The Synovial Fluid of Post-Traumatic Ankle Arthritis Holds Many Potential Inflammatory Cytokine and Metabolite Biomarkers

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Disclosures

• The authors have no disclosures
• This study was funded by a grant from the AOFAS/OEF
Post-Traumatic Ankle Arthritis

• Trauma is the most common etiology of ankle arthritis
• This is in stark contrast to the hip and knee

<table>
<thead>
<tr>
<th>Joint</th>
<th>Incidence of Post-Traumatic Arthritis</th>
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</thead>
<tbody>
<tr>
<td>Hip</td>
<td>1.6%</td>
</tr>
<tr>
<td>Knee</td>
<td>9.8%</td>
</tr>
<tr>
<td>Ankle</td>
<td>65-80%</td>
</tr>
</tbody>
</table>

Post-Traumatic Ankle Arthritis

• Paucity of research dedicated to post-traumatic ankle arthritis (PTAA)

• **Biomarkers of PTAA have not yet been identified**

• The purpose of this study was to use cytokine analysis and metabolic profiling to assess the synovial fluid of PTAA for potential biomarkers
Methods

Sample Acquisition
• Ankle joint synovial fluid obtained from two groups:
  • 20 patients with PTAA
  • 20 patients with no ankle pain or radiographic changes of arthritis (healthy controls)

Global Metabolic Profiling (Metabolon, Inc.)
• Samples analyzed for >3000 metabolites on three platforms:
  • UHLC/MS/MS optimized for acidic species
  • UHLC/MS/MS² optimized for basic species
  • Gas Chromatography/Mass Spectrometry
  • Ultrahigh performance liquid chromatography/tandem mass spectrometry

Cytokine Analysis
• Samples tested for IFN-γ, TNF-α, MIP-1β, MCP-1, IL-1β, IL-1Ra, IL-4, IL-6, IL-8, IL-10, IL-13, and IL-15 using ELISAs

Statistical Analysis
• Unpaired t-tests on cytokines and metabolites between groups
• Random forest supervised classification analysis on metabolic profiles
Results—Metabolic Profiling

- 182 metabolites identified across all samples
- **107 metabolites with mean concentrations significantly different between groups**
  - 106 were significantly elevated in the PTAA group
  - 1 (threonine) was significantly elevated in the control group
- Metabolites from multiple pathways involved:
  - Amino acid metabolism
  - Peptide synthesis
  - Carbohydrate metabolism
  - Energy metabolism (Krebs cycle)
  - Lipid metabolism
  - Redox homeostasis
  - Inflammation
  - Vitamin synthesis
  - Nucleotide synthesis
  - Extracellular matrix turnover
Random Forest Analysis

- **Predictive accuracy of 90% for classifying samples between PTAA and healthy controls using metabolic profiles**
- The top 30 of the most important metabolites to decision tree analysis as well as metabolic pathway and potential importance are shown in Table 1.
Results—Cytokine Analysis

- Mean concentrations of **IL-1Ra, IL-6, IL-8, IL-10, IL-15, and MCP-1** were significantly elevated in PTAA
This study identified inflammatory cytokines and a distinct metabolic profile present in the synovial fluid of PTAA.

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.

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.