INTRODUCTION: Osteoarthritis (OA) is considered to be a complex disease process that involves all components of the joint environment: cartilage, synovium, and subchondral bone. When there is joint injury, such as cranial cruciate ligament (CCL) rupture in dogs or humans, there are abnormal biomechanical stresses placed on the entire joint. Invariably, this leads to loss of the articular cartilage accompanied by osteophyte formation along the margins of the bone surface in an attempt to stabilize the joint. Radiographic evidence of osteophyte formation in typical locations within the canine and human knee along with joint space narrowing has historically been the hallmark of osteoarthritis diagnosis. Classically, biomarkers of bone formation and resorption have been assayed in serum. A common biomarker used to evaluate bone formation is bone alkaline phosphatase (BAP), a cell membrane enzyme present in osteoblasts and osteoblasts. It is an early osteoblastic marker that has been shown in the serum to correlate positively with bone formation, especially in elderly people with osteoporosis (1). However, BAP has been examined in only a few studies of human spinal and peripheral joint OA (2,3). In addition, there is a report of measurement of BAP in the synovial fluid for equine OA (4). Therefore, the purpose of this study was to measure BAP levels in the serum and synovial fluid of a canine CCL transection model of OA, and to compare synovial fluid concentrations to total radiographic osteophyte scores.

MATERIALS AND METHODS: An experimental model of osteoarthritis was created in 19 mature male Walker Hounds that were used as controls in another larger study. The right CCL was transected arthroscopically in all dogs. Synovial fluid samples were collected from the left and right knees using a lavage technique (5 ml of sterile saline) at surgery (Day 0), and then again at 14, 70, and 126 days post-operatively. Serum samples were collected from the jugular vein pre-operatively (Day 0), and then again at day 7, 14, and every two weeks thereafter until day 126 post-operatively. BAP concentrations were measured in the synovial fluid and serum using a commercially available immunoassay. Dorsopalmar and lateral radiographs were taken of the knee pre-operatively (Day 0), and then again at day 14, 28, 70, and 126 days post-operatively. At the conclusion of the study, all radiographs were graded for the presence and severity of osteophytes by a board-certified radiologist. Total radiographic osteophyte scores were derived by grading 9 different locations in which osteophytes commonly form in the osteoarthritic canine knee using a scale of 0-3. Post-operative BAP concentrations in the serum and synovial fluid were compared to baseline, and significant differences between time points were determined using an ANOVA with Tukey’s analysis for multiple comparisons. Spearman Rank correlation analysis was performed to compare BAP concentrations and total radiographic osteophyte scoring. Multiple regression was performed using BAP concentrations as the dependent variable and each individual osteophyte location as the independent variables. A value of p<0.05 was considered significant for all analyses. All procedures were approved by the university’s Animal Care and Use Committee.

RESULTS: Synovial fluid – Right knee (CCL transected) - BAP concentrations significantly increased from baseline (p<0.0001). Compared to baseline, day 14 values were 3.8 times higher (p<0.05), and day 70 and 126 values were both 7.3 times higher (p<0.001, Fig. 1A). There was a significant positive correlation between the BAP concentrations in synovial fluid and the total radiographic osteophyte score (r2=0.46, p<0.0001). Multiple regression identified a significant relationship (p<0.0001) between the presence and severity of osteophytes along the femoral trochlear ridges, tibial intercondylar eminence, and plantar proximal tibia and the concentration of BAP in the synovial fluid. Osteophytes in these three locations account for 50.5% of the variance in BAP concentrations in the synovial fluid.

Synovial Fluid – Left knee (normal) - There was no significant difference in BAP concentrations from baseline (p=0.1953, Fig. 1A).

Discusssion: It has been reported that alkaline phosphatase activity in the synovial fluid in humans with rheumatoid arthritis is primarily bone-specific (5). The concentration of BAP in the synovial fluid from CCL transected joints significantly increased from baseline over time in this study. Since the BAP concentration in the synovial fluid from the opposite joint did not elevate above baseline, the significant elevation in BAP levels must be from some osseous source within the joint itself. Increased BAP concentrations in the synovial fluid of horses with osteochondral fragmentation was believed to be due to early changes occurring to the underlying subchondral bone (4). However, when dealing with the early stages of the canine CCL model of OA in this study, the overall pathological changes that occurred were not severe enough to cause exposure of subchondral bone. Therefore, the likely osseous source of BAP in the synovial fluid from the OA joints in our model was from osteophyte formation. This is supported by a significant positive correlation between the synovial fluid BAP concentration and total radiographic osteophyte score. The increased BAP in the synovial fluid contributing to osteophyte formation may be partially under the regulation of TGF-β, as TGF-β has been shown to be the primary regulator of osteophyte formation (6). In addition, TGF-β has been shown to upregulate alkaline phosphatase activity (7). As noted, the concentration of BAP in serum decreased with progression of OA in the CCL transected joint. Serum is the proximal fluid into which bone components will drain. As is the case with any serum marker, the concentration of BAP from bones of a single injured joint will be diluted and represents BAP from all other bones within the body. Therefore, injury to a single joint and its representative bones must be severe for there to be an increase in serum levels. In addition, BAP may undergo further proteolytic processing in the serum and not be detected by the immunoassay. To the best of our knowledge, this is the first study to document the use of BAP analysis in the synovial fluid and serum of a canine CCL transection model of OA. It demonstrated a positive correlation of synovial fluid BAP levels with osteophyte production in the osteoarthritic joint, as well as a decrease in BAP concentration in the serum.

REFERENCES:

Figure 1: Concentrations (U/L) of BAP in the synovial fluid (A) from the CCL transected (right knee) and opposite normal joints (left knee) and serum (B). Error bars represent the standard error of the means. All post-operative samples were compared to baseline (Day 0) and significant differences are represented as *** p<0.001, and * p<0.05. Significant differences shown in A represent the right synovial fluid samples only.