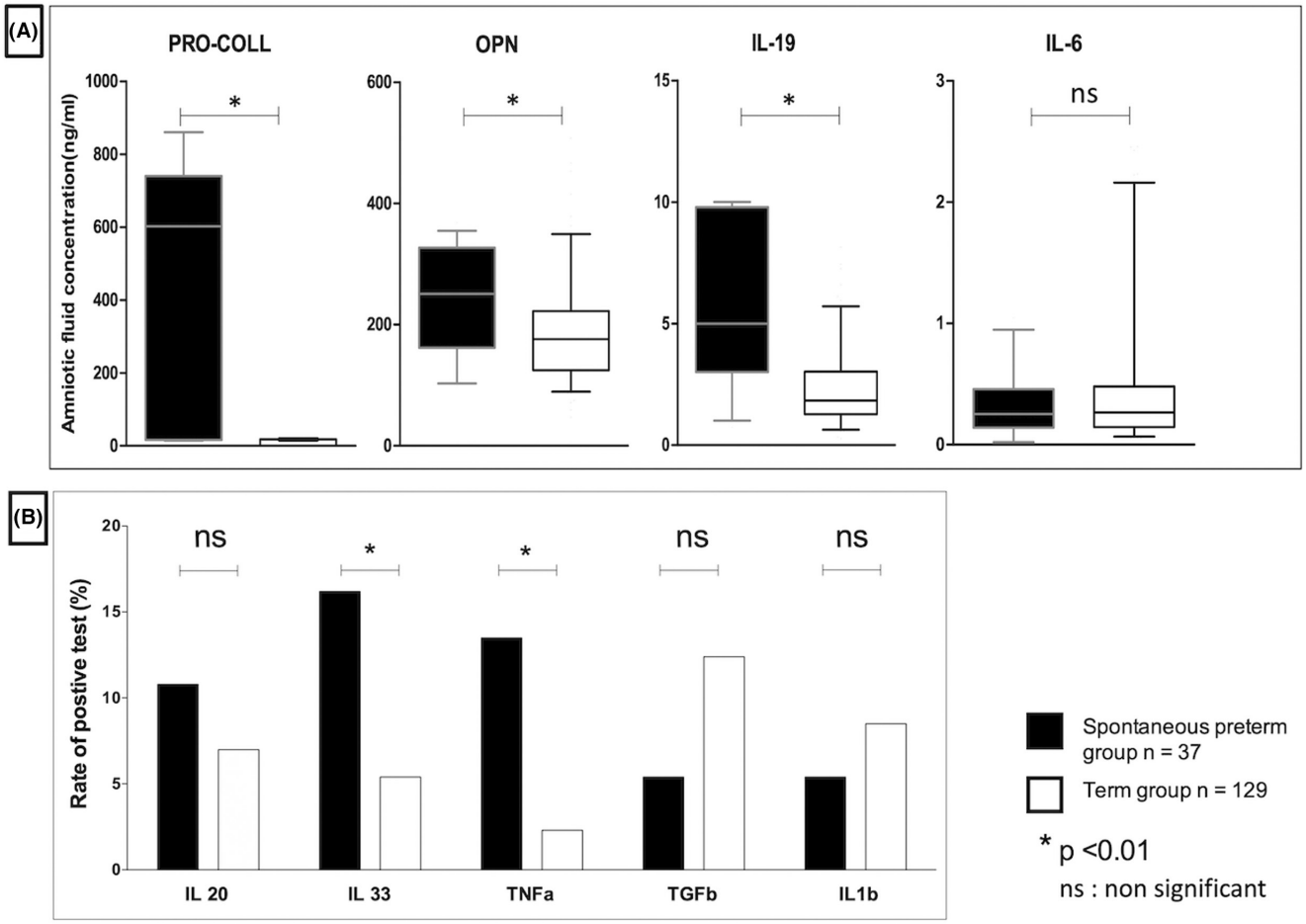

FIGURE 2 Cytokine concentrations obtained from amniotic fluid by amniocentesis at 16–20 weeks of gestation from 166 women: comparison of the preterm group (birth <37 weeks) and full-term group (birth ≥39 weeks), $n = 166$. (A) Comparison of median with interquartile, Mann–Whitney test and (B) comparison of rate of positive test, Chi-2 test.

TABLE 2 Amniotic fluid cytokine concentrations at 16–20 weeks of gestation: comparison of the early preterm (<34 weeks), late preterm ($34^{0/7}$–$36^{6/7}$ weeks) and full-term groups (Birth ≥39 weeks), $n = 166$.

	Preterm group			Full-term group		
	Early preterm birth <34 WG $n = 12$	Late preterm birth $34^{0/7}$–$36^{6/7}$ $n = 25$	p^* Early PTB vs late PTB	Birth ≥39 weeks $n = 129$	p^{**} Early PTB vs FTB	p^{***} Late PTB vs FTB
Quantitative values, median (IQR; ng/mL)						
Procollagen	15 (14–16)	663 (597–790)	<0.01	17 (16–18)	0.002	<0.001
Osteopontin	143 (119–161)	316 (245–333)	<0.01	176 (125–221)	0.09	<0.001
IL-19	3 (2–5)	6 (4–10)	<0.01	2 (1–4)	0.08	<0.001
IL-6	0.4 (0.2–0.5)	0.2 (0.04–0.4)	0.15	0.2 (0.1–0.4)	0.49	0.11
Qualitative positive test *n* (%)						
IL-20	2 (16.7)	2 (8.0)	0.43[k]	9 (7.0)	0.23	0.86
IL-33	1 (8.3)	5 (20.0)	0.37[k]	7 (5.4)	0.67	0.01
TNFα	0	5 (20.0)	0.09[k]	3 (2.3)	0.59	<0.001
TGFβ	0	2 (8.0)	0.31[k]	16 (12.4)	0.19	0.53
IL-1β	0	2 (8.0)	0.31[k]	11 (8.5)	0.29	0.93

Abbreviations: IQR, interquartile; p, k chi-2 or Mann-Whitney test; weeks, weeks of gestation.

*Comparison between early preterm (<34 weeks) group and late preterm group (>34 birth <37).; **Comparison between early preterm (<34 weeks) group and full-term (>39) group.; ***Comparison between late preterm (34 < Birth <37 weeks) group and full-term (>39) group.

We found that IL-19 and TNFα levels were higher in the preterm than the full-term group. IL-19 can activate monocytes to release IL-6 and TNFα[27] and is a potent inducer of the TH2 immune response.[28] Consistent with this, the amniotic fluid TNFα concentration is known to decrease as gestational age advances, and women in spontaneous term labor have a higher median TNFα concentration than those at term not in labor.[29] Taken together, the higher levels of procollagen, osteopontin, IL-33, IL-19, and TNFα in the second-trimester amniotic fluid of women with a third-trimester SPTB are consistent with an early activation process in gestational tissues linked to this type of birth.

The trigger for the onset of labor in women remains elusive. Accumulating evidence suggests that multiple pathologic processes are related to spontaneous preterm labor.[4] The lower level of these cytokine concentrations in the early (<34 weeks) than the late preterm group (34 to less than 37 weeks) is consistent with the hypothesis that the different pathogenetic pathways (or causes) of early preterm delivery are different from those for late preterm delivery and parturition at term. The temporal and topographical events controlling the timing and triggering of spontaneous labor and parturition depend on fetal and gestational tissue maturation.[3,5] One hypothesis is that tissue senescence (in particular, of fetal membranes) initiates a coordinated, redundant signal cascade leading to a laboring uterus phenotype.[3] An accumulation of molecular changes may control the functional decidual "clock" that determines the timing of the onset of labor.[30] The changes in the profile of proteins we studied, including procollagen, osteopontin, IL-33, IL-19, and TNFα, may be a part of this timing mechanism.

Nonetheless, the differences we observed for the early preterm, late preterm, and full-term groups, specifically for IL-6, may be related to different mechanisms of preterm birth, in particular, more cervical processes in the early preterm group. In other words, this second-trimester increase of extracellular matrix-related proteins may be part of a compensatory process that contributes to prolonging pregnancy, but insufficiently, in early preterm births before 34 weeks compared with the later preterm births.

Substantial evidence links pathological inflammation in gestational tissues and preterm delivery.[4] Inflammation may result from innate immunity in cases of infection[2,31,32] or cellular stress, resulting in damage-associated molecular pattern (DAMP) exposure. Inflammation may also result from activation of the adaptive immune system.[3,30,33] In our study, all women who gave birth before 37 weeks had negative PCR for *M. genitalium*, *M. hominis*, and *Ureaplasma* spp. The absence of positive PCR and inflammation in second-trimester amniotic fluid from the women in the early preterm group calls attention to an important point: either inflammatory processes are not the only contributor to the pathogenesis of SPTB, or they are not visible in the second trimester, probably because it is too early to observe them. Therefore, extracellular matrix signaling, with or without infection, has a role in modifying innate or adaptive immunity, by disrupting immunologic dialogues and immunotolerance at the maternal-fetal interface, as previously described.[5,34,35] The absence of inflammation in early preterm delivery strengthens the likelihood that extracellular matrix processes early in pregnancy play a role in the subsequent occurrence of preterm delivery or PPROM. These early extracellular matrix-related protein changes without inflammation suggest the involvement of early events in SPTB, consistent with the observation that inflammation alone does not trigger human parturition.[3] Inflammation may appear later after gestational tissue remodeling and clearance of foreign material,[5] as a consequence and not as a prime mover. These considerations might contribute to open new avenues to identify biological markers to improve prevention, diagnosis, and/or management of SPTB.

5 | CONCLUSION

In amniotic fluid obtained during second trimester amniocentesis, extracellular matrix-related protein concentrations (procollagen, osteopontin and IL-33), together with IL-19 and TNFα, were observed higher in women with later preterm delivery, especially those with late preterm delivery. Furthers studies are needed to establish a link between AF extracellular matrix-related proteins and cytokines concentrations changes and preterm birth.

AUTHOR CONTRIBUTIONS

Concept and design: LM, FB and GK. Acquisition, analysis, or interpretation of data: SC, GK, TS and FG. Drafting of the manuscript: LM and GK. Critical revision of the manuscript for important intellectual content: TS, FB, CM and FG. Statistical analysis: EL. Obtained funding: GK.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371:75-84.
2. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med*. 2000;342:1500-1507.
3. Menon R, Bonney EA, Condon J, Mesiano S, Taylor RN. Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. *Hum Reprod Update*. 2016;22:535-560.
4. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345:760-765.
5. Marcellin L, Schmitz T, Messaoudene M, et al. Immune modifications in fetal membranes overlying the cervix precede parturition in humans. *J Immunol*. 2017;198:1345-1356.
6. Melekoglu R, Yilmaz E, Ciftci O, Kafadar YT, Celik E. Associations between second-trimester amniotic fluid levels of ADAMTS4, ADAMTS5, IL-6, and TNF-alpha and spontaneous preterm delivery in singleton pregnancies. *J Perinat Med*. 2019;47:304-310.
7. Thomakos N, Daskalakis G, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: relationship to intra-amniotic microbial invasion and preterm delivery. *Eur J Obstet Gynecol Reprod Biol*. 2010;148:147-151.
8. Hurst BS, Lange SS, Kullstam SM, et al. Obstetric and gynecologic challenges in women with Ehlers-Danlos syndrome. *Obstet Gynecol*. 2014;123:506-513.
9. Garlow JE, Ka H, Johnson GA, Burghardt RC, Jaeger LA, Bazer FW. Analysis of osteopontin at the maternal-placental interface in pigs. *Biol Reprod*. 2002;66:718-725.
10. Johnson GA, Bazer FW, Jaeger LA, et al. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. *Biol Reprod*. 2001;65:820-828.
11. Huang B, Faucette AN, Pawlitz MD, et al. Interleukin-33-induced expression of PIBF1 by decidual B cells protects against preterm labor. *Nat Med*. 2017;23:128-135.
12. Kayem G, Doloy A, Schmitz T, et al. Antibiotics for amniotic-fluid colonization by *Ureaplasma* and/or *Mycoplasma* spp. to prevent preterm birth: a randomized trial. *PLoS One*. 2018;13:e0206290.
13. Santulli P, Borghese B, Chouzenoux S, et al. Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis. *Hum Reprod*. 2012;27:2001-2009.
14. Gulersen M, Divon MY, Krantz D, Chervenak FA, Bornstein E. The risk of spontaneous preterm birth in asymptomatic women with a short cervix (</=25 mm) at 23-28 weeks' gestation. *Am J Obstet Gynecol MFM*. 2020;2:100059.
15. Galaz J, Romero R, Xu Y, et al. Cellular immune responses in amniotic fluid of women with a sonographic short cervix. *J Perinat Med*. 2020;48:665-676.
16. Cockle JV, Gopichandran N, Walker JJ, Levene MI, Orsi NM. Matrix metalloproteinases and their tissue inhibitors in preterm perinatal complications. *Reprod Sci*. 2007;14:629-645.
17. Challis JR, Patel FA, Pomini F. Prostaglandin dehydrogenase and the initiation of labor. *J Perinat Med*. 1999;27:26-34.
18. Li L, Yang J, Jiang Y, Tu J, Schust DJ. Activation of decidual invariant natural killer T cells promotes lipopolysaccharide-induced preterm birth. *Mol Hum Reprod*. 2015;21:369-381.
19. Frascoli M, Coniglio L, Witt R, et al. Alloreactive fetal T cells promote uterine contractility in preterm labor via IFN-gamma and TNF-alpha. *Sci Transl Med*. 2018;10:eaan2263.
20. Chen Y, Ou Y, Dong J, et al. Osteopontin promotes collagen I synthesis in hepatic stellate cells by miRNA-129-5p inhibition. *Exp Cell Res*. 2018;362:343-348.
21. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Sato S. Serum IL-33 levels are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *Clin Rheumatol*. 2011;30:825-830.
22. Rankin AL, Mumm JB, Murphy E, et al. IL-33 induces IL-13-dependent cutaneous fibrosis. *J Immunol*. 2010;184:1526-1535.
23. Marvie P, Lisbonne M, L'Helgoualc'h A, et al. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med*. 2010;14:1726-1739.
24. Yuge A, Nasu K, Matsumoto H, Nishida M, Narahara H. Collagen gel contractility is enhanced in human endometriotic stromal cells: a possible mechanism underlying the pathogenesis of endometriosis-associated fibrosis. *Hum Reprod*. 2007;22:938-944.
25. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol*. 2010;10:103-110.
26. Luthi AU, Cullen SP, McNeela EA, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity*. 2009;31:84-98.
27. Liao YC, Liang WG, Chen FW, Hsu JH, Yang JJ, Chang MS. IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol*. 2002;169:4288-4297.
28. Jordan WJ, Eskdale J, Boniotto M, et al. Human IL-19 regulates immunity through auto-induction of IL-19 and production of IL-10. *Eur J Immunol*. 2005;35:1576-1582.
29. Maymon E, Ghezzi F, Edwin SS, et al. The tumor necrosis factor alpha and its soluble receptor profile in term and preterm parturition. *Am J Obstet Gynecol*. 1999;181:1142-1148.
30. Norwitz ER, Bonney EA, Snegovskikh VV, et al. Molecular regulation of parturition: the role of the decidual clock. *Cold Spring Harb Perspect Med*. 2015;5:a023143.
31. Gomez-Lopez N, Romero R, Xu Y, et al. Are amniotic fluid neutrophils in women with intraamniotic infection and/or inflammation of fetal or maternal origin? *Am J Obstet Gynecol*. 2017;217:693.e616-693.e691.
32. Combs CA, Gravett M, Garite TJ, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. *Am J Obstet Gynecol*. 2014;210:125.e1-125.e15.
33. Menon R. Human fetal membranes at term: dead tissue or signalers of parturition? *Placenta*. 2016;44:1-5.
34. Xu Y, Romero R, Miller D, et al. Innate lymphoid cells at the human maternal-fetal interface in spontaneous preterm labor. *Am J Reprod Immunol*. 2018;79:e12820.
35. Rinaldi SF, Makieva S, Saunders PT, Rossi AG, Norman JE. Immune cell and transcriptomic analysis of the human decidua in term and preterm parturition. *Mol Hum Reprod*. 2017;23:708-724.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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