SERUM MARKERS DIFFERNTIATE EXERCISE FROM PATHOLOGY AND CORRELATE TO CLINICAL PARAMETERS OF PAIN IN AN OSTEOARTHRITIC MODEL

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Introduction: Biomarkers have been beneficial in assessing osteoarthritic (OA) patients in numerous species including humans, horses and dogs. Questions have been raised concerning the ability of biomarkers to distinguish OA pathology from normal metabolic alterations that occur secondary to exercise. The objective of this study was to determine if serum biomarkers could differentiate subjects undergoing a strenuous exercise protocol from subjects with a solitary OA joint enrolled in a similar exercise protocol. Additionally, the correlations between serum biomarker levels and clinical parameters of pain and histologic articular cartilage pathology were evaluated.

Methods: This study utilized a well-characterized model of OA in the horse (Figure 1).[1] Sixteen 2-year old horses were entered into the study and all horses began the study enrolled in a 21-day strenuous exercise protocol, followed by arthroscopic surgery. Eight of the horses underwent a sham operation (Control group) while the remaining 8 horses had the induction of OA in a solitary joint (OA group). Fourteen days after surgery all horses resumed the exercise protocol for an additional 56 days. Throughout the study serum samples were collected weekly and concentrations of biomarkers were measured for aggrecan synthesis (CS846), glycosaminoglycans (sGAG), type II collagen synthesis (CPII) type I and II collagen degradation (COL2-3/4C_{short}), as well as, bone synthesis (osteocalcin) and degradation (CTX-1). Clinical examinations to assess pain were performed prior to euthanasia. Articular cartilage samples were collected for morphologic assessment using a modified Mankin scoring system. Data were analyzed using a mixed model analysis of variance, discriminate analysis and linear regression. A p-value <0.05 was considered significant and data was transformed using natural logs to fulfill assumptions of normality prior to statistical analysis. The Institutional Animal Care and Use Committee approved all aspects of this experimental study.

Results: All biomarkers increased significantly with exercise during the study. Similarly all biomarkers demonstrated a significant difference between the control and OA groups, the earliest difference was noted 14 days post OA induction (CS846) (Figure 2). Using multivariate analysis CS846, CPII, sGAG and CTX-1 were most useful in predicting if serum was from either a control or OA horse. Furthermore, by day 14 discriminate analysis correctly predicted a sample as being from a control or OA horse 100% of the time (CS846 and sGAG). Serum concentrations of osteocalcin and CS846 provided the best prediction of both the modified Mankin score ($R^2 = 0.72$) and clinical degree of pain ($R^2 = 0.70$) using multivariate linear regression (stepwise model selection). Serum sGAG concentration alone was the best predictor of the degree a horse responded to joint manipulation during clinical examination ($R^2 = 0.60$) (Figure 3)

Discussion: CS846 and sGAG estimates provided the earliest and most useful information on presence of OA and this finding supports proteoglycan concentration as being one of the earliest detectable OA changes. Furthermore, changes in collagen and bone biomarker levels while useful, occurred latter after OA induction as compared to proteoglycan biomarkers. The concentration of CS846, osteocalcin and sGAG appeared to be the most consistent and useful in correlating biomarker levels to clinical signs of pain and articular cartilage pathology. Whether these changes are due to local environmental factors (OA joint) or an abnormal or an altered joint/limb usage pattern will be better-elucidated following analysis of biomarker levels in synovial fluid. In this model the OA is induced using creation of an acute osteochondral fragment and leaving joint tissue debris in the synovial space. Because full-thickness articular cartilage erosions are present very early in these experimental OA joints (within 70 days post surgery), concentrations of biomarkers are likely to be higher than might be present in some naturally occurring early stage OA. Even so, these results suggest that further investigation of biomarkers for the prediction and monitoring of OA is warranted and correlation of biomarker levels to traditional disease monitoring parameters is possible.
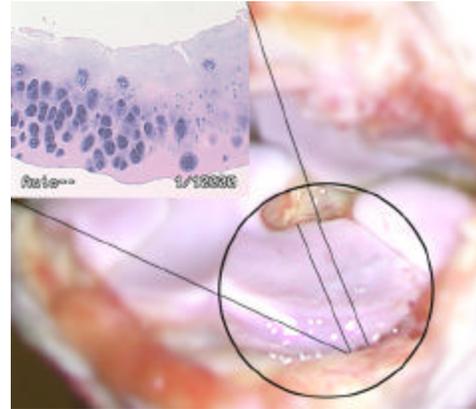

Figure 1 – Gross photo of typical OA lesion with associated histologic appearance (H&E stained section photographed at 10X)
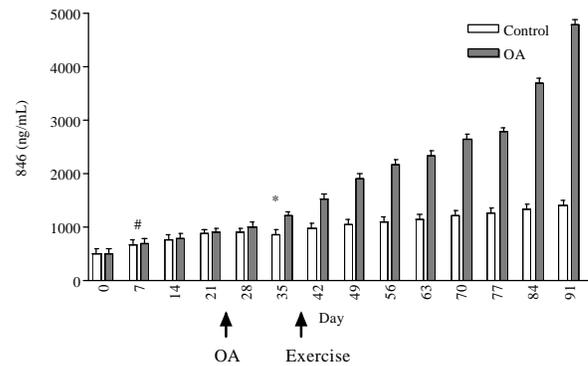

Figure 2 –Serum CS846 concentration (mean ± SEM) was significantly increased in all horses 7 days after the beginning of exercise (#). Within fourteen days after induction of OA a significant difference between the control and OA groups was demonstrated (*).
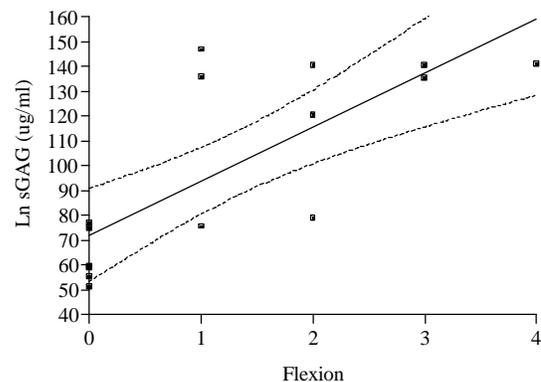

Figure32 - Natural log of sGAG concentration plotted by response to joint manipulation (flexion). The best-fit line and a 95% confidence interval are also presented. The response to joint manipulation is grade 0-4 with a score of zero being no response and a score of 4 representing a severe response.

References:
1. Frisbie DD, McIlwraith CW: Evaluation of gene therapy as a treatment for equine traumatic arthritis and osteoarthritis. Clin Orthop 379S:S273-287, 2000.