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Research paper

# Evaluation of serum cytokines in cats with and without degenerative joint disease and associated pain

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ABSTRACT

Degenerative joint disease is common in cats, with signs of pain frequently found on orthopedic examination and radiographs often showing evidence of disease. However, understanding of the pathophysiology of degenerative joint disease and associated pain remains limited. Several cytokines have been identified as having a role in pain in humans, but this has not been investigated in cats. The present study was performed to use a multiplex platform to evaluate the concentration of 19 cytokines and chemokines in serum samples obtained from cats with and without degenerative joint disease and associated pain. Samples from a total of 186 cats were analyzed, with cats representing a range of severity on radiographic and orthopedic evaluations and categorized by degenerative joint disease scores and pain scores. Results showed that cats with higher radiographic degenerative joint disease scores have higher serum concentrations of IL-4 and IL-8, while cats with higher orthopedic exam pain scores have higher concentrations of IL-8, IL-2, and TNF-α; increased concentration of IL-8 in degenerative joint disease and pain may be confounded by the association with age. Discriminant analysis was unable to identify one or more cytokines that distinguish between groups of cats classified based on degenerative joint disease score category or pain score category. Finally, cluster analysis driven by analyte concentrations shows separation of groups of cats, but features defining the groups remain unknown. Further studies are warranted to investigate any changes in cytokine concentrations in response to analgesic therapies, and further evaluate the elevations in cytokine concentrations found here, particularly focused on studies of local cytokines present in synovial fluid.



## 1. Introduction

Degenerative joint disease (DJD) may be the most common disease in cats, with a prevalence of up to 92%, and increasing radiographic burden with increasing age (Lascelles, 2010). Radiographic evidence of DJD is frequently accompanied by changes in mobility, activity, and social interactions (Lascelles et al., 2010a; Slingerland et al., 2011; Klinck et al., 2012), yet discrepancies exist between radiographic signs, orthopedic exam findings, and owner-reports of impairment (Bennett and Morton, 2009; Freire et al., 2011). Treatment options for this pervasive disease are lacking, in part due to the difficulty in accurately assessing pain and associated disability in cats. There is a need for objective methods to measure

*Abbreviations:* CV, coefficient of variation; DJD, degenerative joint disease; LOQ, limit of quantification; LLOQ, lower limit of quantification.

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DJD-associated pain in order to advance our understanding of the disease and our ability to develop effective treatments. Accelerometry has been useful as a surrogate measure of mobility and activity in cats (Lascelles et al., 2010b; Benito et al., 2013; Guillot et al., 2013), and advances in analytical methods for accelerometry data are being developed. However, these monitors remain costly and data extraction and analysis cumbersome, and offer no insight into the pathophysiology of the disease.

Development of biomarkers is a growing initiative in the fields of osteoarthritis (OA) and pain research in human and veterinary medicine (Bauer et al., 2006; Orita et al., 2011b; Kraus et al., 2015). Biomarkers, generally, may comprise any physical, imaging, or biochemical marker of disease or disease symptomology. Ideally, these markers would be sensitive to the presence of disease, indicative of the severity of disease, and responsive to treatments for the disease. In human DJD/OA research, soluble biomarkers of central interest include markers for structural damage to the joint and inflammation, both of which contribute to the clinical manifestation of pain. Inflammatory cytokines, particularly IL-1β, IL-6, and TNF-α, have been implicated in the pathogenesis of pain and joint disease (Zhang and An, 2007; Kidd and Urban, 2001; Miller et al., 2014; Imamura et al., 2015), and elevated concentrations of cytokines have been detected in synovial fluid samples from human DJD/OA patients (Imamura et al., 2014). Several chemokines have also been demonstrated to be associated with chronic pain in both rodent models (Dawes et al., 2013) and human DJD/OA patients (Orita et al., 2011b; Lee et al., 2013).

Despite their promise, the clinical heterogeneity of the disease in humans and the fluctuations in biomarker concentrations associated with intermittent flaring of symptoms have led to the suggestion that combinations of biomarkers, rather than a single universal marker, will be needed in the study of OA (De Ceuninck et al., 2011). This holds true across the category designations biomarkers are suggested to be useful for – Burden of disease, Diagnosis, Prognosis, Efficacy of intervention, and Investigative markers (Bay-Jensen et al., 2016).

In cats, despite the widespread nature of the disease, and the difficulty in measuring the impact of the disease, soluble biomarkers have not been investigated as indicators of DJD and associated pain. Suggestions from a recent proteomic and genomic study of cats with DJD found that gene expression differences between cats with DJD and an age-matched group of cats without DJD were particularly evident in three main pathways: immune function, apoptosis, and oxidative phosphorylation (Gao et al., 2013). Proteomic analysis of serum found that cats with DJD had an increase in components of the complement system as well as down-regulation of the complement system regulator clusterin (Gao et al., 2013). Up-regulation of the complement system can lead to activation of macrophages and inflammatory cytokine secretion (Haseeb and Haqqi, 2013), and cartilage matrix components can activate complement (Wang et al., 2011). Proteomic analysis of human synovial fluid also found increased expression of complement proteins in DJD/OA patients compared to individuals without OA (Wang et al., 2011). It follows, therefore, that the pattern of cytokine expression may be different in cats with DJD and associated pain compared to cats without DJD, and that this difference could potentially be detected in serum.

The development of multiplex technologies has allowed for the simultaneous quantification of multiple analytes using a very small volume (10–25 μL) of sample. Recently, a feline-specific panel has been developed and validated that allows quantification of 19 cytokines and chemokines using multiplex technology.[1] Given the associations between cytokines and painful DJD/OA in synovial fluid samples from humans (Orita et al., 2011b; Dawes et al., 2013), this study was designed to use the feline-specific panel to evaluate concentrations of cytokines and chemokines in well-phenotyped cats with and without DJD. Furthermore, as a preponderance of cats with clinically evident DJD have concurrent chronic kidney disease (CKD, 68% of cats in the study population in one report) (Marino et al., 2014), we sought to evaluate the interactive effect of pain and CKD status on cytokine concentrations. The effects of age and body condition were also investigated as age-associated inflammation is established in people (Greene and Loeser, 2015) and obesity and increased adipokines have been implicated as risk factors for the development of DJDs (Iannone and Lapadula, 2010).

Our central hypotheses were that cytokine/chemokine profiles in cats with DJD would differ from those of normal cats, that these profiles could be used as surrogate measures of pain in cats with DJD, and in cats with DJD and concurrent CKD, and that combinations of cytokines/chemokines would be able to distinguish between cats with painful DJD and normal cats.

## 2. Methods and materials

### 2.1. Subjects and samples

Samples used in this study were banked serum samples that had been collected from cats presented to the Comparative Pain Research Laboratory over the period from May 2007 to May 2015 (with approval from the North Carolina State University College of Veterinary Medicine Institutional Animal Care and Use Committee). The cats included in this study represented a mix of cats that presented for several studies, including those that were actively recruited as normal controls (where there was a requirement for no owner-noted mobility impairment) and those that presented for studies of treatments for DJD (Lascelles et al., 2010c; Benito et al., 2013; Gruen et al., 2015). Cats recruited to these DJD studies were required to have owner-noted mobility impairment. All cats had been examined by a veterinarian, and had been evaluated for systemic disease, orthopedic pain, and radiographic evidence of DJD as previously described (Lascelles et al., 2010c; Benito et al., 2013; Gruen et al., 2015). Briefly, all cats received a physical examination, including evaluation of body condition score (BCS) (Laflamme, 1997), followed by an orthopedic examination during which each joint or spinal segment was palpated and gently manipulated, and scored for the presence and severity of pain, crepitus, thickening, and effusion. Pain was scored on the following scale: 0 = no resentment; 1 = mild withdrawal, mild resistance to manipulation; 2 = moderate withdrawal, body tenses, may orient to site, may vocalize or increase vocalization; 3 = orients to site, forcible withdrawal from manipulation, may vocalize, hiss, or bite; and 4 = tries to escape or prevent manipulation, bites or hisses, marked guarding of site. Total pain (TPain) scores were calculated as the sum of the scores for individual joints, with a possible range of 0–80, and were further categorized for some analyses as described in the Statistical Analysis section.

Following the physical and orthopedic examinations, cats were sedated using an individually tailored protocol and orthogonal digital radiographs were made of each joint and spinal segment. Radiographs were evaluated by a single investigator (BDXL) and scored for the presence and severity of DJD using previously published criteria established by our laboratory (Lascelles et al., 2010c). Scores were ascribed according to a 10-point scale where 0 = no evidence of DJD and 10 = ankylosis of the joint. Total radiographic DJD (TDJD) scores were calculated as the sum of the scores for individual joints, with a possible range of 0–200, and were further categorized for some analyses as described in the Statistical Analysis section.

[1] FCYTMAG-20K-PMX Feline Cytokine/Chemokine Magnetic Bead Panel Premixed 19-Plex; EMD Millipore, Billerica, MA USA.