Introduction: Congenital hip dysplasia (CHD) in dogs is a well described pathologic condition in which affected individuals develop coxofemoral joint (CFJ) osteoarthritis (OA) with age. The pathogenesis of the condition is well described.\(^1\) Radiography is the standard method by which CFJs are assessed for bony changes characteristic of CHD. The PennHIP radiographic evaluation is one established method.\(^2\) Articular cartilage degeneration in OA has been extensively evaluated. Recent studies have focused on immunological methods to identify epitopes in synovial fluid as a result of articular cartilage changes.\(^3\) Two monoclonal antibodies developed for this use are 3B3(-) (proteoglycan chondroitin sulfate) and COL2-3/4C long (collagen II).\(^4,5\)

The goal of this project was to determine the relationship between canine CFJ OA and synovial fluid concentrations of 3B3(-) and COL2-3/4C long at each age. We hypothesized that synovial fluid concentrations of 3B3(-) and COL2-3/4C long would be elevated in joints destined to develop OA before radiographic evidence of disease was apparent.

Materials and Methods: Twelve hounds from a CHD breeding colony were used for this study. Synovial fluid was collected from all CFJs at 20, 24, 32, 38, 68, and 120 weeks of age. Dogs were euthanized at 120 weeks of age. A competitive equilibrium ELISA was used to quantitate 3B3(-) epitope concentrations.\(^4\) COL2-3/4C long neoeptitope concentrations were monitored by a solution phase ELISA assay.\(^1\) All assays were performed in duplicate, and the recovery of antibodies was determined by running parallel assays after the addition of standards to pooled samples.

PennHIP radiographs were performed at each synovial fluid collection and were scored for OA as none, mild, moderate, or severe by PennHIP personnel using established guidelines. Transverse paraffin sections from the center of each femoral head and acetabulum, collected at 120 weeks of age, were stained with H & E and safranin O stains. The articular cartilage of each section was scored according to a revised Mankin method.\(^6\) Scores from each femoral head and corresponding acetabulum were combined to give one numeric score.

Nullah linear regression analyses were performed to correlate synovial fluid epitope concentrations at each time point with age, radiographic OA, and with final histologic and radiographic OA. ANOVA was used to evaluate differences in epitope concentrations among time points for each final OA score and among final OA scores at each time point. Significant differences among time points or OA scores were evaluated with Tukey’s post hoc t-test. Differences were considered significant at p ≤ 0.05. Trends were considered at p < 0.15.

Results: Radiographs of 24 CFJs were scored for OA by PennHIP personnel at each time point. At 120 weeks of age, 10 joints were scored as none, five were mild, four were moderate, and five were severe. Of the 22 joints available for Mankin scoring, four were normal, 13 were mild, four were moderate, and one was severe.

There was a significant negative correlation between 3B3(-) concentrations and age (Fig. 1A). There were significant linear correlations between 3B3(-) concentrations and OA scores at 24 (r\(^2\)=0.21), 32 (r\(^2\)=0.18), 42 (r\(^2\)=0.31), and 120 (r\(^2\)=0.24) weeks of age. There were linear trends at 20 (r\(^2\)=0.11) and 68 (r\(^2\)=0.09) weeks of age. There were significant linear correlations between 3B3(-) concentrations at 20 (r\(^2\)=0.02), 24 (r\(^2\)=0.19), 32 (r\(^2\)=0.17), 42 (r\(^2\)=0.22), and 120 (r\(^2\)=0.24) weeks of age with final OA scores. There was a significant correlation between Mankin scores and 3B3(-) concentrations at 42 weeks (r\(^2\)=0.18) of age. There were trends at 20 (= 0.14), 24 (= 0.11), and 120 (= 0.16) weeks of age.

There was a very weak but significant positive linear correlation between COL2-3/4C concentrations and age (Fig. 1B). There were significant linear relationships between COL2-3/4C long concentrations and OA scores at 32 (r\(^2\)=0.22), 42 (r\(^2\)=0.24), 68 (r\(^2\)=0.18), and 120 (r\(^2\)=0.26) weeks of age. There were significant linear relationships between COL2-3/4C long concentrations at 24 (r\(^2\)=0.35), 32 (r\(^2\)=0.35), 42 (r\(^2\)=0.44), 68 (r\(^2\)=0.30), and 120 (r\(^2\)=0.26) weeks of age with final OA scores. There was a trend at 20 weeks of age (r\(^2\)=0.12). There was a significant correlation between Mankin scores and COL2-3/4C long concentrations at 32 (r\(^2\)=0.21) and 42 (r\(^2\)=0.38) weeks of age. There were trends at 24 (r\(^2\)=0.10) and 68 (r\(^2\)=0.10) weeks of age. ANOVA analysis of epitope concentrations in synovial fluid among time points for each final OA score revealed significant decreases in 3B3(-) over time (Fig. 2A). The decrease was greatest in joints that did not develop OA and smallest in those that developed severe OA. The only significant differences in COL 2-3/4C long concentrations among time points were in the joints that developed moderate OA, which increased with time (Fig. 2B). The relative relationships of synovial fluid concentrations of both epitopes for each final OA score were fairly well maintained over time. Beginning at 24 weeks of age, there was a significant difference in COL2-3/4C long concentrations between joints that developed severe radiographic OA and those that did not develop OA. There were no significant differences among concentrations at any time point between final radiographic OA scores for 3B3(-).

Discussion: The results of this study show a linear relationship beginning at 20 weeks of age between canine CFJ synovial fluid concentrations of 3B3(-) and the expression of OA at 120 weeks of age, and beginning at 24 weeks of age with synovial fluid concentrations of COL2-3/4C long. Though the synovial fluid concentrations of 3B3(-) and COL2-3/4C long tended to change with age, the relative relationships remained fairly constant, and significant increases in COL2-3/4C long concentrations in joints that developed severe OA were apparent at 24 weeks of age. There may be significant predictive values of synovial fluid concentrations of these markers for the development of CFJ OA. Further studies with larger subject numbers are necessary to define their specific predictive levels. These markers may be useful as an adjunct to other clinical means of joint evaluation to predict disease potential and may pave the way for the development of similar techniques to be used in children predisposed to the development of developmental dysplasia of the hip.


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