Comparison of Cam-Type FAI Versus End-Stage Hip OA Synovial Fluid Biomarker Levels using a Multiplex Platform: A Pilot Study

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INTRODUCTION: Cam-type femoroacetabular impingement syndrome (FAI) is a pre-arthritic hip condition characterized as a bony growth on the femoral head-neck junction, leading to abnormal contact at the impingement site that elicits tissue damage and inflammation. Progression of joint damage to cartilage degeneration and osteoarthritis (OA) occurs when FAI is left untreated, with reports suggesting an etiological role of FAI in up to 50% of all hip OA cases[1]. It has also been reported that articular cartilage from FAI and end-stage OA patients express similar inflammatory and catabolic markers[2]. Synovial fluid is one of the primary components of the local environment of the joint space, serving as a cartilage lubricant that contains biomolecules that are crucial to joint homeostasis. In OA, the expression of interleukins (e.g., IL-6) and catabolic molecules, such as matrix metalloproteinases (e.g. MMP-1, MMP-3, MMP-13), in the synovial fluid may serve as biomarkers of joint degeneration. While studies assessing the inflammatory and matrix protein synovial fluid profiles of OA have been reported[3–5], no studies have been conducted to assess how these profiles may differ from FAI, as a pre-arthritic condition. This study aims to compare synovial fluid profiles of FAI versus end-stage OA patients, using a multiplex platform capable of simultaneous detection of multiple analytes.

METHODS: For this IRB-approved pilot study, 40 synovial samples (20 OA – 9 Male/11 Female, 20 FAI – 4 Male/16 Female) were collected intraoperatively at 3 separate surgery centers. Synovial fluid aspirates were collected during the initiation of surgery from 1) FAIS patients undergoing hip arthroscopic surgery from the high-volume clinical practices of two surgeons (JC, SJN), and 2) end-stage OA patients undergoing total hip arthroplasty from the high-volume clinical practice of a single surgeon (CDV). Only synovial fluid that would be otherwise discarded during the normal course of surgery was procured for this study. Upon procurement, samples were immediately placed at -20°C for immediate storage as at least two separate aliquots of >500 µL, and later transported over dry ice to Rush University Medical Center, where they were cataloged, deidentified, and placed in a -80° freezer until analysis was performed (Figure 1). For multiplex assay preparation, all 40 samples were centrifuged at 1000 g for 5 minutes. The supernatant for all samples were digested using a 4 mg/ml hyaluronidase solution at a 1:1 ratio for 1-hour incubation to decrease sample viscosity and improve accessibility of target antigens for antibody binding, followed by an additional centrifugation at 1000 g for 5 minutes and collection of the resulting supernatant. A ten-fold dilution was then performed for each sample, followed by preparing the assays in accordance with the manufacturer's protocol (Biotechne). All samples were tested in duplicate. A Luminex FlexMap 3D instrument, housed within the Rush Biomarker Development Core at Rush University, was used to assay multiple analytes of interest, including TNF-α, B2M, MMP-1, MMP-3, MMP-13, IL-1ß, IL-6, BMP-7, CCL2, CCL11, CCL19, FGF2, CRP, VEGF, Aggrecan, and TIMP-1. To compare biomarker levels between FAI and OA groups, a Wilcoxon Rank Sum Test was applied to the median fluorescence intensity and concentration data from the assay using MATLAB (α=0.05).

RESULTS: For all samples, bead events per target were all greater than 50. Of all the analytes tested, only IL-6, MMP-1, MMP-3, MMP-13, CCL2, CCL19, FGF2, CRP, and VEGF levels were found to be generally within the limit of quantification of the assay; therefore only these analytes were assessed for group differences (n=20). For these analytes, both FAI and OA synovial fluid levels were found to be highly variable within groups (Figure 1), with OA synovial fluid analyte concentrations mostly spanning the 3rd to 4th quartiles, and the FAI synovial fluid concentrations spanning the lower 1st to 3rd quartiles. The OA group was found to have significantly higher concentrations in the analytes assessed compared to the FAI group ($p<0.05$, Figure 2).

DISCUSSION: These findings indicate that end-stage OA synovial fluid has higher concentrations of cytokines and catabolic factors compared to FAI synovial fluid. While significant differences were identified, both the FAI and OA groups were found to be highly variable. This study has multiple limitations. These samples were not separated from blood content upon collection by centrifugation, due to challenges in sample collection and storage at the three surgery sites from where these samples were procured. Therefore, all samples contained both synovial fluid and blood, which experiences hemolysis upon freezing, and therefore, all results are relative comparisons. Another limitation was that only synovial fluid was collected, as the IRB for this study dictates that only tissue and fluid that would otherwise be discarded during the course of surgery may be collected. Thus, blood, serum, and urine samples could not be collected to determine systemic levels of the biomarkers assessed in this study, which may provide further information into which factors have high/low expression. In future work, we aim to address these limitations by improving our collection workflow, and by collecting additional types of specimens in order to better characterize synovial fluid specific levels of potential biomarkers of cartilage degeneration in FAI and OA patients. Finally, it is important to note that there were FAI and OA patients who did not have sufficient synovial fluid to collect. These subjects were, therefore, excluded from this study, but may also present an important patient group to study in the context of these conditions. In addition, we plan to assess how other factors, such as age, disease severity, sex differences, and surgical outcomes may be associated with biomarker expression in FAI and OA.

SIGNIFICANCE/CLINICAL RELEVANCE: This study serves as an important first step towards establishing differences between FAI and OA synovial fluid biomarker profiles. Understanding the expression of these biomarkers in the synovial fluid may provide insight into which FAI patients are at a greater risk for developing subsequent OA later in life, and for the development of targeted biologic treatments in the long-term.

REFERENCES:1 Ganz, R. *et al. Clin. Orthop.* 466, 264–272 (2008); **2** Haneda, M. *et al. Am. J. Sports Med.* 48, 2481–2488 (2020); **3** Heard, B. J. *et al. BMC Musculoskelet. Disord.* 13, 126 (2012); **4** Meehan, R. T. *et al. J. Clin. Med.* 10, 5027 (2021); **5** Plsikova Matejova, J. *et al. Cartilage* 13, 1421S-1430S (2021)

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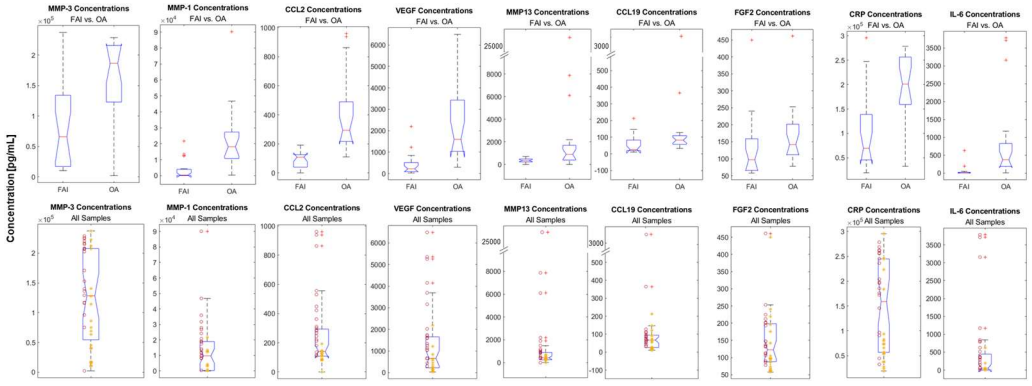

Figure 1: TOP: Comparison of calculated concentration between FAI and OA. BOTTOM: All samples regardless of group are visualized as box plots with FAI (Gold Asterisks) and OA (Red Circles) synovial fluid specimen data points shown.

Analyte	p-value
MMP-3	0.004320
MMP-1	0.000015
CCL2	0.000001
VEGF	0.000006
MMP-13	0.006150
CCL19	0.009787
FGF2	0.013829
CRP	0.002139
IL-6	0.000003

Figure 2: P-values for each analyte, comparing FAI vs. OA concentrations.