Post-traumatic Inflammatory Cytokine Profile in Synovial Fluid Following Intra-Articular Ankle Fracture

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Introduction: Post-traumatic arthritis (PTA) is the etiology of 70-78% of ankle arthritis and is known to occur after intra-articular fracture despite anatomic fracture reduction [1,2]. It has been hypothesized that an early inflammatory response after intra-articular injury could lead to irreversible cartilage damage that progresses to PTA. Therefore, in addition to meticulous fracture fixation, it would be ideal to prevent this initial inflammatory response but little is known about the composition of the synovial environment after intra-articular fracture. Pro-inflammatory cytokines and matrix metalloproteinases (MMPs) have been implicated in the chronic inflammation and degradatory changes of idiopathic osteoarthritis but have yet to be implicated in PTA. The purpose of this work was to characterize the acute-phase inflammatory cytokine and MMP response in the synovial fluid (SF) of patients with acute intra-articular ankle fractures.

Methods: SF was obtained via lavage from bilateral ankles of twenty-one patients with an intra-articular ankle fracture. All patients had a contra-lateral ankle joint that was pain free and had no radiographic evidence of arthritis. Neither ankle had a prior history of trauma. The uninjured ankle served as a matched control. Patients were excluded if they had diabetes, hemophilia, or any systemic inflammatory diseases such as rheumatoid arthritis. Whole blood was obtained simultaneously to SF lavage and centrifuged to obtain serum. Urea has been described as a passive transport marker for arthritis biomarker studies when direct aspiration of joint fluid is difficult [3]. Urea has been shown to have a fixed concentration in SF and serum in both healthy and diseased states [3,4]. Thus, the urea concentrations for both serum and SF were measured using a colorimetric assay (BioAssay Systems, Hayward, CA), and the dilution factor of the SF caused by lavage was calculated by dividing the serum urea concentration by the SF urea concentration for each patient. Cytokine measurements were corrected for dilution factors prior to statistical analysis. SF samples were analyzed for 15 cytokines including GM-CSF, IFN-gamma, IL-1alpha, IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-alpha, MMP-1, MMP-3, MMP-9, MMP-2, and MMP10 as well as the cartilage degradation marker CTXII, sGAG, bilirubin, and biliverdin using immunoassays. Statistical analysis was performed using the Wilcoxon Rank Sum test (alpha<0.05). In addition, correlation analysis was performed for all variables against time from injury and age at injury, and the correlation coefficient (r) was tested for significance against the null hypothesis that Pearson’s Correlation Coefficient (r) = 0 (alpha<0.05).

Results: SF was obtained at a mean of 17 days post-fracture (range 8-40). Of the 18 measured cytokines and soluble factors GM-CSF, IL-10, IL-1beta, IL-6, IL-8, TNF-alpha, MMP-1, MMP-2, MMP-3, MMP-9,
MMP-10, and bilirubin were found to be significantly elevated in the fractured ankles (Figure 1) compared to healthy ankles. Correlation analysis revealed significant inverse relationships between time from injury and concentrations of IL-6 ($r = 0.59$, $P = 0.003$), IL-8 ($r = 0.53$, $P = 0.01$), IL-10 ($r = 0.53$, $P = 0.01$), TNF-alpha ($r = 0.45$, $P = 0.03$), and MMP-10 ($r = 0.46$, $P = 0.3$) in the injury group. There were no significant relationships between cytokines and age at injury for the injury group, and only TNF-alpha ($r = 0.49$, $P = 0.02$) in the healthy group.

**Discussion:** We believe this represents the first study to characterize post-fracture ankle SF during the acute phase with patient-matched controls. We found that levels of 12 inflammatory cytokines were significantly elevated in the fractured ankles compared to the healthy ankles, several of which are previously known to play a role in joint trauma and OA, including IL-1beta, TNF-alpha, IL-6, IL-8, and the MMPs. Additionally, we found a significant correlation of increased inflammatory mediators closer to the time of injury. In addition to anatomic fracture reduction, these data lend credence to reducing acute intra-articular inflammation through the development of antagonists to these pro-inflammatory and degradatory mediators. Likewise, early SF aspiration and lavage, such as at the time of injury, might reduce the intra-articular inflammatory burden.

**Significance:** This study characterizes the acute pro-inflammatory burden in the joint-space after intra-articular fracture defining targets for therapeutic intervention to reduce the progression of post-traumatic arthritis.
**Figure 1.** Median (horizontal line), Interquartile Range (Box), Minimum and Maximum (Whiskers) for measured cytokines in synovial fluid from healthy (white bars) and injured (gray bars) following unilateral ankle fracture.

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