Published in final edited form as:

Clin J Pain. 2020 October ; 36(10): 775–781. doi:10.1097/AJP.0000000000000861.

Plasma concentrations of select inflammatory cytokines predicts pain intensity 48 hours post-shoulder muscle injury

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Abstract

Objectives—The relationship between elevated inflammatory cytokine levels and peak pain intensity following acute musculoskeletal injury has not been fully elucidated in high risk subgroups. Identifying the role that these cytokines have on pain responses may help with developing tailored therapeutic approaches.

Methods—Data were collected from 54 participants who were vulnerable to a robust pain response and delayed recovery following musculoskeletal injury. Participants completed baseline active and resting pain measurements (Brief Pain Inventory) and a blood draw prior to an exercised induced shoulder muscle injury. Participants returned at 24 hours and 48 hours post-injury for follow-up pain measurements and blood draws. Blood plasma was analyzed for IL-1 β , IL-6, IL-8, IL-10 and TNF α . Pearson bivariate correlations were performed between cytokines and pain measurements to identify candidate variables for stepwise multiple linear regression predicting pain intensity reports.

Results—Pearson bivariate correlation identified 13/45 correlations between inflammatory cytokines and resting pain intensity and 9/45 between inflammatory cytokines and active pain ($p < 0.05$, $r = 0.3$ or $r = -0.3$). This led to 5 stepwise multiple linear regression models, of which 4 met the statistical criterion ($p < 0.0167$); including IL-10 baseline plasma concentrations predicting active pain ($r^2 = 0.19$) and resting pain ($r^2 = 0.15$) intensity 48 hours post-injury. IL-6 and IL-10 plasma concentrations at 48 hours were respectively associated with active and resting pain at 48 hours.

Discussion—These findings suggest that elevated concentrations of inflammatory cytokines, specifically IL-10 (at baseline and 48 hours) and IL-6 (at 48 hours), may play a role in heightened pain responses following exercise-induced muscle injury.

Keywords

interleukin-10; interleukin-6; shoulder pain

Introduction

Acute musculoskeletal pain is normally transient and protective in nature, alerting an individual that tissue injury is present, and homeostasis has been disrupted (1). If acute pain persists beyond a normal time-period without intervention, it can transition to chronic pain. Continuous nociception stimulation from acute pain can cause pathophysiological changes at the peripheral site of pain, within the spinal cord, and in the brain. These changes can include a transition from acute to chronic inflammation, expression of chronic pain producing genes and proteins, a reduction of pain threshold and changes in the pain matrix in the brain (2–5). The transition to a chronic pain condition is no longer viewed as a protective mechanism, but instead a symptom of disease (6). Chronic pain is a maladaptive pain response, associated with negative psychosocial behaviors (7–10) and commonly results in prolonged physical disability.

Researchers and clinicians have undertaken efforts to identify individuals who may be at increased risk for a heightened pain response following musculoskeletal injury, which puts this population at a higher risk to transition to chronic pain (11). Previous research has produced encouraging results with the identification of several genetic variants (12–14) and psychological traits (10, 15, 16) that are currently being used to predict individuals who may be at risk for experiencing heightened pain post-injury. Recent investigation into the interaction of genetic and psychological factors has identified a high-risk subgroup vulnerable to a heightened pain response and delayed recovery following musculoskeletal injury. This particular subgroup was comprised of a high pain sensitivity (HPS) catechol-O-methyltransferase (*COMT*) genotype associated with low enzyme activity and pain catastrophizing (17).

COMT is a ubiquitously expressed detoxifying enzyme involved in a number of important biochemical pathways, particularly metabolism of catecholamines, including epinephrine and norepinephrine (18). Individuals with the *COMT* single nucleotide polymorphism (SNP) rs6269 are characterized as having difficulty metabolizing catecholaminergic neurotransmitters, including epinephrine and norepinephrine. These neurotransmitters bind to beta-receptors (β_2 -adrenergic and β_3 -adrenergic) on immune cells stimulating the release of a host of inflammatory cytokines in response to tissue damage (19).

Two pro-inflammatory cytokines, tumor necrosis factor alpha (TNF α) and interleukin –1 beta (IL-1 β), are signaled by the binding of catecholamines to adrenergic receptors following injury. These cytokines are prominent during the acute phase of the inflammatory response, and help with the signaling of other pro- and anti-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8) (19). Increases in concentrations of IL-6 and

IL-8 help recruit additional leukocytes to the site of injury. As more leukocytes infiltrate the injury site, interleukin-10 (IL-10) concentrations rise, inhibiting the expression of several pro-inflammatory cytokines (20). IL-10 is an anti-inflammatory cytokine and is a key downstream regulator of the inflammatory response (21). Increased circulating levels of IL-10 following injury has also been linked with inflammatory resolution and decreased pain levels (22, 23). While difficult to categorize cytokines as only pro- or anti-inflammatory, TNF α , IL-1 β , IL-6, and IL-8 have generally been identified as having pro-inflammatory and hyper-nociceptive properties (24–27). Elevated levels of pro-inflammatory and hyper-nociceptive cytokines downstream of β_2 and β_3 adrenergic receptor stimulation are thought to be one of the drivers of *COMT*-dependent pain through sensitized peripheral nociceptors (19, 28).

To our knowledge, the relationship between cytokine concentrations and peak pain intensity in high-risk subgroups following acute musculoskeletal injury has not been studied. Further investigation is needed to identify the underlying biological mechanisms that contribute to heightened pain experienced in this high-risk subgroup with the long-term goal of developing tailored therapeutic approaches to improve pain management and functional recovery. Therefore, our primary objective was to determine the strength of the relationship between a select group of inflammatory cytokines (TNF α , IL-1 β , IL-6, IL-8, IL-10) and peak pain intensity at baseline (pre-injury), 24 hours and 48 hours post-injury. We hypothesized that plasma levels of inflammatory cytokines would be positively correlated with both active and resting pain-intensity ratings within the 48 hours following an exercise-induced muscle injury. Once relationships were established, our secondary objective was to identify which specific inflammatory cytokines best predict pain intensity ratings following an induced musculoskeletal injury.

Methods

We performed an analysis on inflammatory cytokines as predictors for shoulder pain and disability following an exercise-induced muscle injury. The data for this analysis was obtained from the parent study “Biopsychosocial Influence on Shoulder Pain: A Randomized, Pre-clinical Trial ([NCT02620579](#))”.(17) This study was approved by the Institutional Review Board at the University of Florida and conducted at the Clinical and Translational Science Institute. The parent study is still actively recruiting subjects while this paper reports planned preliminary analyses investigating the role of inflammatory cytokines in predicting pain intensity outcomes. The outcomes reported in this paper are secondary in nature and not related to the primary outcome of the ongoing trial. We are reporting the findings of the relationship between inflammatory cytokine levels and peak pain intensity with the intent to guide future inflammatory cytokine focused analyses in this high-risk cohort.

Participants

To be eligible for the study, participants had to score a minimum of five on the Pain Catastrophizing Scale (PCS) and have the *COMT* polymorphism rs6269, which was the requirement for enrollment into the full parent study (17). Fifty-four eligible participants

[$x=31$, $y=23$; mean age= 21.4 ($\sigma=4.06$); mean weight= 68.89kg ($\sigma=15.6\text{kg}$); mean height= 170.54cm ($\sigma=10.83\text{cm}$)] met these eligibility criteria and were included in the current analysis.

Procedure

The first test day consisted of collecting baseline vitals (blood pressure, heart rate, temperature, and respiratory rate); followed by pain measurements and a blood draw. A maximal voluntary isometric contraction (MVIC) of the external rotator muscle group of the shoulder was then recorded and used to determine a strength value for our experimental muscle injury protocol. Participants were then required to complete a high-intensity resistance exercise protocol for the external rotator muscle group of the shoulder. This protocol was designed to induce a controlled muscle injury characterized by delayed-onset muscle soreness (DOMS), stiffness, and functional impairment.

Follow-up assessments were performed 24 and 48 hours post-shoulder muscle injury and included collection of vitals, blood draw, MVIC, and pain measurements.

Experimental Injury Protocol

Shoulder muscle pain and impairment was induced through high-intensity resistance exercise using a commercial isokinetic testing and exercise device (Biodex System 4 Pro, Shirley, USA, model #850–000). Participants were seated in the Biodex with their dominant shoulder positioned at 45° of abduction and 90° of elbow flexion. The exercise protocol consisted of repeated concentric and eccentric actions of the shoulder external rotators at an angular velocity of $60^\circ/\text{sec}$ for both actions. Participants were instructed to exert maximal effort during the protocol and were given verbal encouragement by the examiner while performing the muscle actions. Participants completed four sets of 10 repetitions separated by 30 seconds of recovery. An MVIC was assessed at the completion of the initial four sets of exercise. If the MVIC was 50% or less of baseline value, the exercise protocol was considered complete. If the MVIC was higher than 50% value, additional sets of 10 repetitions were performed until the aforementioned criteria was fulfilled. We have had success using this model to induce a shoulder muscle injury protocol in our previous studies (29, 30). This type of experimental shoulder injury mimics several conditions we tend to observe in a clinical shoulder injury, such as an increase in musculoskeletal pain that can last for several days, an increase in shoulder disability, and the presence of an inflammatory response (29–33).

Analysis of Inflammatory Cytokines

A registered nurse or phlebotomist drew and collected blood each session using standard EDTA tubes. One teaspoon (tsp) was collected at the beginning of Days 1 – 3. Blood was centrifuged at 3000 rpm for 15 minutes and plasma was stored at -80°C . Plasma samples were then analyzed to determine concentration levels of selected inflammatory cytokines. Analysis of IL- 1β , IL-6, IL-8, IL-10, and TNF α in plasma was performed using a multiplex panel from Millipore Sigma (Milliplex, Human Cytokine/Chemokine panel 1, Millipore Corporation, Billerica, USA) as per standard operating procedure. All sample results below the lower limit of quantitation were classified as missing data and not included in the

analysis. All assays were performed by the Metabolism and Translational Science Core of the Claude D. Pepper Older Americans Independence Center. Absolute values of plasma concentrations were used for comparison in the data analyses. Plasma cytokine concentrations were collected at baseline, 24 hours post-injury, and 48 hours post-injury which totaled 15 samples (5 cytokines $\times$ 3 blood draws).

Brief Pain Inventory (BPI)

The BPI was used to measure pain intensity and duration at the shoulder. The BPI has been found to have solid test-retest reliability over short measurement intervals (34). The BPI makes use of an 11-point numerical rating scale, ranging from 0 (no pain) to 10 (worst pain imaginable). The BPI asks subjects to rate the intensity of their current pain and pain at its worst, best, and average over the past 24 hours. The BPI was used on two different pain measurements: resting pain (when the participant was not contracting the shoulder external rotator muscles); and active pain (immediately after the participant performed an MVIC of the external rotation muscles of the shoulder). BPIs were collected at baseline, 24 hours post-injury and 48 hours post-injury.

Statistical Analysis

All statistical analyses were performed using PASW Statistics 24.0 statistical package (IBM SPSS Inc., Armonk, USA). Pearson bivariate correlation coefficients were calculated to determine the strength of the statistical relationship between inflammatory cytokine concentrations and pain intensity over the 48 hour time period post-injury. We evaluated the correlations to identify the pain time points (baseline, 24 hours and 48 hours post-injury) that were candidates for regression analyses, including statistical evidence of an association ($p < 0.05$), strength of relationship ($r + 0.3$ or $r - 0.3$), and consistency of direction (all positive or all negative) for three or more inflammatory cytokines. We then inserted these candidate cytokines into a stepwise multiple linear regression analysis to predict different pain intensity outcomes, including baseline and at 24 and 48 hours post-injury.

A liberal correction for multiple comparisons was used to control for inflated alpha levels resulting from repeated statistical comparisons and a relatively small sample size for the multiple linear regression analysis. Due to the exploratory nature of the study, we divided $\alpha = 0.05$ by 3, based on the number of predictor groups we investigated (baseline, pain at 24 and 48 hours post-injury). Therefore, the level of significance was adjusted *a priori* to 0.0167 for the predictive analyses. There are more stringent alpha-correction approaches available based on the number of statistical comparisons (e.g. Bonferroni). However, given that the overall purpose of this analysis was to identify candidate markers for future consideration in a larger cohort we selected a liberal way to control for type 1 error. Regression models with the largest R-square value were presented in the results if the stepwise analysis yielded multiple statistically significant models.

Results

Correlations

Descriptive statistics for cytokine concentrations and BPI scores for resting and active pain are presented in Table 1 and Table 2 respectively. Pearson bivariate correlations examined relationships between inflammatory cytokine concentrations and pain measurements. There was a total of 90 correlations investigating inflammatory cytokines and pain (45 inflammatory cytokines and resting pain, 45 inflammatory cytokines and active pain). There were 22/90 correlations between inflammatory cytokine concentrations and pain measurements that met our candidate criterion. Resting pain was correlated with inflammatory cytokine concentrations at 13/45 time points (Table 3), while active pain was correlated with inflammatory cytokine concentrations with 9/45 time points (Table 4). IL-6/IL-10 ratio was also investigated to determine if the proportion of pro- to anti-inflammatory cytokines were related to the outcome measurements, but no correlations were detected that met our candidate criterion.

Identification of prediction models

Candidate inflammatory cytokines were entered into the stepwise regression model as prediction variables for pain. We then reported the stepwise regression models with the largest R-square values that met our statistical criteria. To investigate the stability of the regression models and to account for any biological differences related to sex, sex was added as a predictor variable in all five models. This did not contribute to any of the models in a way that met our statistical criterion ($p > 0.0167$, Table 5).

Regression analysis of inflammatory cytokine concentration with concurrent pain time points

Three models were investigated to determine whether inflammatory cytokine concentrations could be used to predict concurrent shoulder pain intensity following injury.

1. IL-1 β , IL-6, IL-10 and TNF α concentrations 48 hours post-injury with resting pain 48 hours post-injury

This first stepwise regression analysis identified IL-6 plasma concentration 48 hours post-injury as the only inflammatory cytokine to predict resting pain 48 hours post-injury ($R^2 = 0.19$; $p = 0.001$).

2. IL-1 β , IL-6, IL-10 and TNF α concentrations 48 hours post-injury with active pain 48 hours post-injury

The second stepwise regression analysis identified IL-10 plasma concentration 48 hours post-injury as the only inflammatory cytokine identified in this multiple regression to predict active pain 48 hours post-injury ($R^2 = 0.23$; $p = 0.0001$).

3. IL-1 β , IL-6 and IL-10 concentrations on 24 hours post-injury with resting pain on 24 hours post-injury.

The third stepwise regression analysis did not identify a significant contribution ($p > 0.0167$) to resting pain experienced in the shoulder 24 hours post-injury.

Regression analysis of baseline inflammatory cytokine concentrations to predict future pain

Two models were investigated to attempt to identify if baseline concentrations of inflammatory cytokines could predict shoulder pain intensity following injury.

1. IL-1 β , IL-6, IL-10 and TNF α concentrations at baseline with resting pain 48 hours post-injury.

This stepwise regression analysis identified that baseline IL-10 plasma concentrations contributed to explaining resting pain 48 hours post-injury ($R^2=0.15$; $p=0.004$; Figure 1).

2. IL-6, IL-10 and TNF α concentration at baseline with active pain 48 hours post-injury.

This second stepwise regression analysis identified that baseline IL-10 plasma concentrations predicted active pain 48 hours post-injury ($R^2=0.19$; $p=0.001$; Figure 2). Both models identified IL-10 as the only inflammatory cytokine in our regression analysis that met our statistical criteria to predict future pain experienced in the shoulder following a musculoskeletal injury.

Discussion

The positive associations between inflammatory cytokines and pain reported from this analysis are consistent with previous studies (35, 36), and indicate that individuals with higher baseline plasma concentrations of inflammatory cytokines may have higher pain responses up to 48 hours after a standardized exercise induced muscle injury. The association of increased baseline inflammatory cytokines and heightened resting and active pain may be a specific characteristic of our high-risk subgroup. We speculate that the elevated baseline cytokine concentrations contributed to the elevated pain levels at 48 hours post muscle injury by lowering pain thresholds and increasing nociceptive sensitivity in HPS individuals through peripheral or central sensitization effects. We further speculate that the elevated cytokine concentrations stimulated nociceptors post-injury at varying levels of the pain pathway or possibly of an interaction with other pain-producing enzymes and neurotransmitters, such as Substance P and cyclo-oxygenase (37–39). Of particular interest was the concentration of IL-10 at baseline. IL-10 was the only cytokine identified as significantly predicting both resting and active pain during the post-injury recovery period, such that higher IL-10 was associated with greater pain ratings. IL-10 has been characterized in the literature as an anti-inflammatory cytokine (40). However, it appears that it also may have some association with heightened inflammatory pain, and therefore may have both pro- and anti-inflammatory properties.

In this cohort of individuals at higher risk for heightened pain responses, we speculate that elevated IL-10 concentration may further increase reported pain intensity following musculoskeletal injury. One reason for this finding is IL-10 has a strong positive relationship with selected pro-inflammatory cytokines. Individuals that present with higher inflammatory cytokines collectively at baseline may be more at risk for a heightened pain response.

Few studies have investigated baseline IL-10 concentrations, however, evidence suggests that elevated levels of IL-10 prior to injury or infection may lead to poor recovery (41, 42). There is also evidence that the timing of IL-10 release could be essential for proper pain recovery post-injury (43). If IL-10 is released too early or too late during the inflammatory response, secondary tissue damage that perpetuates pain responses could occur. The secondary damage of healthy cells is primarily a result of the reduced effectiveness of the coagulation cascade early in the inflammatory response. Pre-mature cell signaling by IL-10 has been linked to the overproduction of Activated Protein C (APC) (43, 44). APC plays an integral role as an anti-coagulation agent in the inflammatory response, and if activated prematurely may alter the early stages of the coagulation cascade following tissue damage, thus extending the acute inflammatory response (44).

Higher concentrations of baseline levels of IL-10 have been identified as a predictor of long-term adverse cardiovascular outcomes in patients with acute coronary syndrome (45, 46). Higher concentrations of IL-10 have also been shown to delay healing in skin lesions of murine models (47). Elevated levels of IL-10 may delay the recruitment of macrophages to the site of injury. The combination of elevated levels of IL-10 and premature signaling of APC could extend the acute inflammatory response, thus amplifying pain at rest and following movement. Additionally, systemic increases of IL-10 have been shown to mediate both chronic and movement-evoked pain (22, 48).

Psychological and physical stress may also be considered as a cause for any increase of IL-10 concentration at baseline in this study. There is evidence that stress can cause a systemic increase of IL-10 (49). The key characteristics of this high-risk subgroup (i.e. pain catastrophizing and *COMT* polymorphism) could place individuals at higher risk for an exaggerated psychological stress response that could potentially increase IL-10 expression. There were many aspects of the protocol that could be considered a stressor, including the blood draws and the experimental injury protocol. We speculate that some participants may have experienced an elevated psychological response due to heightened levels of stress and anxiety experienced during the early stages of the enrollment period when baseline testing for the parent study was being performed.

In this sample, IL-6 was the only pro-inflammatory cytokine identified to consistently predict concurrent pain in the shoulder muscles at 48 hour post-injury. IL-6 is a known hyperalgesic cytokine (50–52) and is important in signaling the recruitment of IL-10 to the site of injury (24). It is not surprising that IL-6 concentration at 48 hours post-injury was the best predictor of participants' resting pain 48 hours post-injury, as IL-6 is active throughout the inflammation response.

One of the primary limitations of our study was that the time points of our blood draws were limited by the parent study. TNF α , IL-1 β and IL-6 may all reach peak concentrations within the first 24 hours following similar injury protocols (53–56). We did not collect any blood during this time period, and future studies should consider different blood collection time points to observe peak concentrations. This study also did not include a parallel 'control group', defined as a population that was not at high-risk for a heightened pain response. The ability to compare between groups would be beneficial to fully understand the impact that

inflammatory cytokines have on predicting pain, and whether our findings are specific to this high-risk group. This study may also have benefitted with the inclusion of more participants.

Future research should further investigate in the relationships and interactions between pro- and anti-inflammatory cytokines during recovery from musculoskeletal injury. IL-10 was the only “anti-inflammatory” cytokine we examined in this study, including other major anti-inflammatory cytokines such as IL-4, IL-11 and IL-13 (40) would also be worth investigating. Including these cytokines would allow use to see if this prediction is unique to just IL-10 or if including more anti-inflammatory can improve the model. Utilizing a different pain model to examine if inflammatory cytokines have the ability to predict neuropathic pain or musculoskeletal pain at a different location, such as the back, may also warrant further investigation.

Conclusion

Baseline plasma levels of IL-10 contributed to the prediction of heightened pain responses 48 hours following exercise-induced muscle injury in individuals in a high-risk subgroup characterized by pain catastrophizing and a COMT polymorphism. IL-6 plasma concentrations were concurrently linked to pain responses in this injury model, with 48-hour levels being positively associated with resting pain at 48-hours post-injury. Collectively these results provide preliminary evidence supporting the continued investigation of specific biomarkers to help refine the prediction of which individuals are likely to experience heightened pain responses following a standard muscle injury protocol. Future research identifying molecules in clinical populations that are involved with perceived pain will help improve pain treatment outcomes by identify ‘at-risk’ individuals and developing therapeutic interventions to mediate these biomarkers.

Sources of Support

Authors (PB, RF, SG) were supported by AR055899 from the National Institute of Health (NIH)/National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

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Correlation of IL-10 plasma concentration at baseline with resting pain 48 hours post-injury

Figure 1 - Scatterplot between IL-10 blood plasma concentration and resting pain 48 hours post-injury, $r^2=0.15$, $p<0.0167$

Plasma concentration of IL-10 48 hours post-injury and active pain 48 hours post-injury

Figure 2 -

Scatterplot regression analysis between IL-10 blood plasma concentration and active pain 48 hours post-injury, $r^2=0.19$, $p<0.0167$.

Table 1.

Mean inflammatory cytokine concentrations (pg/ml) at baseline, 24 hours post-injury, and 48 hours post-injury

	Mean inflammatory cytokine concentration (pg/ml)		
	Baseline	24 hours post-injury	48 hours post injury
IL-1 β	1.38 (σ =1.58)	1.34 (σ =1.26)	1.50 (σ =1.69)
IL-6	2.84 (σ =3.17)	2.71 (σ =2.49)	3.08 (σ =3.48)
IL-8	3.83 (σ =3.23)	3.97 (σ =2.84)	3.85 (σ =3.00)
IL-10	9.23 (σ =9.38)	8.96 (σ =7.44)	10.00 (σ =10.73)
TNF α	2.22 (σ =1.62)	2.38 (σ =1.39)	2.51 (σ =1.80)

Table 2.

Brief Pain Inventory (BPI) scores for resting and active pain at baseline, 24 hours post-injury, and 48 hours-post injury

	Mean BPI Resting	Mean BPI Active
Baseline	0.11 ($\sigma=0.50$)	1.12 ($\sigma=1.22$)
24 hours post-injury	2.52 ($\sigma=1.75$)	1.95 ($\sigma=1.87$)
48 hours post-injury	2.24 ($\sigma=2.26$)	1.84 ($\sigma=2.01$)

Table 3.R-values of inflammatory cytokines correlated with resting pain (α set; $p < 0.05$)

	Baseline	24 hours post-injury	48 hours post-injury
IL-1 β BL	-	-	0.330
IL-6 BL	-	-	0.376
IL-8 BL	-	-	-
IL-10 BL	-	-	0.390
TNF α BL	-	-	0.346
IL-1 β D2	-	-	-
IL-6 D2	-	-	0.376
IL-8 D2	-	-	-
IL-10 D2	-	-	0.373
TNF α D2	-	-	-
IL-1 β D3	-	0.330	0.358
IL-6 D3	-	0.336	0.430
IL-8 D3	-	-	-
IL-10 D3	-	0.330	0.427
TNF α D3	-	-	0.383

Table 4.R-values of inflammatory cytokines correlated with active pain (α set; $p < 0.05$)

	Baseline	24 hours post-injury	48 hours post-injury
IL-1 β BL	-	-	-
IL-6 BL	-	-	0.390
IL-8 BL	-	-	-
IL-10 BL	-	-	0.434
TNF α BL	-	-	0.332
IL-1 β D2	-	-	-
IL-6 D2	-	-	0.380
IL-8 D2	-	-	-
IL-10 D2	-	-	0.390
TNF α D2	-	-	-
IL-1 β D3	-	-	0.327
IL-6 D3	-	-	0.456
IL-8 D3	-	-	-
IL-10 D3	-	-	0.481
TNF α D3	-	-	0.401

Table 5.

Contribution of sex as a predictor for analyzed multiple linear regression analysis

Multiple Regression Model	P-Value	Beta
Resting pain 48hrs w/ concurrent cytokines	0.814	−0.030
Active pain 48 hrs w/ concurrent cytokines	0.757	−0.039
Resting pain 24hrs w/ concurrent cytokines	0.965	0.006
Resting Pain 48 hrs w/ baseline cytokines	0.934	−0.011
Active Pain 48 hrs w/ baseline cytokines	0.393	−0.109