Expression of proteins in serum, synovial fluid, synovial membrane, and articular cartilage samples obtained from dogs with stifle joint osteoarthritis secondary to cranial cruciate ligament disease and dogs without stifle joint arthritis

Bridget C. Garner, DVM, PhD; Keiichi Kuroki, DVM, PhD; Aaron M. Stoker, PhD; Cristi R. Cook, DVM, MS; James L. Cook, DVM, PhD

Objective—To identify proteins with differential expression between healthy dogs and dogs with stifle joint osteoarthritis secondary to cranial cruciate ligament (CCL) disease.

Sample—Serum and synovial fluid samples obtained from dogs with stifle joint osteoarthritis before (n = 10) and after (8) surgery and control dogs without osteoarthritis (9) and archived synovial membrane and articular cartilage samples obtained from dogs with stifle joint osteoarthritis (5) and dogs without arthritis (5).

Procedures—Serum and synovial fluid samples were analyzed via liquid chromatography–tandem mass spectrometry; results were compared against a nonredundant protein database. Expression of complement component 3 in archived tissue samples was determined via immunohistochemical methods.

Results—No proteins had significantly different expression between serum samples of control dogs versus those of dogs with stifle joint osteoarthritis. Eleven proteins (complement component 3 precursor, complement factor I precursor, apolipoprotein B-100 precursor, serum paraoxonase and arylesterase 1, zinc-alpha-2-glycoprotein precursor, serum amyloid A, transthyretin precursor, retinol-binding protein 4 precursor, alpha-2-macroglobulin, angiotensinogen precursor, and fibronectin 1 isoform 1 preproprotein) had significantly different expression (>2.0-fold) between synovial fluid samples obtained before surgery from dogs with stifle joint osteoarthritis versus those obtained from control dogs. Complement component 3 was strongly expressed in all (5/5) synovial membrane samples of dogs with stifle joint osteoarthritis and weakly expressed in 3 of 5 synovial membrane samples of dogs without stifle joint arthritis.

Conclusions and Clinical Relevance—Findings suggested that the complement system and proteins involved in lipid and cholesterol metabolism may have a role in stifle joint osteoarthritis, CCL disease, or both. (Am J Vet Res 2013;74:386–394)

Osteoarthritis is the leading cause of disability in humans in the United States, with estimated annual health care expenditures totaling >$185 billion.1 Currently, there is no cure for osteoarthritis. Problems preventing identification of a cure for osteoarthritis include an incomplete understanding of disease mechanisms, inability to diagnose the problem in the early stages of disease, and deficiencies in preventive and treatment options.
The mechanisms of disease, determination of a diagnosis may be useful for minimally invasive determination of synovial fluid samples of patients with osteoarthritis. Biomarkers (including proteins) in serum and humans and other animals with that disease. Measurements of protein concentrations in matrix metalloproteinase activities in synovial fluid samples obtained from dogs with stifle osteoarthritis. Interestingly, the proteins identified in that study were associated with inflammation and activation of the immune system; these processes are important in the pathophysiology of osteoarthritis.

Further research to determine (via proteomic analysis) alterations of serum and synovial fluid protein concentrations in animals with osteoarthritis and the roles of such proteins in initiation and progression of osteoarthritis would aid understanding of the inflammatory and immune processes involved in the etiopathogenesis of the disease. Although dogs are commonly included in studies in which osteoarthritis is investigated, results of proteomic analysis of serum and synovial fluid samples obtained from osteoarthritic joint of dogs have not been reported, to the authors’ knowledge. Therefore, an objective of the study reported here was to identify via 1-D gel electrophoresis and LC-TMS-based quantitative proteomic analysis proteins with differential expression in serum and synovial fluid samples of healthy dogs versus dogs with stifle joint osteoarthritis secondary to CCL disease. Our hypothesis was that proteins would be identified via proteomic analysis that had significantly different expression in serum and synovial fluid samples obtained from dogs with stifle osteoarthritis secondary to CCL disease versus samples obtained from dogs with clinically normal stifle joints. Another objective of the study reported here was to analyze archived synovial membrane and articular cartilage samples obtained from stifle joints of dogs with osteoarthritis secondary to CCL deficiency and dogs with clinically normal stifle joints to detect differentially expressed proteins (as determined via proteomic analysis) by use of immunohistochemical techniques.

The hypothesis for this objective was that proteins with differential expression (as determined via proteomic analysis) would be detected in archived synovial membrane and articular cartilage samples of dogs.

Materials and Methods

Animals and sample collection—All procedures were approved by the University of Missouri Animal Care and Use Committee, and informed owner consent was obtained and documented in the medical record for each dog included in this study. Blood and synovial fluid samples were obtained from 10 adult medium-breed and large-breed dogs brought to the University of Missouri Veterinary Medical Teaching Hospital for surgical treatment of unilateral stifle joint osteoarthritis secondary to CCL disease. These dogs ranged in age from 3 to 8 years (mean ± SD age, 5.1 ± 1.8 years) and included 5 castrated male and 5 spayed female dogs. Stifle joint osteoarthritis was confirmed for each dog by a board-certified veterinary surgeon (JLC) on the basis of the detection of joint effusion, periarticular fibrosis, and signs of pain associated with flexion and extension of the affected joint. A board-certified veterinary radiologist (CRC) confirmed that dogs included in the study had radiographic evidence of stifle joint osteoarthritis, including periarticular osteophytosis, stifle joint effusion, and bone sclerosis. A blood sample (approx 3 mL) was collected from each dog via jugular venipuncture within 24 hours before surgery, and serum was harvested for proteomic analysis. Each serum sample was stored in an airtight container at –80°C until analysis. On the day of surgery, each dog was anesthetized and stifle joints were aseptically prepared for surgery. A stifle joint synovial fluid sample (approx 0.2 mL) was collected from each dog via aseptically performed arthrocentesis, and samples were stored at –80°C until proteomic analysis.

During surgery, stifle joints were evaluated and lavaged, and a stabilization procedure was performed for treatment of CCL disease. Eight to 12 weeks after surgery, dogs were brought to the clinic for postoperative assessment. At that time, blood (approx 3 mL) and synovial fluid (approx 0.2 mL) samples were collected to assess changes in protein expression after surgery. All dogs had clinical and functional improvement in affected limbs as determined on the basis of results of physical examination and subjective assessment of gait at a walk and trot. Two female dogs were not returned for follow-up examination; therefore, postoperative blood and synovial fluid samples were obtained from 8 dogs (age range, 3 to 8 years; mean ± SD age, 4.8 ± 1.7 years).

A control group comprised 9 client-owned healthy adult medium-breed and large-breed dogs (age range, 2 to 5 years; mean ± SD age, 2.9 ± 0.9 years); these dogs included 4 castrated males, 2 spayed females, 2 sexually intact males, and 1 sexually intact female. Control dogs had no clinical evidence of osteoarthritis as determined on the basis of results of a physical examination conducted by a board-certified veterinary surgeon (JLC). Radiography of stifle and hip joints of these dogs indicated there were no radiographic signs of osteoarthritis, as determined on the basis of assessment by a board-certified veterinary radiologist (CRC). A blood sample (approx 3 mL) was collected from each control group dog via a method that was similar to the method used to collect blood samples from dogs with osteoarthritis. A synovial fluid sample (approx 0.2 mL) of a stifle joint of each control group dog was collected via aseptic arthrocentesis during sedation with dexmedetomidine hydrochloride (0.012 mg/kg, IV). The serum and synovial fluid samples were stored at –80°C until proteomic analysis.

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analysis. Serum and synovial fluid samples of dogs with osteoarthritis and those with clinically normal stifle joints had been used in another study.4

**Gel electrophoresis**—Serum and synovial fluid samples were thawed. Synovial fluid samples were centrifuged at 32,000 × g for 10 minutes to pellet debris, and supernatant was collected. Synovial fluid samples were incubated with hyaluronidase (2,000 U/mg) at 37°C for 60 minutes to decrease viscosity. Twenty-five microliters of each serum and synovial fluid sample was albumin depleted with an immunoaffinity albumin and IgG depletion kit4 and subsequently concentrated with a 3-kDa centrifugal filter4 via centrifugation at 32,000 × g for 40 minutes. The protein concentration of each sample was determined with a fluorescence-based assay.5 Because proteomic analysis of each serum and synovial fluid sample was cost-prohibitive, samples were pooled for analysis. Volumes of serum and synovial fluid samples with equivalent quantities of protein from 2 to 4 dogs within each group (samples obtained from osteoarthritic dogs before surgery, samples obtained from osteoarthritic dogs after surgery, and samples obtained from control dogs) were pooled; therefore, 9 serum and 9 synovial fluid pooled samples (3 serum and 3 synovial fluid samples for each group) were prepared. Serum and synovial fluid samples for each dog were pooled with samples from other dogs that had similar cytokine and chemokine expression, as determined in another study.4

Each pooled serum and synovial fluid sample was heat denatured, and proteins were separated via 1-D gel electrophoresis in reducing conditions with a 4% to 12% Bis-Tris gel1 at 115 V and 48 mA for 135 minutes. The gel was stained with Coomassie brilliant blue and imaged. The gel lane for each sample was sliced into 8 segments of equal size, diced into approximately 1-mm cubes with a clean scalpel, and transferred to 1.5-mL sterile polypropylene tubes for in-gel trypsin digestion as previously described.11 The extracted peptides were lyophilized and resuspended in 15 µL of a solvent (0.1% [vol/vol] formic acid in ultrapure water) for LC-TMS analyses.

**LC-TMS analyses**—The LC-TMS analysis was conducted with a mass spectrometer4 with integrated high-performance LC.4 Serum and synovial fluid sample enrichment and high-performance LC fractionation was conducted with an in-line column7 prior to final separation by use of a laser-pulled fused silica nanopore analytic column. The fused silica column was prepared by use of a laser-puller4 followed by packing with C18 matrix4 by use of a pressure bomb. The column was then pre-equilibrated for 2 hours with a mixture (60:40 ratio of a solvent [99.9% [vol/vol] acetonitrile and 0.1% formic acid] to 0.1% formic acid in ultrapure water) to ensure proper packing. Six injections of 100 fmol of bovine serum albumin digest7 were used for column quality control prior to sample analyses. The column was then washed with a mixture (80:20 ratio of 99.9% acetonitrile and 0.1% formic acid to 0.1% formic acid in ultrapure water) for 20 minutes, and 2 blank analyses (5-µL injection of 0.1% formic acid in ultrapure water) were conducted prior to sample analyses.

An aliquot (5 µL) of each trypsin-digested sample was then injected onto the trap column with a 20-µL loop and fractionated on the analytic column for 80 minutes by use of a gradient (0% to 45%) of 99.9% acetonitrile and 0.1% formic acid in 0.1% formic acid in ultrapure water. Eluted peptides were analyzed by use of the data-dependent positive acquisition mode of the LC-TMS instrument, with a normal scan rate for precursor ion analysis, and dynamic exclusion enabled (repeat count, 1; repeat duration, 30 seconds; exclusion duration, 30 seconds). Following each full scan (m/z, 300 to 2,000; 3 microscans; resolution, 30,000), data-dependent triggered TMS scans for the 6 precursor ions with the most intense results were acquired.

**Protein identification and quantification**—Acquired TMS spectra were compared against the National Center for Biotechnology Information nonredundant database (taxonomy limited to dogs only) via a data appliance5 with a protein database.12 High-confidence protein identification was performed by requiring a minimum of 2 unique and no overlapping peptides for protein assignment, a minimum peptide probability of 99% for each peptide match, and cross-correlation scores at least 2.7, 3.0, and 3.5 for double, triple, and quadruple charge state peptides, respectively. Output files from the protein database searches were uploaded to computer software9 for spectral counting and automatic performance of statistical procedures. The number of unique spectra was used to estimate the amount of protein in each serum and synovial fluid sample.

**Immunohistochemical analysis**—To identify complement component 3 expression in joint tissues, immunohistochemical analysis was performed for tissue sections of archived formalin-fixed paraffin-embedded synovial membrane and articular cartilage samples of 5 dogs with stifle joint osteoarthritis secondary to CCL deficiency and 5 control dogs with nonosteoarthritic stifle joints; these tissue samples had been used in another study.10 Neither serum nor synovial fluid samples from these dogs were used for the present proteomic analysis. Deparaffinized and rehydrated tissue sections (thickness, 5 µm) were incubated with a polyclonal goat anti-canine complement component 3 antibody.8 After incubation overnight (approx 15 hours) at 4°C, tissue sections were washed in 50mM Tris-HCl (pH, 7.6) with 0.1% Tween 20. Tissue sections were then incubated with biotinylated secondary antibody.8 Bound primary antibody was detected by use of a streptavidin–horse-radish peroxidase method with substrate-chromogen solution.7 Tissue sections stained without primary antibody were used as negative control samples.

**Statistical analysis**—For determination of differences in protein expression between clinically normal dogs and dogs with osteoarthritis secondary to CCL disease before surgery or between dogs with osteoarthritis secondary to CCL disease before surgery and such dogs after surgery, data were considered significantly different for values of P < 0.05 on the basis of results of a t test and > 2.0-fold difference in expression, as calculated with software. The mean ± SD ages of dogs with osteoarthritis and those of control dogs were compared via t test by use of computer software1.
**Results**

Demographic characteristics of dogs—Ages of dogs with osteoarthritis and those of control dogs with clinically normal stifles were compared. The mean ± SD age of dogs with osteoarthritis (5.1 ± 1.8 years) and that of control dogs (2.9 ± 0.9 years) were significantly (P = 0.04) different.

Table 1—Results of proteomic analysis indicating proteins detected in pooled serum samples obtained after surgery from 10 dogs with stifle joint osteoarthritis secondary to CCL disease and pooled serum samples obtained from 9 control dogs without stifle joint osteoarthritis.

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<th>GI No.</th>
<th>Protein</th>
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GI = National Center for Biotechnology Information.

* Database entry to which mass spectrometry data were matched indicates a predicted canine protein determined on the basis of homology to proteins of animals of other species.
Serum sample proteomic analysis—Results of proteomic analysis of serum samples indicated 68 nonredundant proteins that were identified with high confidence in all 3 pooled preoperative serum samples obtained from dogs with osteoarthritis and all 3 pooled postoperative serum samples obtained from dogs with osteoarthritis (Table 1). None of these 68 proteins had significantly different expression between serum samples obtained from dogs with osteoarthritis versus those obtained from clinically normal control dogs or between serum samples obtained from dogs with osteoarthritis before surgery versus those obtained from such dogs after surgery.

Synovial fluid sample proteomic analysis—Results of analysis of synovial fluid samples indicated 76 nonredundant proteins that were identified with high confidence in all 3 pooled preoperative samples obtained from dogs with osteoarthritis and all 3 pooled postoperative samples obtained from such dogs (Table 2). Eleven proteins had significantly different expression (>2.0-fold) between synovial fluid samples collected from dogs with stifle joint osteoarthritis before surgery versus those collected from clinically normal dogs, and 4 proteins had significantly different expression (>2.0-fold) between synovial fluid samples collected from dogs with stifle joint osteoarthritis before surgery versus samples obtained from such dogs after surgery (Figure 1). Concentrations of clusterin were significantly higher in synovial fluid samples obtained from dogs with osteoarthritis versus those in synovial fluid samples obtained from clinically normal dogs (when results for preoperative and postoperative synovial fluid samples of dogs with stifle joint osteoarthritis were combined [data not shown]).

Complement component 3 immunohistochemical analysis—Commercially available antibodies against...
canine proteins with differential expression between synovial fluid samples obtained from dogs with osteoarthritis and synovial fluid samples obtained from clinically normal control dogs in the present study were identified; only anti-complement component 3 antibodies were commercially available. Immunohistochemical analysis of archived synovial membrane and articular cartilage samples was performed for determination of the strength of expression and distribution of complement component 3; results indicated complement component 3 had strong expression primarily on cells of the superficial aspects of all (5/5) synovial membrane samples obtained from osteoarthritic stifle joints. In contrast, only weak expression of complement component 3 was observed in 3 of the 5 synovial membrane samples obtained from nonarthritic stifle joints of dogs (Figure 2). Immunostaining for complement component 3 was weak in osteoarthritic and control articular cartilage samples; only a few chondrocytes in the calcified cartilage of such samples had complement component 3 expression.

Discussion

The development and increased availability of high-throughput and sensitive proteomic techniques have facilitated identification of proteins in fluids and tissue samples from human patients with osteoarthritis; many of those proteins were not previously thought to be associated with osteoarthritis. The objective of the present study was to identify proteins with differential expression between serum and synovial fluid samples obtained from healthy control dogs and dogs with stifle joint osteoarthritis secondary to CCL disease. Results of this study indicated that 11 proteins were differentially expressed between synovial fluid samples obtained from osteoarthritic stifle joints of dogs before surgery and those obtained from clinically normal stifle joints of control dogs; 4 proteins were differentially expressed between synovial fluid samples obtained before surgery from dogs with osteoarthritic stifle joints versus those obtained after surgery from such dogs. Because all synovial fluid samples were obtained from stifle joints of dogs, it was likely that the differences detected among groups were attributable to disease (osteoarthritis or CCL disease or both), suggesting that the differentially expressed proteins may be involved in disease mechanisms of stifle joint osteoarthritis secondary to CCL disease in dogs. Functionally, the proteins identified as differentially expressed were categorized as having functions in the complement system, lipid and cholesterol metabolism, or acute-phase responses.

The complement system has previously been implicated in osteoarthritis. Other investigators found that the complement system has a role in osteoarthritis as indicated by results of proteomic and transcriptomic analyses of synovial fluid and membrane samples obtained from human patients with knee osteoarthritis and experiments including gene knockout mice. Results of the present study indicated that concentrations of precursors of complement component 3 and factor I were significantly higher in synovial fluid samples obtained before surgery from dogs with osteoarthritis versus those obtained from clinically normal control dogs. Complement component 3 is an important part of the complement cascade and a progenitor protein of the anaphylatoxin complement component 3a and opsonin complement component 3b. Factor I, a soluble complement inhibitor, inhibits complement component 3b and complement components 1q, 1s, 2, and 4. Results of the present study also indicated that concentrations of clusterin, another soluble complement inhibitor that inhibits the membrane attack complex, were significantly higher in synovial fluid samples obtained from dogs with osteoarthritis versus those in synovial fluid samples obtained from clinically normal dogs (when results for preoperative and postoperative synovial fluid samples of dogs with stifle joint osteoarthritis were combined [data not shown]). Interestingly, results of another cross-sectional study indicate complement component 3 concentrations are significantly higher in synovial fluid samples obtained from osteoarthritic knees of human patients than they are in samples obtained from clinically normal knees of humans. The finding of the present study that concentrations of complement component 3 and complement inhibitors were higher in synovial fluid samples obtained from dogs with osteoarthritis than they were in synovial fluid samples obtained from clinically normal dogs was similar to results of those other studies including humans; this finding suggested that the complement system may have a role in stifle joint osteoarthritis in dogs. However, the complement system has been implicated in the pathogenesis of CCL disease in dogs; therefore, that finding may be attributable to CCL disease in dogs in the present study.

Results of immunohistochemical analysis indicated complement component 3 was expressed in synovocytes but not in articular chondrocytes of tissue sections of osteoarthritic stifle joints of dogs with CCL in this study; these results suggested that synoviocytes are likely a primary source of complement component 3 in osteoarthritic stifle joints. The finding of the present study that complement component 3 did not have substantial expression in articular chondrocytes of dogs with stifle joint osteoarthritis in the present study was consistent with results of another study that complement component 3 is not detected in articular cartilage of humans (as determined via reverse transcription PCR assay or immunohistochemical analysis). In contrast, components of the classical pathway of complement activation, including complement components 1q, 1s, 2, and 4, are expressed by articular chondrocytes of humans. These findings suggest that complement proteins produced by synovial membrane and articular cartilage cells function together in osteoarthritis disease processes and are present in synovial fluid. Two of the 4 synovial fluid proteins that were found to be differentially expressed between preoperative and postoperative synovial fluid samples of osteoarthritic stifle joints of dogs in the present study were complement proteins (complement component 1q and complement component 4 precursor). Expression of both of these proteins was increased approximately 2-fold in postoperative synovial fluid samples from dogs with osteoarthritis, compared with expression in preoperative synovial fluid samples from such dogs, despite clinical improvement of affected stifle joints of dogs after surgery. These
findings suggested that complement activation via the classical pathway could be accelerated after surgical intervention or during late stages of osteoarthritis.

Apolipoprotein B-100 precursor, PON1, ZAG precursor, and SAA, all of which are involved in lipid and cholesterol metabolism, had significantly higher expression in synovial fluid samples from osteoarthritic dogs (obtained before surgery) versus synovial fluid samples from clinically normal dogs in the present study. Apolipoprotein B-100 is the major protein in LDLs, and >90% of total plasma apolipoprotein B-100 is associated with LDLs. An increased apolipoprotein B-100 concentration is a hallmark of dyslipidemia because the concentration of apolipoprotein B-100 is an indicator of the concentrations of LDLs and a predictor of cardiovascular disease. Low-density lipoproteins are retained in arterial walls during atherosclerosis via interactions between apolipoprotein B-100 and negatively charged glycosaminoglycans in subendothelial proteoglycans. Apolipoprotein B-100 has not been implicated in osteoarthritis; however, apolipoprotein B-100 might play a role in initiation or progression of osteoarthritis via retention of LDLs in glycosaminoglycan-rich articular cartilage, as develops in humans with atherosclerosis. Low-density lipoproteins are retained in arterial walls during atherosclerosis via interactions between apolipoprotein B-100 and negatively charged glycosaminoglycans in subendothelial proteoglycans. Apolipoprotein B-100 has not been implicated in osteoarthritis; however, apolipoprotein B-100 might play a role in initiation or progression of osteoarthritis via retention of LDLs in glycosaminoglycan-rich articular cartilage, as develops in humans with atherosclerosis.

Serum PON1 is associated with HDLs and hydrolyzes lipid peroxidation products and therefore reduces oxidative stress in tissues. Other authors hypothesized that human patients with knee osteoarthritis are more susceptible to lipid peroxidation because such patients have lower serum HDL concentrations and PON1 activities, compared with control humans. Apolipoprotein D precursor concentrations were significantly higher in synovial fluid samples obtained from dogs with osteoarthritis after surgery than they were in synovial fluid samples obtained from such dogs before surgery in the present study. Similar to PON1, apolipoprotein D is primarily associated with HDLs and is capable of reducing lipid peroxidation. The increase in synovial fluid PON1 and apolipoprotein D concentrations detected in dogs with osteoarthritis in the present study could be attributed to a compensatory mechanism that protects joint tissues from oxidative damage. Zinc-alpha-2-glycoprotein stimulates activity of adipocyte adenyl cyclase and induces lipolysis. Supplementation of the diets of humans that have knee osteoarthritis with soy protein causes a decrease in serum concentrations of ZAG. However, to the author's knowledge, the clinical relevance and cause of altered ZAG expression in patients with osteoarthritis are unknown.

Results of the present study indicated synovial fluid SAA concentrations were significantly higher for dogs with osteoarthritis before surgery than they were for clinically normal control dogs and were significantly higher for dogs with osteoarthritis before surgery than they were for such dogs after surgery. Serum amyloid A is a major acute-phase protein primarily produced in the liver. Production of SAA is also induced by inflammatory stimuli in various types of cells that are abundant in joints, such as adipocytes, synoviocytes, and chondrocytes. During an acute-phase response to inflammation, SAA is a major apolipoprotein component of HDL. Although knowledge of the role of SAA in inflammatory responses and its effects on HDL metabolism and cholesterol transport is currently limited, it is thought that SAA is involved in enhancing removal of cholesterol from cells at sites of tissue injury. Impaired cholesterol efflux and abnormal lipid accumulation have been detected in osteoarthritic human chondrocytes. Because SAA was not detected in serum samples of dogs in the present study, increased SAA concentrations in synovial fluid samples obtained from dogs with osteoarthritis were likely attributable to alterations in inflammatory conditions or cholesterol metabolism in osteoarthritic stifle joints.

Transthyretin, RBP4, A2M, and angiotensinogen are acute-phase proteins, and their precursors were differentially expressed in synovial fluid samples obtained from dogs with osteoarthritis prior to surgery versus synovial fluid samples obtained from clinically normal dogs in this study. The clinical relevance and cause of decreased transthyretin and RBP4 precursor concentrations in synovial fluid of dogs with osteoarthritis are unknown, to the authors’ knowledge; however, transthyretin and RBP4 are both negative acute-phase proteins (ie, concentrations of these proteins decrease during acute-phase inflammation). Transthyretin concentrations in synovial fluid samples obtained from human patients with rheumatoid arthritis are lower than those in such samples obtained from healthy control humans. In contrast, both A2M and angiotensinogen are positive acute-phase proteins (ie, concentrations of these proteins increase during acute-phase inflammation). Alpha-2-macroglobulin is a panprotease inhibitor that inhibits proteinases that cause articular cartilage degradation, including A disintegrin and metalloproteinase with thrombospondin motifs-4, -5, -7, and -12. Angiotensinogen is a serine proteinase inhibitor primarily produced in the liver and adipose tissue. Serine proteinases are thought to have an important role in arthritis via extracellular matrix degradation. Increases in A2M precursor and angiotensinogen precursor concentrations in synovial fluid of dogs with osteoarthritis in the present study could have been attributable to a compensatory mechanism that inhibited proteinase activities during osteoarthritis.

Fibronectin 1 isoform 1 preproprotein concentrations were significantly increased in synovial fluid samples obtained from dogs with osteoarthritis before surgery in the present study, compared with synovial fluid sample concentrations for control dogs. High concentrations of this protein were detected in synovial fluid samples obtained from human patients with knee osteoarthritis in another study. Although fibronectin (an extracellular glycoprotein that binds to cells, collagen, and proteoglycans) concentrations were increased in osteoarthritic joints, the role of fibronectin 1 isoform 1 preproprotein is unknown, to the authors' knowledge.
None of the proteins identified in serum samples were differentially expressed between dogs with osteoarthritis and healthy control dogs in the present study. Results of other proteomic studies indicate changes in the proteome profile for serum, synovial fluid, and articular cartilage samples obtained from human patients with knee osteoarthritis, compared with clinically normal humans. Although 1-D gel electrophoresis LC-TMS has been successfully used to detect differences in protein concentrations in synovial fluid and articular cartilage samples between humans with osteoarthritis and clinically normal humans, this technique might not be sensitive enough to detect proteins in serum samples that were produced in osteoarthritic joint tissues; such proteins would presumably be highly diluted in serum. Proteins that were detected via a multiplexed immunoassay in serum and synovial fluid samples obtained from human patients with knee osteoarthritic joint tissues; such proteins would presumably be highly diluted in serum. Proteins that were detectable via a multiplexed immunoassay in serum and synovial fluid samples used in the present study were not detected via 1-D gel electrophoresis and LC-TMS analysis in this study. Because proteins identified by use of multiplexed immunoassays in that other study were detected in picogram concentrations, 1-D gel electrophoresis and LC-TMS analysis was apparently not sufficiently sensitive to detect proteins at such concentrations.

Several other possible limitations of the present study were identified. We do not know whether differential expression of proteins detected in this study was attributable to osteoarthritis, CCL disease, or both. Other authors have suggested that immunoinflammatory processes may be involved in mechanisms of CCL disease. Analysis of synovial fluid samples from dogs with osteoarthritis attributable to various etiologies (such as primary osteoarthritis, post-traumatic osteoarthritis, and osteoarthritis secondary to osteochondritis dissecans) may be necessary to confirm that theory. In addition, results of prospective studies in which synovial fluid samples obtained from subclinically affected stifle joints of dogs with unilateral CCL disease are assayed may improve knowledge regarding mechanisms of initiation of CCL disease. Other investigators have estimated that up to 54% of dogs with unilateral CCL disease will develop clinical CCL disease in the contralateral stifle joint, often within 1 year after the initial diagnosis of CCL disease. Although dogs with osteoarthritis and control dogs in the present study were young adult to middle-aged dogs, the mean ± SD ages of dogs with osteoarthritis (5.1 ± 1.8 years) and control dogs (2.9 ± 0.9 years) were significantly different; therefore, results could have been affected by differences in age of dogs. Determination of differential expression of proteins in synovial fluid samples obtained from stifle joints with CCL disease and contralateral subclinically affected stifle joints (which would result in age-matched and dog-matched control) joints may be a better method for identification of proteins involved in osteoarthritis of CCL disease. Because serum and synovial fluid samples were pooled, protein expression patterns for individual dogs could not be determined in the present study. In addition, the inclusion of 2 dogs with stifle joint osteoarthritis for which postoperative samples were not available made comparisons between results for preoperative versus postoperative synovial fluid samples less reliable than they would have been if postoperative samples had been available for those 2 dogs. Although proteomic analysis of unpooled serum and synovial fluid samples was cost-prohibitive in the present study, and although numbers of samples available for dogs with stifle joint osteoarthritis differed between preoperative and postoperative periods, results indicated differences in protein expression between dogs with osteoarthritis and control dogs. Cytologic examination of synovial fluid samples could have been performed to determine other characteristics of osteoarthritis in dogs in the study. However, such analysis was not performed because a definitive diagnosis of osteoarthritis associated with CCL disease was made for all dogs on the basis of surgical assessments and results of physical and radiographic examinations.

The similarity in results of the present study and those of other proteomic studies of synovial fluid samples obtained from humans with knee osteoarthritis supported the translational research importance of the results of this study. Interestingly, the proteins found to be differentially expressed between synovial fluid samples obtained from dogs with osteoarthritis versus healthy control dogs in the present study have been implicated in the pathogenic mechanisms of atherosclerosis, derangement in functions of the complement system and lipid and cholesterol metabolism might indicate a link between osteoarthritis and other chronic inflammatory disease conditions, such as atherosclerosis. The proteins found to be differentially expressed between dogs with stifle joint osteoarthritis and those without stifle joint osteoarthritis in this study might be important in mechanisms of osteoarthritis and results may be useful for improvement of diagnostic tests and treatments for humans and other animals with osteoarthritis.

References


