Introduction:
Disability resulting from joint pathology is a significant health problem in the United States, resulting in associated costs of over $90 billion annually. Osteoarthritis (OA), the most common degenerative joint disease of the knee, results in pain, dysfunction, and disability. It is now recognized that inflammation plays a significant role in the development and progression of OA. Another factor important to the development of OA is the degradation of the articular cartilage and menisci through mechanical disruptions and matrix metalloproteinases (MMP). Because inflammation has been shown to increase MMP production, we investigated the levels of specific cytokines and MMPs within synovial fluid of normal and osteoarthritic patients so as to understand the dynamics of the inflammatory processes driving OA, and identify potential disease mechanism, diagnostic, and prognostic biomarkers. Identification of viable biomarkers will assist with the establishment of earlier diagnoses, which in turn allow for earlier therapeutic interventions in the setting of clinical OA. In theory, this improves the prognosis for patients.

Objectives:
The objectives of this study were 1) to identify and measure the concentration of specific MMPs and inflammatory cytokines released to the synovial fluid of normal and OA patients undergoing total knee arthroplasty; and 2) to correlate the production of these inflammatory biomarkers with radiographic severity of disease.

Methods:
All procedures were performed with IRB (IRB#1042248) approval. Synovial fluid was aspirated from three “true normal” patients (23, 27, 28 y/o) with no previous knee injury, clinical symptoms of knee pain or OA, or operative procedures performed. Synovial fluid was aspirated from 18 patients (21 knees) with OA immediately preceding their total knee arthroplasty procedure (age range = 44-86 y/o). Equal volumes of hyaluronidase treated synovial fluid samples were analyzed using the Fluorokine MAP human MMP (MMP-1, -2, -9, and -13) and cytokine (Interleukin 1β (IL-1β), IL-6, IL-8, Tumor necrosis factor-α (TNF-α), Macrophage inflammatory protein 1α (MIP-1α), MIP-1β, Monocyte chemotactic protein 1 (MCP-1), RANTES) multiplex panels (R&D Systems). A log transformation was performed to normalize the data for statistical analysis. Results from the normal and OA groups were evaluated using the unpaired t-test and between analytes using the Pearson product moment correlation. Significance was set at p<0.05.

Each patient had a standing AP radiograph performed during preoperative evaluation on the knee which eventually underwent a TKA. The Modified Kellgren and Lawrence scoring system was applied to the medial and lateral compartments then totaled:
- Osteophytes: 0 (none), 1 (small, definite), 2 (moderate), 3 (large)
- Narrowing: 0 (normal), 1 (minimal), 2 (moderate), 3 (bone on bone)
- Subchondral sclerosis: 0 (none), 1 (definite sclerosis)
- Chondrocalcification: 0 (absent), 1 (present)
- Osteophytes of tibial spines: 0 (normal), 1 (sharpened spines)

The radiographic scores were correlated with the log transformed MMP/cytokine data using Spearman rank order correlation with significance set at p<0.05.

Results:
Normal vs. OA (Figure 1): Of the 12 biomarkers tested, MMP-1, IL-6, IL-8, and RANTES were significantly higher in the synovial fluid of OA patients compared to normal patients. Three of the twelve were trending toward significance: MIP-1β, MCP-1, and MMP-2 (not represented in the figure, p=0.105). MMP-9, MMP-13, IL-1β, TNF-α, and MIP-1α were below the detection limits of this assay for all patients. Correlation between Analytes: MMP-1 had a moderate positive correlation with MMP-2 (r=0.43), IL-6 (r=0.52), IL-8 (r=0.43), and RANTES (0.58). IL-6 had a moderate (r=0.79) positive correlation with IL-8 and a moderate (r=0.44) positive correlation with MMP-2. MCP-1 had a moderate (r=0.56) positive correlation with IL-6 and strong (r=0.70) positive correlation with IL-8.

Discussion:
These data provide novel information for the investigation of synovial fluid biomarkers for OA. Importantly, we were able to obtain true normal controls for this study which is often a major limiting factor in human clinical studies. The results from this study suggest that IL-6 and IL-8 are particularly intriguing as potential biomarkers as we showed a significant increase in these two cytokines in OA patients, a strong correlation between the two, and strong correlations to severity of radiographic change. There was also a moderately strong correlation noted between severity of radiographic change and MMP-1 and MCP-1 levels. The correlations to radiographic severity are particularly important as it provides an immediate clinical relevance to the investigation of these proteins as OA biomarkers. Based on previous work in our laboratory and that of others, it is likely that use of a panel of proteins will be necessary to function as accurate and valid biomarkers for OA. IL-6, IL-8, MMP-1, and MCP-1 can readily be assessed as a panel in small volume samples of synovial fluid using a commercially available assay. As such, they have promise for clinical use in patients and ongoing research in our laboratory is aimed at prospectively investigating their utility as part of a biomarker panel for diagnosis, treatment monitoring, and prognostication in OA.