INTRODUCTION

Osteoarthritis (OA) is a widely prevalent, chronic, degenerative disease that affects the articular tissues. Because OA is not typically diagnosed early enough to allow clinical progression of disease to be prevented, development of early OA biomarkers has profound ramifications for treatment planning and prognostication. OA was historically regarded as a non-inflammatory arthritis, but there is now cumulative evidence that inflammation has a prominent role in OA. While other studies have shown that cytokine and chemokine fluctuations occur within the synovial fluid of osteoarthritic patients, comprehensive assessment of the potential clinical significance of these alterations is largely lacking in the literature. Therefore, the study of synovial fluid may elucidate candidates for a diagnostic biomarker panel and also increase our understanding of immune regulation in the pathogenesis of OA. Our objectives for this study were: 1) to delineate the alterations of cytokine and chemokine concentrations in synovial fluid in osteoarthritic and non-osteoarthritic knee joints and 2) to determine if any cytokine and chemokine fluctuations discern OA using receiver operating characteristic (ROC) curve analysis.

METHODS

All procedures were approved by the animal care and use committee (ACUC). Twenty-one adult, intact female, hound dogs >20kg were included in this study. Each dog underwent one of four arthroscopic procedures: transection of the anterior cruciate ligament (ACL-T), transection of the meniscus (MR), creation of two full-thickness grooves in the cartilage of the medial femoral condyle (Groove) or probe manipulation of all joint landmarks without insult (SHAM). The non-operated, contralateral hind limb served as an internal control for each dog. Synovial fluid was collected immediately prior to surgery on the operated limb, and 12 weeks post-operatively from both the operated and contralateral control limbs. These were analyzed using a multiplex canine cytokine and chemokine immunoassay (Millipore) for IL-2, IL-4, IL-7, IL-8, IL-15, IL-18, IP-10, INF-γ, TNF-α, MCP1, KC, and GM-CSF based on the xMAP platform. Results were statistically evaluated with the unpaired t-test or the Mann-Whitney Rank Sum test with significance set at p<0.05. Imaging studies (including ultrasound and magnetic resonance imaging), arthroscopy and histopathologic data were also collected to fully characterize the pathology of all joint tissues.

RESULTS

Synovial Fluid (Figure 1): Monocyte chemoattractant protein 1 (MCP1) was significantly increased in ACL-T joints (n=5) 12 weeks after surgery compared to day 0 (n=21; p=0.001) and the SHAM joints at 12 weeks (n=5; p=0.009). Increased MCP1 was also observed in the Groove (n=6) and MR groups (n=5) at 12 weeks compared to day 0, but statistical significance was not reached. Interleukin-8 (IL-8) was significantly increased at 12 weeks in the ACL-T and MR dogs compared to day 0 (n=21; A: p=0.001, M: p=0.018), the non-operated limb at 12 weeks (n=21; A: p=0.002, M: p=0.018), and the SHAM group (n=3; A: p=0.019, M: p=0.049) at 12 weeks. Keratinocyte-derived chemokine (KC) was significantly decreased in the Groove group at 12 weeks (n=6) compared to day 0 (n=21; p=0.009). The remaining cytokines and chemokines were below detectable levels in the synovial fluid of these animals.

DISCUSSION

Using canine models of OA, we have shown that changes in cytokine and chemokine levels occur within the synovial fluid after OA induction. ROC curves for diagnostic tests with perfect discrimination between normal and OA have an AUC=1.0. The results demonstrated that the combination of monocyte chemoattractant protein 1 (MCP1/CCL2), interleukin-8 (IL-8/CXCL8) and keratinocyte-derived chemokine (KC/CXCL1) demonstrates strong discriminatory ability for distinguishing OA dogs from normal dogs (AUC= 0.88).

Chemokines were first identified as chemoattractants for inflammatory cells. However, their potential roles in the pathogenesis of OA including induction of proteinase expression and inhibition of proteoglycan synthesis have been reported. There is very limited information in the literature linking MCP1, IL-8 or KC to OA in dogs or humans, and to our knowledge their combined use in an OA diagnostic biomarker panel is unreported.

ROC curve analyses of synovial fluid concentrations of MCP1, IL-8 and KC show these analytes provide strong discrimination between induced OA and non-osteoarthritic joints. It is possible that synovial fluid fluctuations of one or a combination of these chemokines could serve as a constituent of a successful diagnostic OA biomarker panel, and additional studies are currently underway in our laboratory. These include determining the intra-articular tissues responsible for the chemokine production and prospective investigation of these chemokines in naturally occurring OA patients before and after surgical intervention to address their clinical symptoms.