Synovial Fluid Biomarkers are Indicative of Synovial Inflammation Following Articular Fracture

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Introduction: Joint injuries, such as ACL or meniscal tears in the knee and articular fracture of any joint, can progress to post-traumatic arthritis (PTA). Even with surgical restoration of the joint, articular fractures can develop PTA within one year of injury[1]. The resulting pain, disability, and potential need for a joint replacement can be particularly devasting to this patient population that may be younger and in their prime income-earning years. Currently, there are no disease-modifying treatments for articular fracture to prevent PTA. Acute local inflammation in patients and in animal models are reported and likely drive degradative joint changes[2, 3]. Levels of some cytokines, chemokines, and matrix-degrading proteases (MMPs) are reported to be increased acutely (1-7 days) post-fracture (fx) and may serve as biomarkers of joint inflammation[4-6]. Additionally, the severity of the fracture has been shown to increase the level of synovial inflammation in mice and levels of inflammatory biomarkers in synovial fluid following fracture in patients[5]. We proposed to determine if biomarkers previously identified in patient fracture samples reflect the severity of acute synovial inflammation following articular fracture in a mouse model of closed articular fracture.

Methods: All procedures were performed under an IACUC-approved protocol. Adult C57BL/6 mice (4.5 mos. age, male, JAX # 005304) were subjected to a moderate closed articular fracture of the left knee[3, 7]. Mice were sacrificed on day 3 (n=4) and day 7 (n=4) post-fx and compared to uninjured pre-fx mice (n=4). Fracture energy was calculated from the area under the load-displacement curve (Acumen 3, MTS). At the time of sacrifice, serum and synovial fluid[8] were collected from all mice. Hind limbs were harvested for formalin-fixed paraffin histologic assessment of synovitis[9] from 8µm thick H&E stained sections. Synovial fluid and serum concentrations of analytes previously identified as being acutely elevated in patients with articular fracture were quantified using a custom biomarker multiplex panel (MSD 10-plex: IL-1β, IL-6, IL-10, KC/GRO, IL-33, IP-10, MCP-1, MIP-1β, VEGF, MMP-9). Statistical tests are reported in the methods and figure legends.

Results: Inflammation of the joint capsule in the fractured knee was increased on both day 3, initially on the lateral side near the fracture, and day 7 throughout the joint (Fig 1A), with the highest synovitis scores (reflecting increased cellularity of the lining and stroma) observed at day 7 (Fig 1B). The total joint synovitis scores (sum of medial and lateral) were significantly correlated (Spearman) with fracture energy (r=0.82, p=0.002). For synovial fluid biomarkers, 7 of 10 were significantly increased in the fractured knee at day 3: IL-1β, IL-6, KC/GRO, IP-10, MCP-1, VEGF, and MMP-9. Additionally, the following SF biomarkers were significantly correlated (Spearman) with the total joint synovitis scores: IL-1β (r=0.64; p=0.03), IL-6 (r=0.74; p=0.009), KC/GRO (r=0.62; p=0.03), VEGF (r=0.65; p=0.03), and MMP-9 (r=0.62; p=0.03). For serum biomarkers, only IL-1β and IP-10 were significantly increased at day 3 post-fx compared to pre-fx. No significant correlations were found between serum biomarkers and synovitis scores.

Discussion: We found several candidate biomarkers that may reflect of the degree of synovial inflammation following an articular fracture. The concentrations of these inflammatory factors in synovial fluid peaked at day 3, whereas tissue inflammation, as reflected by synovitis scores of hypercellularity, was greatest at day 7 post-fracture. This pattern of synovial fluid biomarker changes may indicate an early window during which interventions would need to be administered to optimally mitigate soluble factors within the joint to reduce joint pathology. These preclinical data are consistent with a shared pathophysiology of PTA following a joint injury in humans and mice, as these murine data appear to recapitulate the intra-articular response seen in patients with articular fractures.

Significance/Clinical Relevance: Defining the acute response to articular fracture and how it relates to the development of PTA is important criteria necessary for translating potential therapies. Success would aid in both identifying the optimal timing for interventions and also for identifying those patients at high risk of developing PTA.

References: **1.** Brown TD. *J Orthop Trauma.* 2006; **2.** Furman BD. *Arthritis Rheumatol.* 2015; **3.** Lewis JS. *Osteoarthritis Cartilage.* 2011; **4.** Adams SB. *Foot Ankle Int.* 2017; **5.** Haller JM. *J Bone Joint Surg Am.* 2015; **6.** Haller JM. *Clin Orthop Relat Res.* 2015; **7.** Furman BD. *J Orthop Res.* 2007; **8.** Seifer DR. *Osteoarthritis Cartilage.* 2008; **9.** Krenn V. *Histopathology.* 2006

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Figure 1.

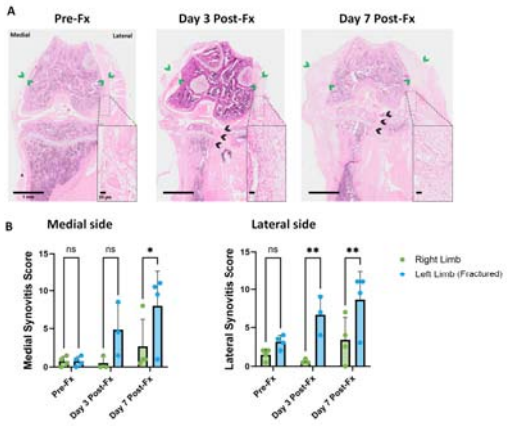

Figure 1. A. H&E stained histology of left knee with green arrows showing joint capsule thickness and black arrows indicating fracture (Fx) location with insert showing increased cellularity of the synovium (20x). **B.** Synovitis scores of the medial and lateral sides of the joint. Two-way ANOVA with Sidak post-hoc test (*p=0.014, **p=0.006)

Figure 2.

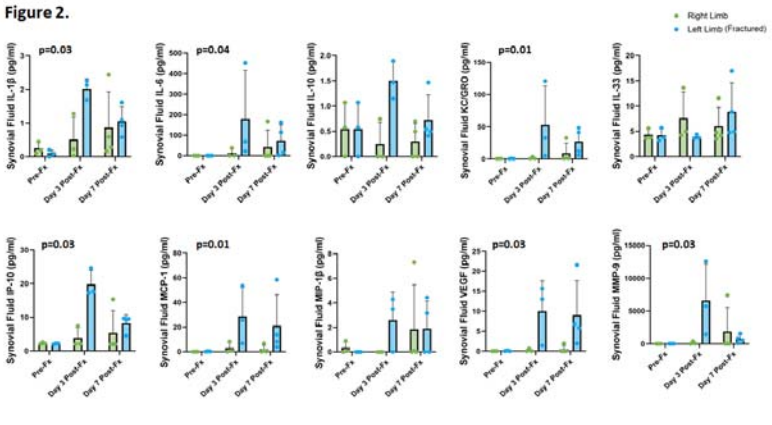

Figure 2. Synovial fluid concentrations of biomarkers following articular knee fracture. Kruskal-Wallis nonparametric test.