Does Intra-Amniotic Inflammation Influence Pregnancy Outcome After Cerclage Orprogesterone (17OHP-C) Therapy For Midtrimester Short Cervix?

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CYTOKINE INFLAMMATORY SCORE PREDICTS PREGNANCY OUTCOME IN WOMEN WITH MIDTRIMESTER SHORT CERVIX

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Abstract

OBJECTIVE:
We propose a novel amniotic fluid (AF) cytokine score based on a comprehensive cytokine analysis of patients with mid-trimester short cervix.

STUDY DESIGN:
AF was collected from singleton gestations (n=44) with a cervical length of ≤25mm at 16-24 weeks gestation and assayed for 25 cytokines simultaneously using the Bio-Plex™ system. Univariate and regression analyses identified which mediators were detected in substantial quantities and predictive of delivery. The distribution of the inflammatory score, clinical characteristics, and delivery outcome are reported.

RESULTS:
Univariate analysis identified 13 cytokines that were undetected in at least 25% of patients. Patients were assigned 1 point for each of these cytokines if their level was >2 times the level of detection. Levels of IL-6, IL-8, G-CSF, Interferon-γ, MCP-1, MIP-1β, or RANTES in the upper quartile were predictive of delivery. Patients were assigned 1 point for each of these cytokines in the upper quartile. 5 cytokines were detected in almost all patients, but were not predictive of delivery. The inflammatory score ranged from 0-20 and was bimodally distributed (Figure1). Patients with a score <10 (n=18) had a shorter cervical length and delivered significantly earlier (Table 2, P<0.001 for each, Wilcoxon). A score ≥10 had a 77% sensitivity, 100% specificity, 100%PPV, 81%NPV for delivery <32 weeks.

CONCLUSION:
A cytokine inflammatory score is related to delivery outcome & clinical characteristics. If shown to be predictive in future studies, this score may aid in choosing therapies based on the presence or absence of inflammation.

Introduction

- Many, but not all, patients with mid-trimester short cervix (≤25mm) have elevated levels of cytokines. However, most studies have only examined one or two cytokines at a time.
- We propose a novel amniotic fluid (AF) cytokine score based on a comprehensive cytokine analysis of patients with midtrimester short cervix.

Materials and Methods

- Study cohort:
  - Singleton pregnancies
  - Gestational age 16-24 weeks
  - Transvaginal cervical length of ≤25mm
  - Patients who received no surgical or hormonal treatment for short cervix
  - No known fetal chromosomal or structural abnormality, vaginal bleeding, or ruptured membranes
  - Patients underwent amniocentesis with an aliquot frozen for cytokine analysis. Amniotic fluid (AF) samples were simultaneously analyzed for 25 inflammatory mediators using the Bio-Plex™ array system (Bio-Rad Laboratories, Hercules, CA). See Table 1 for a listing of the cytokines utilized in our analysis.

Statistical Analysis

- For each cytokine, univariate analysis was used to examine the distribution, define the upper quartile, and determine the proportion of samples which had cytokine levels below the level of detection for the assay.
- The ability of cytokine levels in the upper quartile to predict interval to delivery (within 28 days of amniocentesis and before 34 weeks gestation) was calculated from patients who delivered near-term for each cytokine separately using odds ratios.

- Data from the above analysis was used to determine which cytokines should be included in the AF cytokine score.
- The AF cytokine score was determined for each patient.
- The distribution of the score, its relationship to other clinical characteristics, and its ability to predict perinatal outcome were then examined.
- A P value <0.05 was required for statistical significance, with utilization of the Bonferroni correction where appropriate to control for simultaneous examination of 25 cytokines.

Table 1. Univariate Analysis

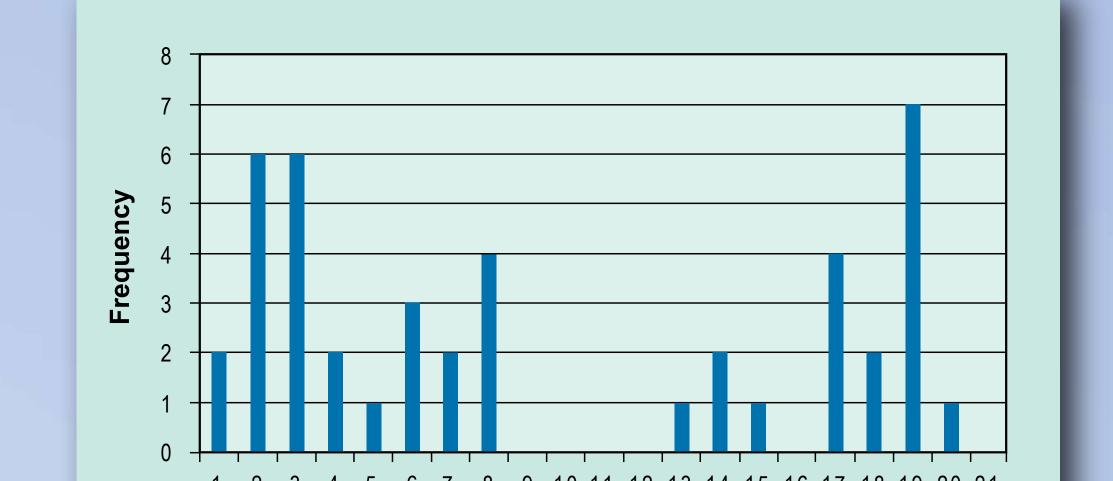| Cytokine	Median (pg/ml)	Lower limit of detection (pg/ml)	% of samples below level of detection	Odds (99.8% CI) Ratio to Predict Delivery within 28 days	Odds (99.8% CI) Ratio to Predict Delivery before 34 weeks
Cytokines with >25% undetected; 1 point given for each cytokine if level was >2 times the lower limit of detection					
IL-1β	0.9	0.8	47.7	21.60 (1.81 - 258.13)	40.00 (2.65 - 604.53)
IL-2	1.4	1.1	47.7	5.67 (0.73 - 43.99)	6.00 (0.75 - 48.15)
IL-4	0.5	0.5	52.3	6.33 (0.72 - 55.96)	7.80 (0.76 - 79.72)
IL-5	0.8	0.8	93.2	NE	NE
IL-10	1.1	0.9	45.5	17.00 (1.51 - 191.60)	15.20 (1.49 - 155.14)
IL-12	1.1	0.5	27.3	17.00 (1.51 - 191.60)	15.20 (1.49 - 155.14)
IL-13	2.1	2.1	86.4	6.87 (0.20 - 235.51)	5.56 (0.16 - 189.69)
IL-15	6.6	4.2	27.3	12.04 (1.26 - 115.26)	11.52 (1.25 - 106.52)
IL-17	0.2	0.2	61.4	70.40 (2.05 ->999.99)	NE
Eotaxin	18.2	14.6	36.4	7.08 (0.87 - 57.45)	7.31 (0.88 - 60.56)
MIP-1α	2.4	2.1	59.1	93.50 (2.56 - >999.99)	NE
TNF-α	3.0	3.0	68.2	35.74 (1.13 - >999.99)	NE
VEGF	4.7	0.5	27.3	2.13 (0.29 - 15.74)	2.46 (0.32 - 19.09)
Cytokines levels in upper quartaile were predictive of delivery; 1 point given for each cytokinein upper quartile					
IL-6	515.9	1.1	4.6	26.92 (1.76 - 411.03)	21.33 (1.78 - 255.52)
IL-8	576.7	0.5	6.8	17.00 (1.51 - 191.60)	15.20 (1.49 155.14-)
G-CSF	308.4	1.1	4.6	26.92 (1.76 - 411.03)	21.33 (1.78 - 255.52)
INF-γ	86.6	19.3	2.3	9.00 (1.04 - 78.10)	9.71 (1.06 - 89.07)
MCP-1	539.1	6.7	2.3	45.71 (1.43 - >999.99)	70.40 (2.05 - >999.99)
MIP-1β	60.2	1.1	4.6	13.71 (1.27 - 48.45)	21.33 (1.78 - 255.52)
RANTES	20.2	1.2	2.3	17.00 (1.51 - 191.60)	.20 (1.49 - 155.14)
Cytokines detected in most patients, but were not predictive of delivery; No points were given for these cytokines					
IL-1ra	5414.3	1.4	4.6	3.71 (0.517 - 26.69)	6.00 (0.75 - 48.15)
IL-7	3.9	0.5	13.6	2.92 (0.325 - 26.25)	2.27 (0.26 - 20.17)
IL-9	17.1	0.7	4.6	3.71 (0.52 - 26.69)	6.00 (0.748 - 48.15)
IL-10	26147.2	6.5	4.6	0.89 (0.10 - 7.670)	1.129 (0.130 - 9.78)
PDGF-bb	327.9	1.0	2.3	3.05 (0.43 - 21.50)	4.98 (0.63 - 39.12)

Results

- Forty-four patients met eligibility criteria.
- Univariate analysis showed 13 of the 25 cytokines were below the level of detection for the assay in at least 25% of patients (Table 1). However, in some patients, levels of these cytokines were comparatively high. Therefore, patients were assigned 1 point for each of these 13 cytokines if their level was ≥2x the lower limit of detection.
- There were seven cytokines (IL-6, IL-8, G-CSF, Interferon-γ, MCP-1, MIP-1β, or RANTES) that were detected in almost all patients, and levels in the upper quartile were predictive of delivery (both within 28 days of amniocentesis and prior to 34 weeks' gestation). Patients were assigned 1 point for each of these cytokines in the upper quartile.
- There were five cytokines (IL-1ra, IL-7, IL-9, IP-10, PDGF-bb) that were detected in almost all patients, but were not predictive of delivery. No points were assigned for these cytokines.
- Therefore, the AF cytokine score could range from 0-20. The Inflammatory Score was bimodally distributed as shown in Figure 1. Sixteen patients had a score <10 and 18 had a score ≥10.
- An Inflammatory score ≥10 had:
  - 77% sensitivity, 100% specificity, 100% PPV, 81% NPV for delivery <32 weeks.
  - 81% sensitivity, 96% specificity, 94% PPV, 85% NPV for delivery <28 weeks
- The Inflammatory score was inversely related to cervical length (Figure 2) (-0.590, P<0.0001, Spearman)
- All patients with Inflammatory Score ≥10 delivered prior to 30 weeks' gestation (Figure 3).

Table 2. Comparison of Clinical Characteristics by Score

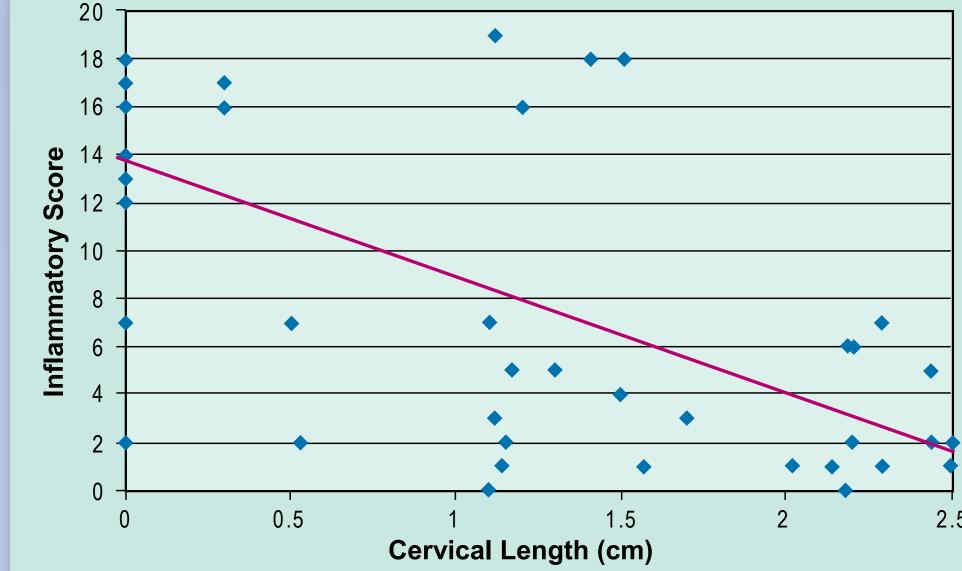| Variable	Low Inflammatory Score (<10) N=16	High Inflammatory Score (≥10) N=18	P Value*
Demographic Characteristics			
Maternal Age (yrs)	30 (16-41)	24 (16-35)	0.11
Parity	1 (0-3)	0.5 (0-2)	0.03
Gestational Age at Admission (weeks)	20 (17-24)	21.5 (18-24)	0.08
Clinical Characteristics			
Cervical Length (mm)	16 (0-25)	0 (0-55)	<0.0001
AF Glucose	41 (22-63)	25.8 (1-40)	0.003
AF WBC Count	22 (0-400)	80 (5-1400)	0.06
Pregnancy Outcome			
Delivery Interval (days)	116 (92-129)	4 (2-14)	<0.0001
pPROMn (%)	4 (15.4%)	10 (55.6%)	0.007
Gestational Age at Delivery (weeks)	38 (34-39)	23 (21-25)	<0.0001

* Wilcoxon Rank-Sum for continuous data; Fisher's Exact for proportions
** Data are presented as median (range)
***Data are presented as median (interquartile range) or n(%)

Figure 1. Distribution of AF Cytokine Inflammatory Score

Figure 2. Correlation of AF Cytokine Score with Cervical Length
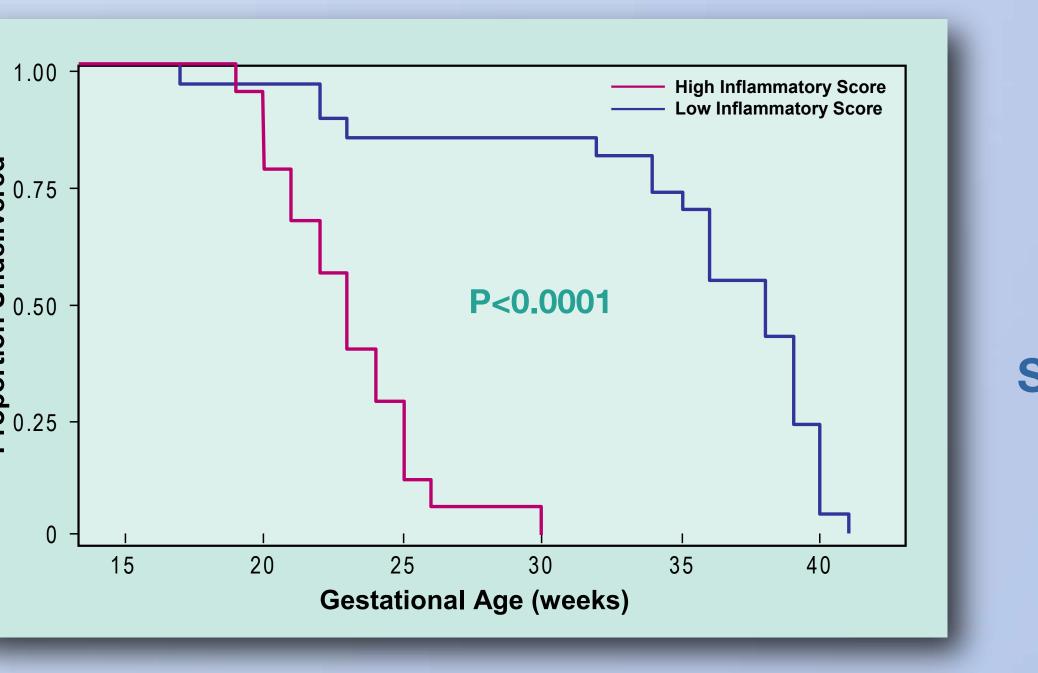

Figure 3. Kaplan-Meier Pregnancy Survival Curve by Inflammatory Score

Conclusions

- An amniotic fluid cytokine inflammatory score is related to clinical characteristics & delivery outcome.
- If shown to be predictive in future studies, this score may aid in choosing therapies based on the degree of inflammation.