INTRODUCTION: Unlike other weightbearing joints, the majority (80%) of ankle arthritis is post-traumatic. Reports have estimated approximately a 20 year time-period of progression from injury to end-stage ankle arthritis. Thus, there is the potential for many years of intervention with disease modifying agents if the correct targets are identified. However, very little is known about the disease process of post-traumatic ankle arthritis.

Cytokine analysis, used as a tool to identify biomarkers, has been widely performed on knee joint synovial fluid but not in the ankle. Metabolic profiling is an emerging field of research concerned with the comprehensive characterization of the metabolites in biological systems. Metabolites are the end-products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to environmental changes such as those that might occur in the cartilage and synovium of post-traumatic arthritis. Metabolites have served as biomarkers in cardiovascular disease and prostate cancer. To date, there has been no literature identifying key metabolites or describing the metabolic state of post-traumatic ankle arthritis.

The purpose of this study was to define the cytokine composition and metabolic profile of post-traumatic ankle arthritis, potentially identifying altered metabolic pathways that could be used as targets for disease modification or individual metabolites or cytokines that could serve as biomarkers of post-traumatic ankle arthritis.

METHODS: This study was approved by our Institutional Review Board and comprised two groups of patients: 20 patients with end-stage post-traumatic ankle arthritis and 20 patients with no ankle pain and no radiographic evidence of ankle arthritis (control group). The patients in the end-stage arthritis group underwent a terminal operation for arthritis: arthrodesis or arthroplasty. The patients in the control group had no history of hind foot trauma and underwent forefoot surgery only. Patients with systemic inflammatory diseases and diabetes were excluded. Ankle joint synovial fluid aspirates were acquired during the surgical procedure.

Synovial fluid samples were analyzed for IFN-γ, TNF-α, MCP-1, IL-1β, IL-1Ra, IL-6, IL-10, IL-13, and IL-15 using a Multiplex® Human Cytokine Panel (Millipore, Billerica, MA) on a flow cytometry based Luminex™ 200 system (Luminex Corporation, Austin, TX). Unpaired t-tests were used to compare the concentrations of the cytokines between the end-stage arthritis and control groups.

Synovial fluid samples were analyzed by a mass spectrometry protocol that allowed for global profiling of more than 2000 identified metabolites (Metabolon, Inc; Durham, NC). This protocol included data collection from liquid chromatography/mass spectroscopy (LC/MS), with positive and negative mode electrospray ionization (ESI), and gas chromatography/mass spectroscopy (GC/MS) on all samples. Identification of metabolites was based on comparison to metabolomic library entries of purified standards. Welch’s unpaired t-tests were used to identify metabolites that differed significantly between the arthritic and control groups. Identified metabolites were fit into known biological pathways. Additionally, a random forest (RF) analysis was performed on the identified metabolites to determine whether the control and end-stage arthritis samples could be differentiated from one another based on their metabolic profile, and to determine which metabolites were most integral to differentiation between groups.

RESULTS: Several cytokines were found to be significantly higher in the end-stage arthritis group: IL-1α, IL-6, IL-8, IL-10, IL-15, and MCP-1.

Metabolic profiling identified 182 metabolites across all synovial fluid samples. Of these, 106 (58%) were found to be significantly elevated in the end-stage arthritic group and one was found to be significantly higher in the control group (threonine).

RF analysis yielded a predictive accuracy of 95% when using the metabolic profiles to distinguish between control and end-stage arthritic samples. The top 30 metabolites (all significant) important to this supervised classification analysis are shown in Figure 1. It is beyond the scope of this abstract to list all significant metabolites, but several warrant mentioning. Glutamate, which has previously been associated with arthritis, was significantly elevated (>7 fold) and ranked at the top of the RF plot.

Significantly elevated levels of proline, trans-4-hydroxyproline, and the dipeptide prolyl-hydroxyproline in the arthritis group, support increased extra-cellular matrix (ECM) turnover and collagen breakdown. Lactate was significantly elevated in the arthritis group.

Interestingly, lactate dehydrogenase (LDH), the enzyme required for the formation of lactate from pyruvate, has been implicated as an arthritis biomarker. This previous work has suggested that LDH is released from chondrocytes and the ECM as a result of collagen degradation.

Evidence of an increased oxidative environment in post-traumatic arthritis is provided by the significantly increased levels observed for oxidized glutathione (GSSH), for the cysteine disulfide, cystine, cysteine-glutathione disulfide, threonate, and alpha-tocopherol.

Another study identified inflammatory cytokines and a distinct metabolic profile present in the synovial fluid of end-stage post-traumatic ankle arthritis. Several of the inflammatory cytokines have previously been implicated in rheumatoid arthritis and osteoarthritis in other joints. The RF analysis indicated that the identified metabolites could be used to identify synovial fluid from end-stage arthritic ankle joints with 90% accuracy.

DISCUSSION: With respect to arthritis research, the ankle joint has largely been ignored. Knowledge about arthritis in other joints, such as the knee or hip, cannot simply be inferred to the ankle joint, as the cause of ankle arthritis is largely (80%) post-traumatic, whereas only 10% and 2% of knee and hip arthritis, respectively, is post-traumatic.

This study identified inflammatory cytokines and a distinct metabolic profile present in the synovial fluid of end-stage post-traumatic ankle arthritis. Several of the inflammatory cytokines have previously been implicated in rheumatoid arthritis and osteoarthritis in other joints. The RF analysis indicated that the identified metabolites could be used to identify synovial fluid from end-stage arthritic ankle joints with 90% accuracy.

SIGNIFICANCE: The identified cytokines and metabolites can be used as biomarkers for post-traumatic arthritis diagnosis or to monitor disease progression or therapeutic response. Metabolic profiling may have potential as a diagnostic tool for arthritis.

![Figure 1. The 30 most influential metabolites to RF sample classification between control and end-stage arthritic groups.](image)