The Interplay of the Interleukin 1 System in Pregnancy and Labor

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Yujing Jan Heng, PhD^{1,2,3}, Stella Liong, BSc(Hons)^{1,2},
Michael Permezel, MD^{1,2}, Gregory E. Rice, PhD⁴,
Megan K. W. Di Quinzio, MD^{1,2}, and Harry M. Georgiou, PhD^{1,2}

Abstract

This work assessed the temporal coexpression of interleukin 1 (IL-1) and its inhibitor, IL-1 receptor antagonist (IL-1ra), in the cervicovaginal fluid (CVF) beyond 24 weeks gestation including women in spontaneous term labor. Two cohorts of women were recruited at 24 to 35 weeks' gestation ($n = 65$) and in late pregnancy (>36 weeks' gestation; $n = 88$). The CVF was serially collected either every 4 weeks between 24 and 35 weeks' gestation ($n = 123$ samples) or weekly during late pregnancy ($n = 240$ samples). The IL-1 and IL-1ra were quantitated by enzyme-linked immunosorbent assay, and the effect of vaginal microflora and unprotected sexual intercourse were also investigated. The IL-1 β and IL-1ra remain unaltered between 24 and 35 weeks' gestation. At late pregnancy, IL-1 α and β concentrations peak at 4 to 14 days prior to labor onset, while IL-1ra decreases with approaching spontaneous term labor ($P < .05$, 2-way analysis of variance). The IL-1 and IL-1ra were significantly correlated ($P < .001$, Pearson r). A combined biomarker model of IL-1 α , IL-1 β , and IL-1ra can predict term labor with 86% sensitivity and 92% specificity. This study indicates a shifting inflammatory balance in the gestational tissues prior to labor onset.

Keywords

cervicovaginal fluid, interleukin-1, interleukin-1 receptor antagonist, labor, pregnancy

Introduction

The cervicovaginal fluid (CVF) proteome reflects the local biochemical milieu and is influenced by the physical changes occurring in the cervix and adjacent overlying fetal membranes.^{1,2} The current gold standard test to predict preterm birth (PTB) is the detection of fetal fibronectin (fFN > 50 ng/mL) in the CVF with low sensitivity and a high negative predictive value (NPV).³ As preterm delivery requires medical intervention, an alternative biomarker to predict PTB with improved sensitivity and a high positive predictive value (PPV) is warranted. The development of a PTB diagnostic test faces many challenges, including the difficulty in obtaining a sufficient number of prospective samples from women who subsequently deliver preterm and to discover and validate biomarkers.

As preterm and term labor share a final common pathway of cervical ripening, myometrial activation, and membrane rupture leading to birth, it is hypothesized that biomarkers predictive of term labor could potentially be utilized in a preterm setting. In addition, it is worth considering the benefits of predicting postterm labor as postterm pregnancy is associated with considerable health risks to the fetus and mother.⁴ The ability to predict postterm labor may permit the tailoring of obstetric management to reduce costs and minimize perinatal and maternal morbidity associated with unnecessary induction of labor or cesarean section delivery.^{5,6}

Using 2-dimensional polyacrylamide gel electrophoresis, we discovered that the naturally occurring inhibitor of interleukin 1 (IL-1), IL-1 receptor antagonist (IL-1ra), was significantly decreased in the human CVF in association with spontaneous term labor.⁷ Our IL-1ra finding was subsequently validated using enzyme-linked immunosorbent assay (ELISA).⁸ It is widely acknowledged that human labor is an acute inflammatory process. Elevated levels of maternal circulating leukocytes,⁹ influx of leukocytes, and expression of proinflammatory cytokines, including IL-1, in the cervix, myometrium, and fetal membranes before or during parturition have been reported.¹⁰⁻¹³ The IL-1ra inhibits the biologic responses induced by IL-1 by competing for

¹ Department of Obstetrics & Gynaecology, The University of Melbourne, Parkville, Victoria, Australia

² Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, Victoria, Australia

³ Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

⁴ University of Queensland Centre for Clinical Research, Herston, Queensland, Australia

Corresponding Author:

Yujing Jan Heng, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 25 Orde Street, 6-1001, Toronto, Ontario M5T 3H7, Canada.
Email: heng@lunenfeld.ca

Table 1. Demographic and Obstetric Characteristics of Women in Cohorts A and B.

	Cohort A (24-35 Weeks' Gestation)	Cohort B (≥ 36 Weeks' Gestation)
Women (n)	65	88
Maternal age, years (mean $\pm$ SD)	31.9 $\pm$ 4.1	32.0 $\pm$ 5.2
Gravidity (mean $\pm$ SD)	3.7 $\pm$ 2.0	3.1 $\pm$ 1.3
Parity (mean $\pm$ SD)	1.1 $\pm$ 1.4	1.5 $\pm$ 0.8
Gestational age at delivery, weeks (mean $\pm$ SD)	39.5 $\pm$ 1.2	40.1 $\pm$ 0.9
Fetal birth weight, g (mean $\pm$ SD)	3436.5 $\pm$ 505.2	3626.5 $\pm$ 422.5
Fetal gender, n (%)		
Male	27 (42)	50 (57)
Female	38 (58)	38 (43)

Abbreviation: SD, standard deviation.

cell surface receptors (IL-1 receptor types I and II) without activating target cells.¹⁴

The IL-1 and IL-1ra concentrations in the CVF have been investigated by others to determine their utility as predictors of preterm^{15,16} and/or term labor.¹⁶⁻¹⁹ Most of these previous studies quantified only IL-1 β ¹⁶⁻¹⁸; while 2 other studies measured IL-1 α and IL-1 β ¹⁹ or IL-1 β and IL-1ra.¹⁵ This current study aims to provide a comprehensive screen of IL-1 and IL-1ra in serially collected CVF beyond 24 weeks' gestation. Therefore, the specific aims of our study are to investigate (1) the quantitative temporal expression IL-1 β and IL-1ra between 24 and 35 weeks of gestation; (2) the quantitative temporal expression of IL-1 α , IL-1 β , and IL-1ra in late pregnancy (>36 weeks' gestation) until spontaneous term labor; and (3) the utility of the IL-1 system to predict spontaneous term labor.

Materials and Experimental Design

Patient Recruitment

This longitudinal observational study of CVF in pregnancy was approved by the Mercy Health, Research Ethics Committee. All participants provided written informed consent. Pregnant women who attended the Antenatal Clinic at the Mercy Hospital for Women (Heidelberg, Victoria, Australia) were prospectively recruited by a research midwife beyond 24 weeks gestation. Detailed inclusion and exclusion criteria are described in the following sections. After obtaining the participants' delivery outcomes, women were retrospectively selected, focusing on those who experienced spontaneous onset of labor. Two experimental groups were investigated and for convenience are referred to as cohort A and cohort B. Temporal changes in the concentration of IL-1 β and IL-1ra in the CVF between 24 and 35 weeks' gestation were investigated in samples from cohort A, while temporal changes in IL-1 α , IL-1 β , and IL-1ra in the CVF during late pregnancy (>36 weeks) until spontaneous onset of term labor were investigated in samples from cohort B (Table 1). Women were questioned at each CVF sampling as to whether they had

unprotected sexual intercourse in the preceding 48 hours, an internal ultrasound within the preceding 6 hours, experienced any recent vaginal bleeding, or were taking any medication.

Cohort A Participants

Women in cohort A were recruited at approximately 24 weeks gestation. Serial CVF swabs were obtained every 4 weeks where possible until 35 completed weeks of gestation. Inclusion criteria were healthy women with a singleton pregnancy who delivered spontaneously between 37 and 42 weeks' gestation. Exclusion criteria were multifetal gestation, PTB (delivery <37 weeks' gestation), prelabor rupture of fetal membranes (PROMs), elective cesarean section delivery, induction of labor, bacterial vaginosis, cervical cerclage, and progesterone pessary treatment. Samples were not collected if the women had a digital vaginal examination or transvaginal ultrasound within the preceding 6 hours. Two women reported an isolated incidence of mild transient vaginal bleeding. Neither woman was actively bleeding at the time of CVF sampling.

Cohort B Participants

Cohort B consisted of women recruited at 36 weeks' gestation. Serial CVF swabs were obtained weekly starting from 36 weeks' gestation and, when possible, an in-labor swab was obtained before the rupture of fetal membranes. Labor was defined as regular painful uterine contractions that lead to the effacement and dilation of the cervix (>3 cm). Inclusion criteria were healthy women with a singleton pregnancy and spontaneous onset of labor at 37 to 42 weeks' gestation. Exclusion criteria were multifetal gestation, <37 or >42 weeks' gestation at delivery, PROM, elective cesarean section, induction of labor, and bacterial vaginosis. Likewise, CVF was not collected if the women had a digital vaginal examination or transvaginal ultrasound within the preceding 6 hours. One woman reported an incident of mild transient vaginal bleeding but was not actively bleeding at the time of CVF sampling. Two women were taking antibiotics for mild respiratory tract infections.

Sample Collection

The CVF was collected adhering to our previously published protocol.^{1,7,8,20-22} Briefly, CVF was collected using a sterile speculum and a DUO-TRANSTUBE double-tipped rayon swab (Medical Wire & Equipment Co Ltd, Corsham, Wilts, England). The swab was placed into the posterior vaginal fornix for 30 s and immersed in 1 mL of chilled "extraction" buffer (50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate, 1 mmol/L EDTA, and 1 mmol/L Pefabloc SC 4-(2-aminoethyl)benzene sulfonyl fluoride; Roche Diagnostics GmbH, Mannheim, Germany). The tubes were left on ice and transported to the laboratory for processing within 2 hours. Samples were centrifuged, and the supernatant fluid was collected and stored at -80°C .

Table 2. Microbiology Results for Cohorts A and B.^a

	No Pathogens Isolated	Group B <i>Streptococcus</i> Colonization	<i>Candida</i> spp Colonization	<i>Ureaplasma</i> spp Colonization	Mixed Colonization	Two-Way ANOVA P Value
Cohort A (total of 91 samples from 65 women)						
Samples (n)	57	13	6	13	2	
Women (n)	47	11	4	11	2	
IL-1 β median, pg/mg protein	52.21	185.80	1615.09	0.00	227.82	.036 ^b
IL-1ra median, μ g/mg protein	2.97	7.73	2.46	1.97	1.78	.157
Cohort B (total of 59 samples from 59 women)						
Samples (n)	29	4	7	10	9	
Women (n)	29	4	7	10	9	
IL-1 α median, pg/mg protein	145.32	232.43	0.00	219.95	207.27	.243
IL-1 β median, pg/mg protein	132.11	397.42	65.47	567.81	334.05	.227
IL-1ra median, μ g/mg protein	0.92	2.14	0.60	1.18	0.99	.178

Abbreviations: ANOVA, analysis of variance; HSD, honestly significant difference; IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist.

^a For cohort A, microbiologic assessment was performed at each sampling, where possible. For cohort B, microbiologic assessment was performed once at 36 weeks' gestation.

^b Tukey HSD post hoc tests are reported in Results section.

Vaginal Microflora

Upper vaginal microbiology assessment and culture were performed by the clinical microbiology laboratories at St Vincent's Hospital and Austin Pathology, Austin Health (Melbourne, Victoria, Australia). The presence of group B *Streptococcus* (GBS), *Candida* spp, and *Ureaplasma* spp in the vaginal tract are generally not considered as pathogens; their detection is termed as "colonization." Microbiology reports were divided into 5 groups, no significant pathogens, GBS colonization, *Candida* spp colonization, *Ureaplasma* spp Colonization, and mixed colonization (consisting of 2 or more of these groups). Microbiologic results were matched to the IL-1 α , IL-1 β , and IL-1ra concentrations of the CVF sample collected on the same day of testing.

For cohort A, microbiologic analysis was generally performed at every sampling. A total of 91 microbiology results were obtained from the 65 women recruited. One woman was treated with clindamycin for abnormal vaginal discharge, and 9 women were prescribed erythromycin for *Ureaplasma* spp colonization. For cohort B, microbiologic assessment was performed only once, usually on the day of recruitment (n = 59 women). One woman allocated into the mixed colonization group had *Mycoplasma* sp and *Candida* spp. One woman diagnosed with candidiasis was treated with clotrimazole pessaries.

Quantitation of IL-1 and IL-1ra

The CVF samples in cohort B were quantitated prior to the completion of sample collection in cohort A. The ELISA kit used to analyze samples from cohort B was discontinued by the manufacturer; therefore, a different ELISA kit was used for sample analysis for cohort A. For cohort A, IL-1 β was quantitated using the IL-1 β /IL-1F2 DuoSet ELISA (R&D Systems, Minneapolis, Minnesota). The sensitivity, interassay, and intraassay coefficient of variations (CVs) were 3.91 pg/mL, 6.5%, and 6.2%, respectively. Samples below the detectable

IL-1 β assay range were assigned as 0. The IL-1ra was quantitated using the IL-1ra/IL-1F3 DuoSet ELISA (R&D Systems). The sensitivity, interassay, and intraassay CVs for the IL-1ra assay were 3.6 pg/mL, 6.0%, and 5.6%, respectively.

For cohort B, IL-1 α was quantitated using the IL-1 α CytoSet ELISA (BioSource International, Camarillo, California). The sensitivity, interassay, and intraassay CVs for the IL-1 α assay were 2.49 pg/mL, 3.5%, and 5.7%, respectively. The IL-1 β was quantitated using the IL-1 β singleplex express assay (Bio-Rad, Hercules, California). The linear range of the assay was 3.2 to 3261 pg/mL; the sensitivity of the assay was 0.6 pg/mL, and the interassay and intraassay CVs were 8% and 6%, respectively. The IL-1ra was quantitated using the IL-1ra CytoSet ELISA (BioSource International). The sensitivity of the assay was 4.0 pg/mL; interassay and intraassay CVs were 5.2% and 4.1%, respectively. Samples below the detectable range were assigned as 0.

Data presented in the results section were corrected for total protein and expressed as per milligram of protein. The total protein of all CVF samples was determined using the Bicinchoninic acid protein assay (Pierce, Rockford, Illinois).

Statistical Analyses

Statistical analyses were performed using SPSS (v17.0; SPSS Inc, Chicago, Illinois). Data for each cohort were assessed for homogeneity of variance and were not normally distributed ($P < .05$, Kolmogorov-Smirnov test). In order to utilize the parametric 2-way analysis of variance (ANOVA), data were transformed to normality. The IL-1 α and IL-1 β were cube root transformed, while IL-1ra was square root transformed. All statistical comparisons between the groups were performed using 2-way ANOVA with post hoc testing using Tukey honestly significant difference (HSD), where appropriate. To account for multiple sampling from the same woman, each patient was included as a random factor in all statistical models. The presence of specific vaginal microflora and the potential influence of unprotected sexual

Figure 1. In cohort A (65 women, $n = 123$), IL-1 β displayed no differential expression between the groups (A; $P = .799$, 2-way ANOVA) nor linear trend (B; $P = .234$, 2-way ANOVA) with advancing gestational age from 24 to 35 weeks. There was no differential expression in IL-1ra concentration between the groups (C; $P = .145$, 2-way ANOVA) despite a mild increasing linear trend for IL-1ra was observed (D; $P = .032$, 2-way ANOVA). ANOVA indicates analysis of variance; IL-1 β , interleukin 1 β ; IL-1ra, IL-1 receptor antagonist.

intercourse on the concentrations of IL-1 or IL-1ra were investigated with “gestational age” or “days from labor” included as a covariate factor in statistical analyses.

Temporal changes in the concentration of IL-1 α , IL-1 β and IL-1ra were analyzed between the groups (cohort A: 24-25, 26-27, 28-29, 30-31, 32-33, and 34-35 weeks; cohort B: in-labor, 0-3, 4-14, and >14 days before labor). Median values were used to calculate the fold changes. The box and whiskers plots were produced using GraphPad Prism v5.02 (GraphPad Software, San Diego, California); the boxes represent the median and interquartile range, while the whiskers represent the 5th and 95th centiles.

Linear regression analysis (2-way ANOVA) was used to examine the linear relationship of IL-1 and IL-1ra with advancing gestational age or sampling-to-labor intervals. Scatterplots were graphed using square- or cube root-transformed values and GraphPad Prism v5.02.

Correlation was performed using Pearson r . Receiver-operator characteristic (ROC) curves were plotted for IL-1 α , IL-1 β

and IL-1ra, and the threshold values were determined for each variable to evaluate their utility as individual predictors of labor. Binary logistic regression (patients included as a categorical variable for multiple sampling) provided the sensitivity, specificity, PPV, and NPV of a 3-cytokine model to predict labor onset. Statistical significance was assumed when $P < .05$.

Results

Cohort A

Each woman contributed 1 to 4 CVF samples ranging from 16 to 117 days prior to spontaneous labor onset. As the samples were collected remote from labor onset, they were grouped according to the gestational age as follows: 24 to 25 (22 women, $n = 22$ samples), 26 to 27 (18 women, $n = 18$), 28 to 29 (27 women, $n = 27$), 30 to 31 (22 women, $n = 22$), 32 to 33 (25 women, $n = 25$), and 34 to 35 (9 women, $n = 9$) weeks' gestation.

Figure 2. IL-1 α , IL-1 β , and IL-1ra concentrations obtained from cohort B during late pregnancy. A, IL-1 α at 4 to 14 days was 1.45-fold significantly higher than 0 to 3 days before labor but did not display any linear trend with approaching labor onset (B; 84 women, $n = 215$, $P = .378$, 2-way ANOVA). C, IL-1 β at 4 to 14 days was 4.55- and 1.91-fold significantly higher than both 0 to 3 days and >14 days but did not display any linear trend with approaching labor onset (D; 81 women, $n = 211$, $P = .145$, 2-way ANOVA). E, IL-1ra was significantly decreased in labor and 0 to 3 days before labor onset compared with 4 to 14 days (3.0- and 1.6-fold, respectively) and >14 days prior to labor (3.7- and 2.0-fold, respectively). IL-1ra demonstrated a significant linear decreasing trend toward labor (F; 88 women, $n = 240$, $P = .005$, 2-way ANOVA). P values in (A), (C), and (E) represent Tukey HSD post hoc tests. ANOVA indicates analysis of variance; HSD, honestly significant difference; IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist.

Figure 3. Receiver–operator characteristic curves for IL-1 α (AUC = 0.649), IL-1 β (AUC = 0.533), and IL-1ra (AUC = 0.693), and the combination of all the 3 biomarkers (AUC = 0.963, 80 women, $n = 204$) to predict the onset of term labor within 3 days. AUC indicates area under the curve; IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist.

In cohort A, IL-1 β concentrations were significantly different between women with no pathogens isolated, colonized by GBS, *Candida* spp, *Ureaplasma* spp, or mixed colonization (Table 2; $P = .036$). The IL-1 β concentrations associated with *Candida* spp colonization were significantly higher than no pathology (6.1-fold, $P < .001$), GBS (7.3-fold, $P = .008$) and *Ureaplasma* spp colonization (14.3-fold, $P < .001$, Tukey HSD). The IL-1ra concentration were not affected by the different pathogens (Table 2; $P = .157$).

There was no significant difference in IL-1 β and IL-1ra concentrations in samples collected from women who reported having unprotected sexual intercourse in the preceding 48 hours ($n = 14$) versus those that did not ($n = 55$; IL-1 β $P = .576$, IL-1ra $P = .767$).

The IL-1 β and IL-1ra were assayed in 123 samples collected from 65 women. There was no linear trend for IL-1 β with advancing gestational age ($P = .234$, Figure 1B) nor between the gestational age groups ($P = .799$, Figure 1A). The IL-1ra displayed a weak significant increasing linear trend with advancing gestational age ($P = .032$, Figure 1D) but was not different between the groups ($P = .145$, Figure 1C). The IL-1 β and IL-1ra were significantly correlated ($P < .001$, Pearson $r = .460$).

Cohort B

Each woman contributed 1 to 6 CVF samples. Samples collected ranged from in-labor to 47 days prior to spontaneous

labor onset and were stratified into 4 groups: in-labor (20–25 women, $n = 20$ –25 samples), 0 to 3 days (17–22 women, $n = 17$ –22), 4 to 14 days (52–58 women, $n = 74$ –83), and >14 days (58–66 women, $n = 98$ –110) before spontaneous labor.

The IL-1 α , IL-1 β , and IL-1ra concentrations were not different between women with no pathogens isolated, colonized by GBS, *Candida* spp, *Ureaplasma* spp, or with mixed colonization (Table 2; IL-1 α $P = .243$, IL-1 β $P = .227$, and IL-1ra $P = .178$).

There was no difference in IL-1 α , IL-1 β , and IL-1ra concentrations in samples collected from women who reported having unprotected sexual intercourse in the preceding 48 hours ($n = 26$ –29) versus those that did not ($n = 122$ –129; IL-1 α $P = .408$, IL-1 β $P = .802$, and IL-1ra $P = .443$).

A total of 216 IL-1 α measurements were performed on 84 women. The IL-1 α displayed no linear trend with approaching labor ($P = .378$, Figure 2B). However, the IL-1 α concentration in the CVF was significantly different between the groups ($P = .001$). The IL-1 α concentration at 4 to 14 days was significantly higher ($\uparrow 1.45$ -fold) than 0 to 3 days before labor onset ($P = .024$, Tukey HSD; Figure 2A).

The IL-1 β was assayed in 211 samples collected from 81 women. There was no linear trend with approaching labor ($P = .145$, Figure 2D). The IL-1 β concentration in the CVF was significantly different between the groups ($P = .050$). The IL-1 β concentration at 4 to 14 days was significantly higher than 0 to 3 days ($\uparrow 4.55$ -fold, $P = .012$) and >14 days ($\uparrow 1.91$ -fold, $P = .005$, Tukey HSD) prior to labor onset (Figure 2C).

A subset of IL-1ra data presented in this study has been previously published.⁸ We corrected the IL-1ra data for total protein in this current study and have included additional samples. In total, IL-1ra was assayed in 240 samples collected from 88 women. There was a significant linear decreasing trend toward labor onset ($P = .005$, Figure 2F). The IL-1ra concentrations were significantly different between the groups ($P < .001$). The IL-1ra was significantly decreased in labor compared with 4 to 14 days ($\downarrow 2.96$ -fold, $P < .001$) and >14 days ($\downarrow 3.66$ -fold, $P < .001$, Tukey HSD) prior to labor onset. The IL-1ra concentration at 0 to 3 days before labor onset was also significantly lower than at 4 to 14 days ($\downarrow 1.62$ -fold, $P = .023$) and >14 days ($\downarrow 2.01$ -fold, $P = .004$, Tukey HSD; Figure 2E). All correlations were significant ($P < .001$, Pearson r): IL-1 α and IL-1 β ($r = .546$, $n = 204$); IL-1 α and IL-1ra ($r = .491$, $n = 216$); and IL-1 β and IL-1ra ($r = .543$, $n = 211$).

Predicting Term Labor

Individual ROC curves IL-1 α , IL-1 β , and IL-1ra as well as the combination of all 3 cytokines (using predicted probabilities derived from binary logistic regression) were generated to predict spontaneous onset of term labor within 3 days of sampling (Figure 3). A total of 204 samples from 80 women in cohort B screened for all 3 cytokines were used in this part of the analysis. The combined cytokine model yielded 86% sensitivity, 92% specificity, 69% PPV, and 97% NPV to predict labor (Table 3).

Table 3. Efficiency of IL-1 α , IL-1 β , and IL-1ra and the Combination of All the 3 Biomarkers to Predict Spontaneous Term Labor Within 3 days of Onset in 80 Women From Cohort B (Total n = 204 Samples; Samples Ranged From In-Labor and up to 47 Days Prior to Spontaneous Labor Onset).

	IL-1 α	IL-1 β	IL-1ra	IL-1 α , IL-1 β , and IL-1ra
Concentration thresholds	95 pg/mg protein	120 pg/mg protein	0.37 μ g/mg protein	–
Probability cutoff value	–	–	–	0.7
Area under the curve	0.649	0.533	0.693	0.963
95% confidence interval	0.551-0.747	0.428-0.637	0.598-0.788	0.938-0.989
P value	.005	.540	<.001	<.001
Sensitivity, %	52.8	52.8	52.8	86.1
Specificity, %	77.4	66.1	76.2	91.7
Positive predictive value, %	33.3	25.0	32.2	68.9
Negative predictive value, %	88.4	86.7	88.3	96.9
False positive rate, %	22.6	33.9	23.8	8.3
False negative rate, %	47.2	47.2	47.2	13.9

Abbreviations: IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist.

Discussion

This study has examined the interplay of the IL-1 system beyond 24 weeks' gestation in the human CVF. From 24 to 35 weeks' gestation, IL-1 β and IL-1ra appear to maintain a relatively consistent baseline expression. The IL-1ra may play a role in the dampening of the proinflammatory response of IL-1 during this period of pregnancy to maintain uterine quiescence. It should be emphasized that the bioavailability of IL-1ra is several thousand times greater than the combined bioavailability of IL-1 α and IL-1 β . The IL-1 α and IL-1 β concentrations remained unaltered in late pregnancy, while IL-1ra displayed a significant linear decrease with approaching labor. The significant correlations between these 3 cytokines provide evidence of their well-established biologic interdependence.

A subset of the IL-1ra data obtained from women in late pregnancy and included in the current study has been published previously.⁸ In that publication, IL-1ra was similarly found to significantly decrease with approaching spontaneous labor and demonstrated a similar 3- to 4-fold decrease in labor. Data from the previous publication were not corrected for total protein. In our experience, the total yield of CVF protein can vary widely between women. To accurately quantify the measurement of any biomarker, we have normalized the data by correcting for total protein. We found that correcting the IL-1ra data for total protein in the current study did not differ from our initial IL-1ra interpretation in late pregnancy.⁸

Numerous studies have investigated the biologic effects of IL-1 during parturition and its increase in serum, gestational tissues, and amniotic fluid.²³⁻²⁹ Similarly, previous studies of the CVF have described impending parturition and labor onset to be associated with an increase in IL-1 β concentration.¹⁶⁻¹⁹ We failed to observe an increase in IL-1 β in the CVF in our study. This conflicting IL-1 β result may partly explain why IL-1 β is yet to be translated into clinical use to predict spontaneous labor or the successful induction of labor. In contrast, our IL-1 α data are consistent with Imseis et al,¹⁹ where they also reported no change in CVF IL-1 α with labor onset.

The constant level of IL-1 and the decrease in IL-1ra with impending term labor may augment the actions of IL-1 in the

myometrium, cervix, and fetal membranes as the bioavailability of IL-1ra is reduced.^{26,30-33} We previously reported that IL-1ra appears to be in lower concentrations in women with PROM and regular contractions occurring ≥ 1 hour after compared with women with spontaneous onset of labor with intact membranes.⁸ In addition, we recently found that IL-1ra was significantly decreased by 40% in the CVF of women who subsequently experienced preterm PROM compared with gestation-matched controls.³⁴ Future studies elucidating the direct role of IL-1ra in fetal membrane physiology could be of interest.

The complex physiology of parturition precludes the likely opportunity of finding a single biomarker that reliably predicts human labor. A multiple biomarker diagnostic test with improved sensitivity and specificity would therefore be more reliable and efficacious in predicting labor.^{20,35,36} In addition, the final pathway of cervical remodeling, myometrial activation, and membrane rupture common to term and preterm labor suggests the potential translation of term labor biomarker(s) to predict preterm labor. Compared with each individual cytokine, the combination of all the 3 cytokines to predict term labor improved the area under the curve and predictive values. For term pregnancies where labor is expected, a highly specific test with a high NPV may be a useful indicator of "failure to labor," which would invariably require medical intervention.

The fFN predicts term labor within 2 to 7 days of sampling with a wide range of sensitivity, specificity, PPV, and NPV,^{5,17,37-41} while IL-1 β predicts term labor within 7 days of sampling with 76% sensitivity, 55% specificity, 50% PPV, and 79% NPV.¹⁷ Although the predictive properties of IL-1 and IL-1ra in this current study (either individually or in combination) cannot be directly compared with these previous fFN and IL-1 β studies due to different sample-to-delivery time frames and study populations, we believe the combination of the 3 biomarkers (IL-1 α , IL-1 β , and IL-1ra) to predict spontaneous labor compares favorably to fFN with improved PPV.

An ideal diagnostic test should be robust to the high phenotypic variation between women caused by genetic, environmental, health condition, and lifestyle factors. Moreover, with regard to

human CVF, any diagnostic test should also be robust to the influence of vaginal microflora, semen, and vaginal bleeding. The wide range in upper vaginal microflora did not influence CVF's IL-1 and IL-1ra concentrations at term. The microflora between 24 and 35 weeks gestation did not affect IL-1ra concentration, whereas IL-1 β was significantly increased when *Candida* spp was detected. These data must be interpreted with caution due to small sample size. It is also possible that erythromycin treatment may have influenced IL-1 or IL-1ra CVF concentrations associated with *Ureaplasma* spp. colonization between 24 and 35 weeks. Unprotected sexual intercourse did not influence CVF IL-1 and IL-1ra concentrations in this and other studies,^{42,43} but it is known to influence fFN testing.⁴⁴

In conclusion, this study describes the differential expression of IL-1 α , IL-1 β , and IL-1ra beyond 24 weeks' gestation and until spontaneous term labor onset. Although IL-1 α and IL-1 β showed little or no change, IL-1ra concentration remained fairly constant throughout gestation followed by a rapid decline in the final days before spontaneous labor. These changes may be indicative of a shifting inflammatory balance in the gestational tissues prior to labor onset. In the future, it may also be beneficial to include fFN and/or insulin growth factor-binding protein 1⁴⁵ in a multiple marker model to predict labor.

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Authors' Note

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Declaration of Conflicting Interests

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References

- Di Quinzio MK, Oliva K, Holdsworth SJ, et al. Proteomic analysis and characterisation of human cervico-vaginal fluid proteins. *Aust N Z J Obstet Gynaecol*. 2007;47(1):9-15.
- Pereira L, Reddy AP, Jacob T, et al. Identification of novel protein biomarkers of preterm birth in human cervical-vaginal fluid. *J Proteome Res*. 2007;6(4):1269-1276.
- Honest H, Bachamann LM, Gupta JK, Kleijnen J, Khan KS. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review. *BMJ*. 2002;325(7359):301.
- Norwitz ER, Snegovskikh VV, Caughey AB. Prolonged pregnancy: when should we intervene? *Clin Obstet Gynecol*. 2007;50(2):547-557.
- Lockwood CJ, Moscarelli RD, Wein R, et al. Low concentrations of vaginal fetal fibronectin as a predictor of deliveries occurring after 41 weeks. *Am J Obstet Gynecol*. 1994;171(1):1-4.
- Lagrew DC, Freeman RK. Management of postdate pregnancy. *Am J Obstet Gynecol*. 1986;154(1):8-13.
- Di Quinzio MK, Georgiou HM, Holdsworth-Carson SJ, et al. Proteomic analysis of human cervico-vaginal fluid displays differential protein expression in association with labor onset at term. *J Proteome Res*. 2008;7(5):1916-1921.
- Heng YJ, Di Quinzio MK, Permezel M, Rice GE, Georgiou HM. Interleukin-1 receptor antagonist in human cervicovaginal fluid in term pregnancy and labor. *Am J Obstet Gynecol*. 2008;199(6):656.
- Efrati P, Presentey B, Margalith M, Rozenszajn L. Leukocytes of normal pregnant women. *Obstet Gynecol*. 1964;23:429-432.e1-7.
- Liggins GC. Cervical ripening as an inflammatory reaction. In: Ellwood DA, Anderson ABM, Embrey MP, eds. *The Cervix in Pregnancy and Labour*. Edinburgh, IN: Churchill-Livingstone; 1989:1-9.
- Haddad R, Tromp G, Kuivaniemi H, et al. Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature. *Am J Obstet Gynecol*. 2006;195(2):394.
- Osman I, Young A, Ledingham M, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. *Mol Hum Reprod*. 2003;9(1):41-45.
- Thomson AJ, Telfer JF, Young A, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. *Hum Reprod*. 1999;14(1):229-236.
- Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol*. 1998;16:27-55.
- Rizzo G, Capponi A, Rinaldo D, et al. Interleukin-6 concentrations in cervical secretions identify microbial invasion of the amniotic cavity in patients with preterm labor and intact membranes. *Am J Obstet Gynecol*. 1996;175(4 pt 1):812-817.
- Tanaka Y, Narahara H, Takai N, Yoshimatsu J, Anai T, Miyakawa I. Interleukin-1beta and interleukin-8 in cervicovaginal fluid during pregnancy. *Am J Obstet Gynecol*. 1998;179(3 pt 1):644-649.
- Imai M, Tani A, Saito M, Saito K, Amano K, Nisijima M. Significance of fetal fibronectin and cytokine measurement in the cervicovaginal secretions of women at term in predicting term labor and post-term pregnancy. *Eur J Obstet Gynaecol Reprod Biol*. 2001;97(1):53-58.
- Cox SM, King MR, Casey ML, MacDonald PC. Interleukin-1 beta, -1 alpha, and -6 and prostaglandins in vaginal/cervical fluids of pregnant women before and during labor. *J Clin Endocrinol Metab*. 1993;77(3):805-815.
- Imseis HM, Greig PC, Livengood CH III, Shunior E, Durda P, Erikson M. Characterization of the inflammatory cytokines in the

- vagina during pregnancy and labor and with bacterial vaginosis. *J Soc Gynecol Investig.* 1997;4(2):90-94.
20. Heng YJ, Di Quinzio MK, Permezel M, Rice GE, Georgiou HM. Temporal expression of antioxidants in human cervicovaginal fluid associated with spontaneous labor. *Antioxid Redox Signal.* 2010;13(7):951-957.
 21. Heng YJ, Di Quinzio MK, Permezel M, Rice GE, Georgiou HM. Cystatin A protease inhibitor and cysteine proteases in human cervicovaginal fluid in term pregnancy and labor. *Am J Obstet Gynecol.* 2011;204(3):254. e251-e257.
 22. Heng YJ, Di Quinzio MK, Liong S, et al. Temporal investigation of matrix metalloproteinases and their inhibitors in human cervicovaginal fluid in late pregnancy and labor. *Reprod Sci.* 2012;19(1):55-63.
 23. Unal ER, Cierny JT, Roedner C, Newman R, Goetzel L. Maternal inflammation in spontaneous term labor. *Am J Obstet Gynecol.* 2011;204(3):223. e221-e225.
 24. Markovic D, Vatish M, Gu M, et al. The onset of labor alters corticotropin-releasing hormone type 1 receptor variant expression in human myometrium: putative role of interleukin-1 β . *Endocrinology.* 2007;148(7):3205-3213.
 25. Schmitz T, Leroy MJ, Dallot E, Breuiller-Fouche M, Ferre F, Cabrol D. Interleukin-1 β induces glycosaminoglycan synthesis via prostaglandin E₂ pathway in cultured human cervical fibroblasts. *Mol Hum Reprod.* 2003;9(1):1-8.
 26. Ämmälä M, Nyman T, Salmi A, Rutanen EM. The interleukin-1 system in gestational tissues at term: effect of labour. *Placenta.* 1997;18(8):717-723.
 27. Opsjøn SL, Wathen NC, Tingulstad S, et al. Tumor necrosis factor, interleukin-1, and interleukin-6 in normal human pregnancy. *Am J Obstet Gynecol.* 1993;169(2 pt 1):397-404.
 28. Keelan JA, Marvin KW, Sato TA, Coleman M, McCowan LM, Mitchell MD. Cytokine abundance in placental tissues: evidence of inflammatory activation in gestational membranes with term and preterm parturition. *Am J Obstet Gynecol.* 1999;181(6):1530-1536.
 29. Romero R, Brody DT, Oyarzun E, et al. Infection and labor. III. Interleukin-1: a signal for the onset of parturition. *Am J Obstet Gynecol.* 1989;160(5 pt 1):1117-1123.
 30. Romero R, Sepulveda W, Mazar M, et al. The natural interleukin-1 receptor antagonist in term and preterm parturition. *Am J Obstet Gynecol.* 1992;167(4 pt 1):863-872.
 31. Mitchell MD, Edwin SS, Silver RM, Romero RJ. Potential agonist action of the interleukin-1 receptor antagonist protein: implications for treatment of women. *J Clin Endocrinol Metab.* 1993;76(4 pt 1):1386-1388.
 32. Brown NL, Alvi SA, Elder MG, Bennett PR, Sullivan MH. Regulation of prostaglandin production in intact fetal membranes by interleukin-1 and its receptor antagonist. *J Endocrinol.* 1998;159(3):519-526.
 33. Kniss DA, Zimmerman PD, Garver CL, Fertel RH. Interleukin-1 receptor antagonist blocks interleukin-1-induced expression of cyclooxygenase-2 in endometrium. *Am J Obstet Gynecol.* 1997;177(3):559-567.
 34. Liong S, Di Quinzio MK, Heng YJ, et al. Proteomic analysis of human cervicovaginal fluid collected before preterm premature rupture of the fetal membranes. *Reproduction.* 2013;145(2):137-147.
 35. Goldenberg RL, Iams JD, Mercer BM, et al. The preterm prediction study: toward a multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol.* 2001;185(3):643-651.
 36. Sanchez-Ramos L, Delke I, Zamora J, Kaunitz AM. Fetal fibronectin as a short-term predictor of preterm birth in symptomatic patients: a meta-analysis. *Obstet Gynecol.* 2009;114(3):631-640.
 37. Ahner R, Kiss H, Egarter C, et al. Fetal fibronectin as a marker to predict the onset of term labor and delivery. *Am J Obstet Gynecol.* 1995;172(1 pt 1):134-137.
 38. Ahner R, Kub-Csizi P, Heinzl H, et al. The fast-reacting fetal fibronectin test: a screening method for better prediction of the time of delivery. *Am J Obstet Gynecol.* 1997;177(6):1478-1482.
 39. Mouw RJ, Egberts J, Kragt H, van Roosmalen J. Cervicovaginal fetal fibronectin concentrations: predictive value of impending birth in postterm pregnancies. *Eur J Obstet Gynecol Reprod Biol.* 1998;80(1):67-70.
 40. Mouw RJ, Egberts J, van Roosmalen JJ, Kragt H. High cervical fetal-fibronectin concentrations and birth within 3 days in pregnancies of 41 weeks or more. *N Engl J Med.* 1995;332(16):1105.
 41. Luton D, Guibourdenche J, Sibony O, et al. Fetal fibronectin in the cervical secretion predicts accurately the onset of labor at term. *Eur J Obstet Gynecol Reprod Biol.* 1997;74(2):161-164.
 42. Donders GG, Vereecken A, Bosmans E, Spitz B. Vaginal cytokines in normal pregnancy. *Am J Obstet Gynecol.* 2003;189(5):1433-1438.
 43. Agnew KJ, Aura J, Nunez N, et al. Effect of semen on vaginal fluid cytokines and secretory leukocyte protease inhibitor. *Infect Dis Obstet Gynecol.* 2008;2008:820845.
 44. Leitich H, Egarter C, Kaider A, et al. Cervicovaginal fetal fibronectin as a marker for preterm delivery: a meta-analysis. *Am J Obstet Gynecol.* 1999;180(5):1169-1176.
 45. Kekki M, Kurki T, Kärkkäinen T, Hiilesmaa V, Paavonen J, Rutanen EM. Insulin-like growth factor-binding protein-1 in cervical secretion as a predictor of preterm delivery. *Acta Obstet Gynecol Scand.* 2001;80(6):546-551.