

# Coll2-1 and Coll2-1 NO<sub>2</sub>: Markers of Early Disease in the Hartley Guinea Pig Model of Spontaneous OA

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## Introduction

Several different type II collagen epitopes have been described as potential biomarkers of onset and/or progression of OA (1). Among the degradative markers, the C-telopeptide fragment, CTXII, has the most abundant data supporting its use as an arthritis marker (2,3). More recently, two serum immunoassays have been developed to quantify a sequence derived from the triple helical region of type II collagen (Coll2-1) and its nitrated form (Coll2-1 NO<sub>2</sub>) that can be used to quantify both cartilage degradation as well as oxidative damage, respectively (4,5). In this study, we investigated the utility of type II collagen catabolism serum biomarkers: Coll2-1, Coll2-1NO<sub>2</sub>, C2C, and CTXII, in a well characterized cohort of Hartley guinea pigs; a strain of guinea pig shown to spontaneously develop OA of the knee, for the purpose of comparing the usefulness of the biomarkers of degradation in the model system.

## Methods

Forty six male Hartley guinea pigs were sacrificed at 3 weeks, 2, 4, 7, 10, 12 (n=6) and 18 months (n=10) of age, at which time blood samples were obtained. Histological severity of OA was determined using a semi-quantitative grading scheme described previously (6). Serum Coll2-1 and Coll2-1 NO<sub>2</sub> were quantified by competitive ELISA described previously (4), serum C2C were quantified by a commercially available ELISA (IBEX, Montreal, Quebec) and serum CTXII were quantified using the Serum Pre-clinical Cartilaps ELISA (Nordic Bioscience) as per the manufacturers' protocols. This assay was designed to detect degradation products of C-terminal telopeptides of type II collagen in animal sera. For detailed locations of all epitopes, see Figure 1 below. Statistical analyses using Prism GraphPad 4.0 included: the non-parametric Mann Whitney test to compare the levels of Coll2-1 at 3 weeks and 4 months of age, and correlations were estimated by the non-parametric Spearman's rank correlation coefficient. Total histological scores were separated into quartiles and corresponding levels of biomarkers were analyzed by ANOVA followed by non-parametric Kruskal-Wallis post-hoc test. Data were considered statistically significant at p value less than 0.05.

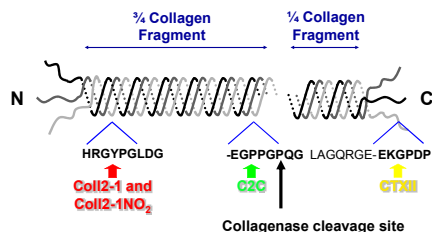


Figure 1. Schematic of the molecular origin of the type II collagen degradation epitopes, Coll2-1 and Coll2-1NO<sub>2</sub>, and degradation epitopes, C2C and CTXII.

## Results

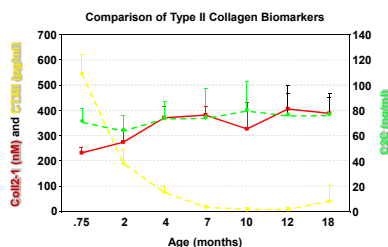


Figure 2. Comparison of type II collagen biomarkers (Coll2-1, C2C, and CTXII) in a cross-sectional cohort of Hartley guinea pigs aged 3 weeks to 18 months of age (mean values  $\pm$  SD). Both Coll2-1 and C2C display completely different time course profiles compared to CTXII. At 3 weeks of age, levels of CTXII are elevated and rapidly decline to barely detectable levels by 7 months of age, showing very little change from 7-18 months of age. In contrast, although levels of C2C decrease slightly from 3 weeks to 2 months of age, there were no differences in the mean concentrations coincident with the development of histological OA (7). However, levels of Coll2-1 are lowest at 3 weeks of age and increase by 65% from 3 weeks to 4 months of age ( $p=0.002$ ) with consistently elevated levels thereafter to 18 months of age. The serum profile for Coll2-1NO<sub>2</sub> was similar, but concentrations equaled 1-2% the serum concentrations of Coll2-1 throughout the time course.

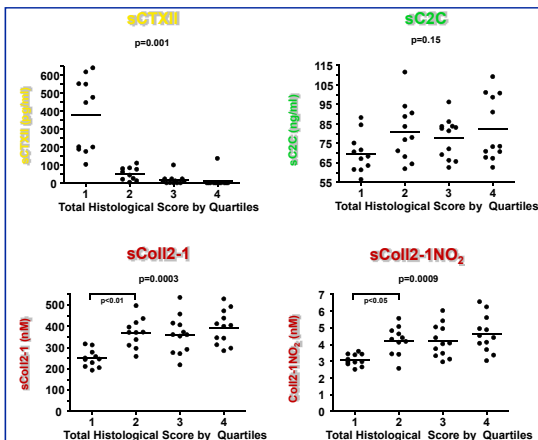


Figure 3. Distribution of serum biomarker levels by quartiles of total histological score. Total histological scores were divided into quartiles (Q1: 0-9; Q2: 10-18; Q3: 19-32) to examine the distribution of levels of serum biomarker by severity of histological OA. Levels of sCTXII were elevated in Q1, represented by young animals with no histological OA, and decreased significantly in subsequent quartiles representing increasing severity of histological OA, thus it appears to be reflective of the rapid turnover associated with growth and development in this model and not sensitive to the collagen breakdown associated with the progression of OA. There were no significant differences in levels of serum C2C with increasing severity of histological OA. There was a statistically significant increase in both serum Coll2-1 and Coll2-1NO<sub>2</sub> from Q1 to Q2 ( $p<0.01$  and  $p<0.05$ , respectively) and levels remained elevated with increasing severity. The same trend was observed for sC2C, however, this increase was not significant due to the increased scatter of values observed for this biomarker in each quartile.

	sColl2-1	sColl2-1NO <sub>2</sub>	sC2C	sCTXII
sColl2-1	—	0.9441 ( $<0.0001$ )	0.3788 (0.0094)	-0.4076 (0.0055)
sColl2-1NO <sub>2</sub>		—	0.4577 (0.0014)	-0.3782 (0.0104)
sC2C			—	-0.1710 (0.2813)
sCTXII				—

Figure 4. Correlation between serum levels of Coll2-1, Coll2-1NO<sub>2</sub>, C2C, and CTXII. Each cell contains Spearman  $r$  followed by the  $p$ -value (two-tailed). There was no significant correlation between CTXII and C2C, however there were significant negative correlations between CTXII and both Coll2-1 and Coll2-1NO<sub>2</sub>. Correlations between C2C and both Coll2-1 and Coll2-1NO<sub>2</sub> were significant and the correlation between Coll2-1 and Coll2-1NO<sub>2</sub> was highly significant, suggesting that these are reflective of interrelated biological processes in this model.

## Conclusions

•sCTXII, sC2C, sColl2-1, sColl2-1NO<sub>2</sub> reflect different biological processes in this animal model, probably as a result of differential expression of collagen degradative enzymes in different joint tissue compartments.

•Serum CTXII profile in the guinea pig is most compatible with collagen II turnover in the growth plate cartilage, which ceases at 4 months of age in this model, and is barely measurable during the period of development of histological OA.

•Levels of sC2C appear to remain essentially constant throughout the development of OA, but are reflective of inter-related biological processes measured by Coll2-1 and Coll2-1NO<sub>2</sub> in this model.

•Levels of Coll2-1 and Coll2-1NO<sub>2</sub> are not confounded by growth plate cartilage turnover, and are in fact at their lowest levels during the most active period prior to 4 months of age.

•The marked increase in sColl2-1 from 3 weeks to 4 months of age occurs concomitantly with collagen disruption, as measured by collagen birefringence, and levels remain elevated during the course of disease development.

•Thus, Coll2-1 and Coll2-1NO<sub>2</sub> epitopes are likely generated from articular cartilage and may be useful as quantitative biomarker outcomes for early disease prevention and treatment in this model system.

## References

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