INTRODUCTION: The CTX II assay has been developed to examine the collagen type II telopeptide that is released from articular cartilage into the systemic circulation. In human osteoarthritis (OA) patients, the Urine Cartilaps® ELISA has been reported to be useful in the prediction of progression of OA. Collection of urine samples is readily achieved in human patients, but can be difficult to obtain in animal models of joint disease, especially when compared to other fluids such as serum and synovial fluid. From a biomarker perspective, the horse is a unique model of joint disease because urine, serum, and synovial fluids can be readily obtained, allowing biomarker comparisons to determine which fluid or fluids best predict disease. We hypothesized that the combination of urinary and serum CTX II concentrations may show better correlation with radiographic and arthroscopic signs of disease severity than the use of urinary or serum CTX II alone. Thus, the purpose of this study was to correlate both urinary and serum CTX II with these clinical markers of joint injury.

METHODS: Forty-seven horses were divided into one of two groups: (1) control horses that demonstrated no apparent symptoms of joint disease (n=19) and (2) joint disease horses that had osteochondritis dissecans (OCD), osteochondral fragmentation and/or OA in one or more limbs (n=28). Urine was collected via catheterization from all 47 horses, and serum was collected via jugular venipuncture from 20 (11 controls and 9 joint disease) horses. All procedures were approved by institutional animal care and use committees. Radiographic and arthroscopic scores were determined as previously reported. If multiple limbs were affected, the highest radiographic or arthroscopic scores were used for analyses. Urine Pre-Clinical Cartilaps® and Serum Pre-Clinical Cartilaps® ELISAs (IDS Nordic a/s) were used to analyze the urine and serum samples respectively. Both assays have been previously validated for use in the horse. Samples were not digested and were appropriately diluted. To correct urine concentrations for creatinine, urine samples were analyzed using the MicroVue Creatinine assay kit (Quidel Corporation). Mann-Whitney t-tests were performed to determine differences between groups and Spearman correlations were performed to determine relationships between CTX II concentrations and radiographic and arthroscopic scores. P values <0.05 were considered significant.

RESULTS: Urinary CTX II concentrations were significantly higher (P<0.05) in horses with joint disease compared to control horses (Figure 1A). However, serum CTX II concentrations demonstrated no significant differences between groups (Figure 1B). There was a strong correlation (R=0.90, F=0.001) between urine and serum CTX II concentrations (Figure 1C and Table 1). Both urine and serum CTX II concentrations significantly correlated with radiographic scores, but not arthroscopic scores (Table 1). When combining the CTX II concentrations into a ratio of urine to serum, significant correlations between urine:serum and the radiographic (R=0.79, P<0.0001) and arthroscopic scores (R=0.54, P=0.044) were identified (Table 1).

DISCUSSION: Urinary CTXII has been used extensively to determine existence and progression of joint disease in humans. Therefore it is important to be able to demonstrate that this biomarker responds in a similar fashion in animal models of joint disease. We demonstrated in an equine model of joint disease that urinary CTX II concentrations were significantly higher in horses with joint disease compared to controls. Serum CTX II concentrations are rarely used in humans, but are often used in animal models of joint disease. Serum concentrations were not significantly different between groups in our study which may be due to low power. However, the urinary and serum CTX II concentrations were significantly correlated (Figure 1C). To our knowledge, this is the first report demonstrating correlation between urinary and serum CTX II concentrations. Both urinary and serum CTX II concentrations correlated with radiographic scores, but not arthroscopic scores. However, the ratio of urinary CTX II to serum CTX II (urine:serum) correlated to both radiographic and arthroscopic scores of joint disease severity. Thus, our study demonstrates that urinary CTX II concentrations can be used in animal models of joint disease to distinguish disease from controls. In addition, the relationship between biochemical and clinical markers of joint disease may be strengthened by the evaluation of more than one body fluid.


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