most strongly associated with activated macrophages in the capsule of the tibiofemoral (TF) compartments (p=0.0063) and the whole joint synovium (p=0.0017). sCD163 was also associated with severity of radiographic OA, specifically joint space narrowing (p=0.0554), but not osteophyte severity. The use of the markers in combination improved the prediction of activated macrophages in the capsule of the PF knee compartment (p=0.013) and joint space narrowing (p=0.0099) independent of osteophyte severity.

Conclusions: These findings suggest that cell-surface markers shed from activated macrophages may be used as biomarkers to identify a subtype of OA patients with an active inflammatory disease state. The combined analysis of both CD14 and CD163 indicate synergism between the markers for identifying the presence of activated macrophages in the capsule of the PF knee and joint space narrowing. Furthermore, these results contribute to a growing literature identifying macrophages and macrophage-activation pathways as potential targets for new OA therapies.

Acknowledgements: Samples were obtained with support of a grant from Lilly USA, Inc; support for the assays was provided by laboratory research funds of the PI (VBK), and support for personnel was provide by NIH 5T32AI007217-30 (HND) and P01 AR050245.

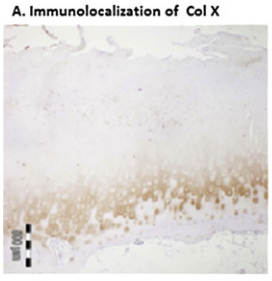
127
CHONDROCYTE HYPERTROPHY, MEASURED BY THE SECRETION OF COLLAGEN TYPE X, IS A HALLMARK OF PATHOLOGICAL CHANGES IN OSTEOARTHRITIS

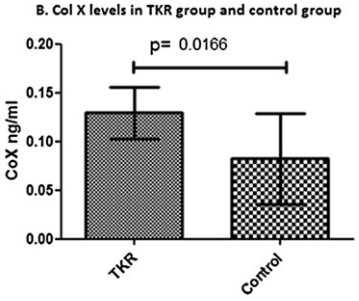
Y. He[†], N. Urik Brndt-Hansena[†], J. Wang[‡], D. Su[‡], Q. Zheng[‡], O. Simonsen[§], K. Petersen[‖], M. Karsdal[†], A.-C. Bay-Jensena[†]. [†]*Nordic BioSci., Herlev, Denmark;* [‡]*Nordic BioSci. China, Beijing, China;* [§]*Dept. Orthopedic Surg, Aalborg Univ. Hosp., Aalborg, Denmark;* [‖]*Ctr. for Sensory-Motor Interaction, Dept. of Hlth.Sci. and Technology, Aalborg Univ., Aalborg, Denmark*

Purpose: Collagen type X (Col X) is a short chain and network-forming collagen, which is specifically synthesized by hypertrophic chondrocyte in the growth plate during endochondral ossification, the calcified cartilage in normal adult or the deep zone of osteoarthritic (OA) cartilage; thus Col X may play an important role in cartilage calcification. The aim of the study was to measure serum levels Col X in OA patients as well characterize the presence of the ColX in OA cartilage section.

Methods: A monoclonal antibody (mAb) was raised against the 10aa at C-terminus of NC1 domain of Col X. This mAb was characterized by western blot using U2-OS osteosarcoma cell as positive control, and by immunohistochemistry on 5 human OA knee cartilages sections. A solid-phase competitive ELISA was developed applying the mAb and tested in a small cohort including: 47 OA patients undergoing total knee replacement surgery (mean age ± SD: 64±15), and 13 controls with knee problems having arthroscopy (mean age ± SD: 34±21), all women. Mann-Whitney test was applied to compare the serum Col X levels in TKR and control group. Pearson correlation was done on log transformed data.

Results: The mAb, NB509-11G8, was specific for the immunogen; it did not recognize truncated or nonsense peptides when tested in the ELISA. The mAb detected the monomeric Col X (59KDa) and dimeric Col X (120KDa) in the U2-OS cell lysate. Moreover, these bands were blocked by the specific peptide. NB509-11G8 stained the extracellular matrix of chondrocytes in the deep zone and calcified cartilage of human OA samples but not the middle or upper zone (fig. A). The competitive ELISA had a measuring range of 0.02-5 ng/ml, with intra- and inter-assay CVs of 4.19% and 10.62%. The mean serum Col X level was significantly higher in the TKR than the control group (P=0.017, fig. B). Serum Col X level did not correlate with age (R^2=0.055), but weak relation with BMI (R^2=0.17). The AUC for serum Col X for discriminating TKR from controls was 0.72 (P=0.017).





Conclusions: Secretion of Col X as a marker of cartilage hypertrophy may serve as an early biomarker for OA burden of disease.

128
CTX-II IS A MARKER OF CARTILAGE DEGRADATION BUT NOT OF BONE TURNOVER

J. Catterall[†], S. Dewitt Parr[‡], K. Fagerlund[§], B. Caterson[‡]. [†]*Duke Univ., Durham, NC, USA;* [‡]*Cardiff Univ., Cardiff, United Kingdom;* [§]*Pharmatest Services Ltd, Turku, Finland*

Purpose: The CTx-II biomarker has been widely investigated and used to determine the status of cartilage turnover in osteoarthritis and rheumatoid arthritis. Recent publications have discussed the possibility that CTx-II release may partially reflect bone turnover rather than being a specific marker of cartilage turnover. As CTx-II has been used so widely, it is clear that the specificity of CTx-II for cartilage needed to be re-evaluated. To this end, we decided to investigate the specificity of CTx-II for cartilage in comparison to the gold standard CTx-I bone degradation marker. We investigated the specificity of the ELISAs for their respective epitopes, release of CTx-I and CTx-II from bone and cartilage explant cultures, and levels of CTx-II and CTx-I in a small acute injury and osteoarthritis (OA) cohort.

Methods: ELISA specificity for CTx-I and CTx-II was determined by depletion of the epitopes from urine and by affinity purification of the epitopes. Release of CTx-I and CTx-II from bone and cartilage was investigated by digesting both cartilage and bone with either cathepsin K or matrix metalloproteinases, and release of CTx-I and CTx-II determined by ELISA. We also investigated the release of CTx-I and CTx-II in explant culture from both bone by osteoclasts mediated bone degradation and from pro-inflammatory cytokine-stimulated cartilage explant cultures. Finally, we investigated the changes in CTx-I and CTx-II in patient cohorts.

Results: *In vitro*, cathepsin K digestion of bone released CTx-I alone while digestion of bone with activated MMPs was unable to release either CTx-I or CTx-II from bone. Only digestion of cartilage by activated MMPs released CTx-II, while digestion of cartilage by either MMPs or cathepsin K was unable to release detectable CTx-I. Osteoclast digestion of bone slices released only CTx-I and no detectable CTx-II, while the reverse was true in chondrocyte-mediated cartilage degradation, with high CTx-II release and no detectable CTx-I. In our OA patient cohorts we observed an increase in CTx-II levels when compared to healthy controls but no change in CTx-I, while acute injury patients demonstrated a reduction in CTx-I levels after injury and no change in CTx-II.

Conclusions: Our data strongly suggest that CTx-I reflects bone degradation and CTx-II reflects cartilage degradation, and that in acute injury and in osteoarthritis, bone turnover and cartilage degradation/repair processes differ. As CTx-II reflects cartilage degradation, it is a valid biomarker of cartilage breakdown and not a biomarker of bone turnover, although in some cases it may reflect the connected pathology of bone and cartilage in joint diseases.

129
CTX-II LEVELS ARE ASSOCIATED WITH PERIPHERAL BONE DENSITY, SUGGESTING CTX-II EPITOPE RELEASE FROM BONE: DATA FROM COHORT HIP AND COHORT KNEE (CHECK)

W.E. van Spil, K.L. Vincken, W.F. Lems, F.P. Lafeber. *Univ. Med. Ctr. Utrecht, Utrecht, The Netherlands*

Purpose: Recent data have suggested that CTX-II (C-terminal telopeptide of collagen type II) may not only originate from articular cartilage but also from bone changes. Therefore, the purpose of this study was to look for cross-sectional associations between urinary CTX-II levels and bone density distant from the subchondral bone area.

Methods: This study was performed in 366 subjects with early-stage symptomatic knee and/or hip osteoarthritis from Cohort Hip and Cohort Knee (CHECK). In urine (u-) and serum (s-) samples from these subjects, uCTX-II, the cartilage markers sCOMP, sPIIANP, and sCS846 (cartilage oligomeric matrix protein, N-terminal propeptide of procollagen type IIA, and chondroitin sulphate 846), and the bone markers uNTX-I, uCTX-I, sPINP, and sOC (N and C-terminal telopeptides of collagen type I, N-terminal propeptide of procollagen type I, and osteocalcin) were assessed by enzyme-linked immunosorbent assay or radioactive immunoassay. Bone density was determined at the tibia and