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Human Synovial Fluid Interleukin-6, but not Type II Collagen Breakdown, Positively Correlated with Pain After ACL Injury and Reconstruction

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Abstract

Anterior cruciate ligament (ACL) injury initiates a biochemical cascade thought to contribute to the onset and progression of posttraumatic osteoarthritis (PTOA). Interleukin-1ß (IL-1ß), IL-6, and C-telopeptide fragments of type II collagen (CTX-II) are implicated in joint inflammation and cartilage degradation following ACL injury; however, their association with pain is still being explored. The purpose of this study was to evaluate the associations between synovial fluid concentrations of IL-1ß, IL-6, and CTX-II with pain following ACL injury and reconstruction. We hypothesized that greater IL-1ß, IL-6, and CTX-II would correlate with greater Pain Visual Analogue Scale (VAS) scores. This was a secondary analysis of 23 patients (mean age=18.4 y, BMI=27.4, 13 Females/10 Males) with acute ACL tears who participated in a pilot randomized trial. Synovial fluid and VAS scores were collected on the day of initial presentation, at ACL reconstruction, and 1- and 4-weeks after surgery. Synovial fluid concentrations of IL-1ß, IL-6, and CTX-II were assessed using enzyme linked immunoabsorbent assays (ELISA), and repeated measures correlations were used to assess the relationships between pain and synovial IL-1ß, IL-6, or CTX-II after ACL injury and reconstruction. Pain was positively correlated with synovial fluid IL-6 concentrations ($r=0.52$, $p<0.001$); however, pain was inversely correlated with CTX-II ($r=-0.39$, $p=0.002$). IL-1ß had no significant correlation with pain.

Keywords

anterior cruciate ligament; knee; cartilage; pain; biomarker

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Introduction

Post-traumatic osteoarthritis (PTOA) develops following anterior cruciate ligament (ACL) injuries, and the incidence of PTOA following ACL injury is as high as 87%.[1] Because knee injuries commonly occur in young, active patients, the onset of PTOA is often earlier than idiopathic OA.[1] Those with a history of joint trauma are diagnosed with OA 10 years earlier than those without a history of joint injury[2], indicating that PTOA can be an especially debilitating disease with the potential to affect younger patients earlier in life.

OA was previously thought to be a mechanical "wear and tear" disease, but inflammation may play a key role in the pathogenesis of knee OA and its associated pain.[3] Several of the inflammatory mediators that have been implicated as potentially contributing to OA pathogenesis include interleukins and tumor necrosis factors (TNF).[4] As part of the inflammatory response following acute ACL injury, intra-articular levels of pro-inflammatory cytokines interleukin-1ß (IL-1ß) and IL-6 increase. The initial acute phase of inflammation after an injury is followed by a subacute phase, which can last more than three months following initial injury.[5,6] When inflammation does not resolve, the joint enters a low grade chronic inflammatory state that may last months to years and is characterized by elevated levels of pro-inflammatory cytokines, proteolytic enzymes, markers of tissue injury, and complement components.[2] This prolonged inflammatory response may play a role in precipitating cartilage injury.[6,7] One marker of joint degradation, specifically of the cartilage extracellular matrix, is C-telopeptide of type II collagen (CTX-II).[8,9] CTX-II levels increase acutely after joint injury and can remain elevated more than 2 years post-injury.[2,10] Taken together, these results show that joint injuries can lead to a prolonged inflammatory environment in the joint, which is associated with cartilage degradation, and therefore, likely play a significant role in the progression to PTOA.[2]

While IL-1ß, IL-6, and CTX-II are elevated following injury, their associations with perioperative and/or OA pain are still being explored. Recent studies have had contradicting results; some studies have shown positive correlations between synovial fluid IL-1ß and IL-6 with various pain scores, while others show no association.[11–13] The relationship between CTX-II and pain is also unclear. The purpose of this study is to evaluate the associations between perioperative pain following ACL injury and reconstruction with synovial fluid concentrations of IL-1ß, IL-6 and CTX-II. We hypothesized that greater synovial fluid IL-1ß, IL-6 and CTX-II would correlate with greater Pain Visual Analogue Scale (VAS) scores.

Methods

This prospective study is a secondary analysis (Level of evidence: III) of skeletally mature patients with acute ACL tears who consented to participate in an IRB-approved pilot randomized trial (NCT03429140)[14]. Isolated ACL injury was determined via clinical exam and once identified, patients were screened for enrollment in the study. Inclusion criteria included: patients between the ages of 14 and 32 with no history of previous traumatic ipsilateral knee injury, no clinical evidence of posterior cruciate ligament injury, and no more than grade 1 medial or lateral collateral ligament injury. Exclusion criteria included:

injury occurring more than 12 days prior to enrollment, previous ipsilateral knee surgery, or a history of any inflammatory disease.

In the original pilot study, patients were randomized to receive either an intraarticular hyaluronate injection or saline placebo one week post reconstruction. It has been previously reported that IL-6, IL-1β, and CTX-II did not differ between the hyaluronate and placebo groups[14], and we further demonstrate in the current study that perioperative pain also did not differ between groups (p=0.48). As such, data was collapsed across groups for the current analyses.

Arthrocentesis and collection of Pain Visual Analogue Scale (VAS) scores were performed on the day of initial presentation (mean 6 days post-injury), pre-operatively on the day of ACL reconstruction prior to the patient receiving any medication or anesthesia, and 1- and 4-weeks after surgery (Table 1). Joint aspiration was performed through a superolateral suprapatellar approach with local cutaneous anesthesia. Synovial fluid samples were immediately centrifuged at 3,500 rpm for 10 minutes. The supernatant was collected, aliquoted and stored at −80°C. Synovial fluid IL-1ß (Meso Scale Discovery), IL-6 (Meso Scale Discovery), and CTX-II (Immunodiagnostic Systems) were assessed using commercially available enzyme linked immunoabsorbant assays. Assays were completed per the manufacturer's instructions and were run in duplicate. Any samples outside of the limits of detection or quantification were rerun. For all plates, intra-assay coefficients of variance were < 9.5. All samples were assessed on a single plate to avoid issues with inter-assay variation (ie 1 plate each IL-6, IL-1β, and CTX-II).

Statistical Analyses

The change in pain over the four study time points was assessed with a repeated measures analysis of variance (ANOVA), with Bonferroni post hoc analyses used to identify pairwise differences. Biomarker concentrations were not normally distributed, and were log transformed for analysis. Using the methods described by Bakdash and Marusich[15], repeated measures correlations were used to assess the relationships between pain and synovial fluid IL-1ß, IL-6, or CTX-II after ACL injury and reconstruction. Analyses were performed in R using the rmcorr package[16] and an α-level of $p \leq 0.05$ was considered statistically significant.

Results

ACL reconstructions were performed by 3 experienced, board-certified sports medicine surgeons. All procedures were performed arthroscopically, with 19 treated with a bone-patellar tendon-bone autograft and 4 treated with a hamstring autograft. Synovial fluid samples from 23 patients were included in the study. Demographic information can be found in Table 2. Concomitant meniscus injuries are presented in Table 3. Of the 23 patients, only 3 had no meniscus pathology; 8 had isolated medial meniscus tears, 5 had isolated lateral meniscus tears, and 7 patients had both medial and lateral meniscus tears. All meniscal repairs and partial meniscectomies were performed arthroscopically. No full thickness articular cartilage lesions were noted at the time of surgery. Synovial fluid was

successfully collected in 79 of 92 possible aspirations (86%), with most dry aspirations occurring on the day of surgery (n=5) or the 1-month postoperative follow-up (n=4, Table 1).

Pain at initial presentation was 54.7 ± 28.3 and significantly improved by the day of surgery (28.3 ± 5.1, p<0.001). Pain then significantly increased 1 week after surgery (55.7 ± 5.8, p=0.001), and again demonstrated a significant decrease 4 weeks postoperatively (27.0 ± 5.4, p<0.001). Pain was positively correlated with synovial fluid IL-6 concentrations (r = 0.52 [95%CI: 0.30 to 0.69], p = 0.00003, Figure 1); however, pain was inversely correlated with CTX-II (r = −0.39 [95%CI: −0.60 to −0.14], p = 0.003, Figure 2). IL-1ß was not significantly correlated with pain (r = −0.09 [95%CI: −0.35 to 0.19], p = 0.53, Figure 3).

Discussion

The primary findings of this study were that patient reported pain was significantly and positively correlated with synovial fluid IL-6, and pain was negatively correlated with CTX-II. These results suggest that IL-6 may play a role in the pain signaling pathways following ACL injury and subsequent reconstruction. A more novel finding is that increased early cartilage breakdown was present despite being associated with less pain.

Early PTOA has been described as a “silent killer” of the knee joint[17] with progressive, irreversible cartilage degradation occurring in the years following injury but often with little or no pain. The current results further support this concept as there was a negative association between CTX-II and pain. There may be additional temporal differences in pain signaling pathways across the spectrum of PTOA. Synovial IL-6 has been associated with pain early after ACL injury and reconstruction but has not consistently correlated with pain for those with moderate to severe knee OA.[7,11,12] Conversely, synovial CTX-II did not correlate with pain early after ACL injury or reconstruction. However, urinary CTX-II (uCTX-II) was predictive of pain progression for those with mild to moderate knee OA and was also demonstrated to be increasingly elevated for those with short-term and longer-term OA pain.[18,19]

IL-6 and Pain

Our findings are congruent with those of Gupta et al. which found a positive correlation between pre-operative IL-6 synovial fluid concentrations and postoperative VAS pain scores at one, two, six and twelve months after ACL reconstruction.[20] Since IL-6 was only collected preoperatively in this previous study, the preoperative inflammatory state of the joint may affect postoperative pain[20]. The results of the present study add to this understanding by demonstrating that the inflammatory environment of the joint and intra-articular IL-6 levels may contribute to pain both prior to and following surgery. After ACL injury, synovial fluid IL-6 concentrations have demonstrated a more than 10-fold increase during the acute phase post-injury (0-48 hours) and then decreased in the days to months following injury.[7] Although IL-6 levels decreased relative to the acute phase, they remained elevated in the chronic phase (3 months or more after injury or surgery) compared to reported low normal levels.[7,21] In a study by Watt et al., higher pre-operative synovial fluid IL-6 was associated with a lower (worse) Knee Injury and Osteoarthritis Outcome Score 4 ($KOOS_4$) measured at baseline.[21] Another study of patients with acute knee injuries found a

significant negative correlation (p=0.017) between synovial fluid IL-6 and $KOOS_4$ scores at 2 years following surgery.[22] The pain subdomain of the $KOOS_4$ score (KOOS pain) highly correlated with overall $KOOS_4$ scores at 2 years,[22] indicating a likely positive correlation between IL-6 and pain, agreeing with the results of the present study. However, Watt et al. also showed that at 3 months, IL-6 was associated with a greater improvement in $KOOS_4$. [21] This demonstrates that the relationship between IL-6 and pain at different time points is not fully understood. Different pathways may be involved with pain signaling at more prolonged follow-ups. Synovial IL-6 has also been reported to continue to be elevated 5 years after ACL reconstruction when compared to healthy control subjects;[23,24] however, synovial fluid IL-6 concentrations 2 years after ACL injury do not correlate with patient-reported outcomes at 5 years.[25] When taken with the current results, this suggests that different pain signaling pathways are involved over the time course of PTOA progression.

The relationship between synovial fluid IL-6 and VAS pain scores observed in the current study indicates that IL-6 may play a role in pain signaling following joint injury, which has been observed in other studies. IL-6 and soluble IL-6 receptor (sIL-6R) were injected into rat knee joints, and action potentials of afferent fibers supplying the knee joint were then recorded in response to innocuous and noxious stimuli (rotation of the tibia against the femur).[26] Injection of IL-6 alone and IL-6 plus sIL-6R both led to sensitization of unmyelinated C fibers within 1 hour.[26] Sensitization of these fibers has two implications in pain production and perception: 1) sensitization lowers the threshold of high-threshold nociceptors to mechanical stimulation, such that previously innocuous stimuli can then cause pain and 2) increases the responsiveness of low-threshold fibers[26,27]. Low-threshold fibers produce low-discharge rates in response to innocuous stimuli and higher discharge rates in response to noxious stimuli.[26,27] These results demonstrate that mechanical stimulation in the presence of IL-6 produces pain and leads to mechanical hypersensitivity, which may be a responsible pain mechanism when loading the knee following ACL reconstruction.

CTX-II and Pain

Another finding of the present study was that synovial fluid CTX-II levels were inversely correlated with VAS pain scores, disproving our hypothesis. This finding suggests that CTX-II, unlike IL-6, is likely not involved in the joint pain signaling pathway early after ACL reconstruction. Previous studies investigating pain and uCTX-II have had varying results. uCTX-II is a breakdown product of articular cartilage that is excreted in the urine.[28] Many studies measure uCTX-II in lieu of serum or synovial CTX-II because it less invasive to obtain urine samples.[29] Although it is not a direct measure of the intra-articular environment, uCTX-II is accepted as a measure of cartilage breakdown and may therefore have diagnostic and prognostic value for patients with OA.[28,29]

The inverse relationship between synovial fluid CTX-II and joint pain observed in the present study indicates that, while inflammation may contribute to joint pain, early cartilage degradation following joint trauma may be painless. Placed in the context of early PTOA progression, this observation makes sense. Cartilage itself is avascular and aneural[30], so its early destruction may not directly lead to pain. Thus, PTOA joint pain likely arises from other joint structures, such as the synovium, bone and surrounding soft tissue.[30] For

older individuals with radiographic OA, uCTX-II has been found to be predictive of pain progression and is increasingly elevated for those with no pain, short-term pain (<15 days) and longer-term OA pain (> 15 days).[18,19] These differences indicate that more studies investigating CTX-II and pain are needed to establish the relationship and the role of CTX-II on pain production and perception at the different stages of disease progression.

The inverse relationship between pain and synovial fluid CTX-II may also be interpreted as early cartilage breakdown potentially being greater for those with less pain in the perioperative period. Future prospective longitudinal studies are necessary to determine if increased pain may in fact play a protective role, whereas reduced pain may be associated with increased weightbearing thereby increasing cartilage degradation in the early post-injury or postoperative periods. To date, little is also known about the optimal joint loading in the immediate post-injury and/or postoperative periods. Recent animal models of PTOA suggest that unloading the limb early after injury via tail suspension can be protective against early PTOA changes[31]; however, clinically, the opposite has been reported. In a cross-sectional study of patients one month after ACL reconstruction, Wellsandt et al. reported that relative underloading of the operative limb was associated with greater cartilage composition changes on MRI.[32] Similarly, there is debate in the literature as to whether under- vs. over-loading of the operative knee 6-12 months after ACL reconstruction is associated with serum and imaging biomarkers of early cartilage degradation.[33–35] The potential interplay between pain, biological factors, biomechanical factors and cartilage degradation requires additional study but offers the potential for novel multimodal treatment algorithms to optimize joint health throughout the first year after ACL injury.

IL-1ß and Pain

Though our study did not show any significant relationship between synovial fluid IL-1ß and VAS pain scores in the perioperative period for ACL reconstruction, previous studies have shown that IL-1ß may play a role in the pathophysiology of idiopathic OA pain. In a study investigating the effects of IL-1ß and TNFα on rabbit articular chondrocytes, IL-1ß was found to stimulate prostaglandin E_2 (PGE_2) production.[36] A synergistic effect was observed when TNFα was added with IL-1ß.[36] This is substantial as PGE_2 is believed to be a major contributor to inflammatory pain in arthritis.[37] The current results suggest that IL-1ß is not a predominant factor in early post-injury or postoperative pain signaling. Future studies are necessary to determine if different pain signaling pathways are involved over the time course of PTOA progression, as well as whether pain pathways differ between PTOA and idiopathic OA.

Limitations

Our study with a relatively small sample size was not without limitation. First and foremost, pain is undoubtedly multifactorial and may be influenced by surgical technique, graft type, presence and magnitude of subchondral bone marrow edema, and multimodal perioperative pain protocols, as well as by psychosocial factors and each individual's personal experiences. The present study assessed one specific area and evaluated the potential associations of synovial fluid cytokine concentrations and early evidence of type II collagen breakdown with pain. The present study examined inflammatory and

chondrodegenerative markers up to 4 weeks post-operatively, and so it is unknown if markers remain elevated and contribute to pain long-term following ACL reconstruction. Only acute ACL injuries were included in the present study (within 12 days of injury), so inflammatory and chondrodegenerative markers and pain may differ in subacute or chronic ACL injuries. Similarly, the study involved a younger patient population; thus, the results may not be generalizable to older ACL-injured patient populations. Additionally, including other biomarkers may have led to a greater understanding of the role that inflammatory and chondrodegenerative biomarkers may play in joint injury and subsequent pain. Finally, since synovial fluid biomarkers were investigated in this study, the results may be biased as the patients with persistent effusions would be potentially overrepresented when compared to those without effusions leading to dry aspirations. Fortunately, synovial fluid samples were collected for the majority of patients at all 4 time points. However, the results may not be generalizable to ACL reconstruction patients without effusions, who may have different amounts of inflammation and cartilage breakdown that was not able to be measured in the present study.

Conclusion

Synovial fluid IL-6 concentrations positively correlated with pain after ACL injury and reconstruction; however, pain was inversely correlated with the synovial biomarker of type II collagen breakdown, CTX-II. PTOA has been described as a “silent killer” and these results suggest that there may be pathway differences in early PTOA that are not primarily pain driven, but still lead to progressive cartilage loss despite minimal symptoms.

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Statement of Clinical Relevance:

PTOA has been described as a “silent killer” and these results suggest that early PTOA may have pro-inflammatory pathways that are not primarily associated with pain but still lead to progressive cartilage loss.

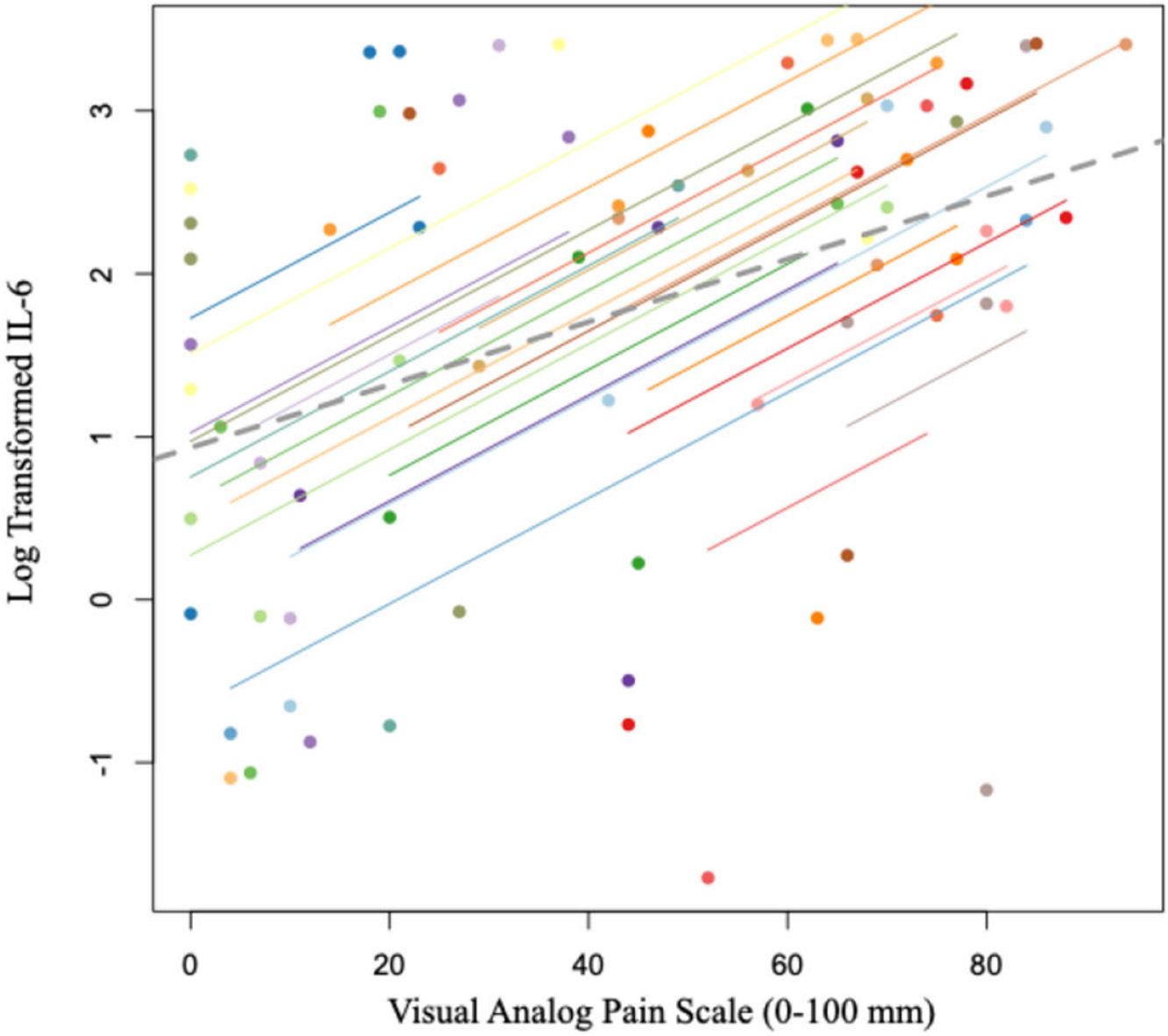

Figure 1:
Scatterplot of log transformed IL-6 values and VAS Pain Scores. Participants' data and corresponding lines are shown in different colors, and the dashed grey line represents the regression line if each participant's data was averaged across the four time points.

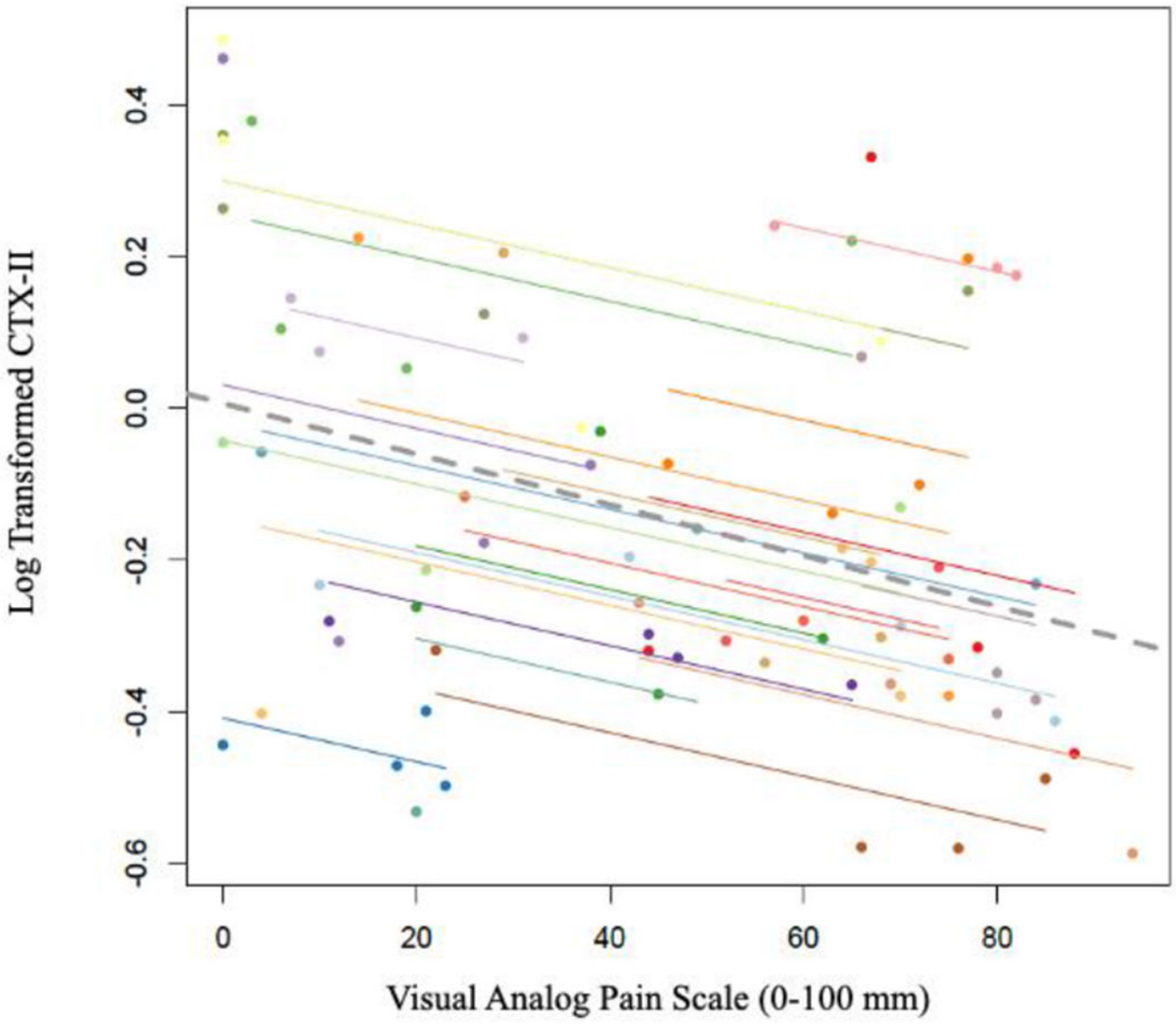

Figure 2:
Scatterplot of log transformed CTX-II values and VAS Pain Scores. Participants' data and corresponding lines are shown in different colors, and the dashed grey line represents the regression line if each participant's data was averaged across the four time points

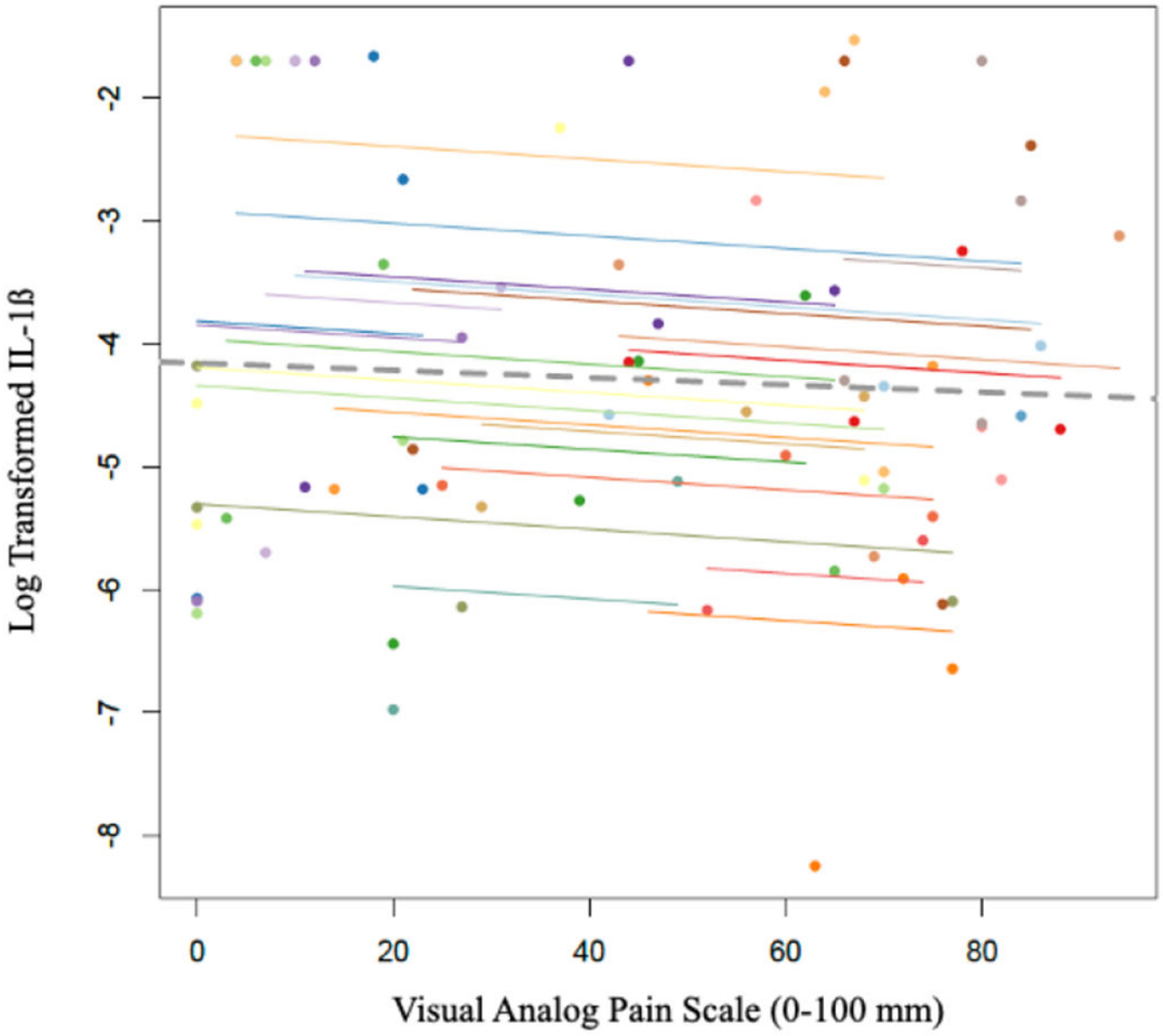

Figure 3:
Scatterplot of log transformed IL-1ß values and VAS Pain Scores. Participants' data and corresponding lines are shown in different colors, and the dashed grey line represents the regression line if each participant's data was averaged across the four time points.

Table 1.

Description of study visits and arthrocentesis success

Visit	Study tasks performed	Successful Arthrocentesis
Preoperative enrollment	Informed consent, arthrocentesis and VAS scores	21/23 (91.3%)
Day of Surgery	Arthrocentesis and VAS scores (collected preoperatively)	18/23 (78.3%)
1 week postoperative	Arthrocentesis and VAS scores	21/23 (91.3%)
4 weeks postoperative	Arthrocentesis and VAS scores	19/23 (82.6%)

VAS=Visual Analog Scale

Table 2.

Demographic information of subjects

Variable	Frequency
Male	10
Female	13
	Mean ± SD
Age	18.4± 2.6 y
BMI	27.4 ± 4.9 kg/m^2

Table 3.

Concomitant meniscus injuries and treatment

	Medial Meniscus	Lateral Meniscus
Not Injured	8 (34.8%)	11 (47.8%)
Meniscus Repair	13 (56.5%)	9 (39.1%)
Partial Meniscectomy	2 (8.7%)	3 (13.0%)