A Novel, High Sensitivity Marker, hsPro-C2, Of Cartilage Formation, Was Developed And Tested In A Phase II Clinical Trial Of PTH

Luo, Yunyun; He, Y; Byrjalsen, I; Henriksen, K; Gudmann, N S; Mobasher, A; Hansen, G; Karsdala, M; Bay-Jensen, A-C

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Different times (day 0, 15, 30, 60, 90 and 120). Samples were frozen and collected from the jugular vein in vacutainer tubes with EDTA on six occasions.

Type 2 Cleavage (CIIC), Procollagen II C-Terminal Propeptide (PIICP), and Keratan Sulfate (KS) were analyzed. These biomarkers were canine-specific and showed promise for assessing OA.

**Methods:** Twelve dogs with clinical and radiographic hip dysplasia were included. Hip dysplasia severity was graded according to the American Kennel Club (AKC) and the Canadian Orthopaedic Research Society (CORS) criteria. The dogs were divided into two groups: control (C) and dysplasia (D).

**Results:** The dysplasia group had significantly lower levels of Type II collagen cleavage (CIIC) and Procollagen II C-Terminal Propeptide (PIICP) compared to the control group. There was a significant difference in the Keratan Sulfate (KS) levels between the two groups, with the dysplasia group showing higher levels.

**Conclusions:** These findings suggest that biomarkers can be used to assess the severity of hip dysplasia and may be useful in monitoring the progression of the disease. Further studies are needed to validate these findings in a larger population.
for proof of concept, we used an osteoporosis (OP) trial testing teriparatide (human parathyroid hormone (PTH) 1–34). Teriparatide has been shown to have potential chondro-protective and chondro-regenerative effects on articular cartilage in vitro and in vivo. However, it remains unclear whether the pro-anabolic effect of teriparatide translates to human OA.

**Methods:** A high sensitivity competitive electro-chemiluminescence immunoassay for detection of PIBNP (hsPro-C2 ECLIA) was developed and the technical performance evaluated. From a randomized, double-blind placebo-controlled study with an open-label active comparator/positive control (teriparatide) in postmenopausal women with OP (clinicaltrials.gov: NCT01321723), the biomarker sub-study included 64 Caucasian postmenopausal women (age 45–80 years) with OP duration of at least 5 years. Thirty-one women were treated with teriparatide, and 29 with placebo. Biomarkers of bone formation (PINP; procollagen type I N-terminal propeptide) and cartilage formation (hsPro-C2) were analyzed retrospectively at baseline, week 4, 12 and 24. Correlation between PINP and hsPro-C2 at baseline and change at week 4 relative to baseline were investigated by Pearson’s correlation.

**Results:** The technical performance of hsPro-C2 ECLIA was summarized in Table 1. The intra-assay CV was 5.4% and the inter-assay CV was 5.5%. The measurement range was 1–32 ng/ml. The spiking and dilution recovery tested in human serum were 100±20% within the measurement range of the assay. The mean percent change in serum hsPro-C2 level was higher in teriparatide treated group compared to the placebo group at week 4 (9%), 12 (3%) and 24 (10%), although it was not statistically significant (Fig.1A). There were no statistically significant correlation between hsPro-C2 and PINP before treatment initiation (Fig.2A), however an increase in hsPro-C2 was significantly associated with increase in PINP in the teriparatide treated group at week 4 ($R^2 = 0.1976$, $p < 0.05$, Fig.2B). This was not observed in the placebo group (data not shown).

**Conclusions:** In spite of the small sample size of this study there was a clear trend toward increased cartilage formation in the PTH treated group over time. Furthermore, hsPro-C2 changes correlated with the changes in PINP, which is believed to be a pharmacodynamics biomarker of teriparatide treatment, indicating that hsPro-C2 reflect a possible chondro-anabolic effect of PTH. It is concluded that hsPro-C2 may be a promising and novel marker of cartilage formation to be used in DMOAD development.

**Table 1**

<table>
<thead>
<tr>
<th>Summary of technical performance for two biomarkers assays</th>
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<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Linear range of standard (ng/ml)</td>
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<tr>
<td>LLOD (ng/ml)</td>
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<tr>
<td>Intra-assay % CV</td>
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<tr>
<td>Inter-assay % CV</td>
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<tr>
<td>Spiking Recovery, % range</td>
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<td>Dilution Recovery, % range</td>
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LLOD: lower limit of detection

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**Figure 1** Response as median percent change from baseline (± interquartile range) in hsPro-C2 (A) and PINP (B) in women receiving teriparatide (●) or placebo (○). Teriparatide was administered subcutaneously with 20 mg/day.

**Figure 2.** Correlation between the serum procollagen type IIB N-terminal propeptide (Pro-C2) change and serum procollagen type I N-terminal propeptide (PINP) change in patients receiving teriparatide (A, B) and placebo (A). Person’s correlation coefficient ($R^2$).

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**141 POST TRANSLATIONAL MODIFIED LUBRICIN PRESENT IN OSTEOARTHRITIS PATIENTS’ SYNOVIAL FLUID AND PLASMA.**

N.G. Karlsson, C. Jin, S. Huang. *Univ. of Gothenburg, Gothenburg, Sweden*

**Purpose:** Lubricin has been implicated to be a key molecule to sustain a healthy joint by providing lubrication and chondroprotection. Lubricin is extensively post translational modified. The modifications include proteolytic cleavage of the protein backbone, glycosylation of the mucin domain as well as covalent complexation with matrix proteins. All these modifications have been indicated to be altered in osteoarthritis. We wanted to investigate the extent of these alterations and if they could be correlated with the pathological condition of an osteoarthritic joint.

**Methods:** Synovial fluid and plasma were collected from osteoarthritis patients subjected to joint replacement surgery. Western blot of synovial fluid using antibodies for lubricin together with molecular weight correlation of the staining using lectins and antibodies against matrix proteins was performed. This allowed us identify sandwich ELISA pair that could be used for screening of post translational modified lubricin in patient plasma. A validated sandwich ELISA methods was used to screen patients’ synovial fluid and plasma.

**Results:** We could show that lubricin was glycomodified both in synovial fluid and plasma. Lectins targeting sialic acid (Sambucus nigra and Maackia Amurensis II lectins) as well as peanut agglutinin targeting Galβ1-3GalNAcα1- were selected to match the major type of glycans found on the mucin domain and inflammation related L-selectin was successfully used to detect minor glycan components on lubricin. We could also show that lubricin in complex with COMP was present in patients synovial fluid and circulating plasma of the patients.

**Conclusions:** Appropriate posttranslational modification of lubricin is important to fine tune its function in addition to a regulating of its expression to sustain the healthy joint. The sandwich ELISA data suggest that the post translational modifications on lubricin can be used to improve the specificity of lubricin as a biomarker for detecting early stage OA before chronic damage of the joint has occurred.

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**142 CARBOXYLATED AND UNCARBOXYLATED OSTEOCALCIN LEVELS ARE NOT LINKED WITH INSULIN RESISTANCE IN OSTEOARTHRITIS PATIENTS**

E. Abed, J. Martel-Pelletier, J.-P. Pelletier, F. Mineau, P. Delorme, D. Lajeunesse, CRCHUM, Montréal, QC, Canada

**Purpose:** Normal 0 21 false false false FR-CA X-NONE X-NONE An association between knee osteoarthritis (OA) and the metabolic syndrome (MetS) has been proposed and in particular between OA and type 2 diabetes (T2D). Altered glucose metabolism and insulin resistance (IR) are observed in T2D individuals. In animal studies, a link between glycaemia/insulin resistance (IR) and circulating levels of either carboxylated and uncarboxylated osteocalcin (OC) has been observed. Previous studies reported an increased production of osteocalcin by OA osteoblasts. Herein, we evaluated if OA patients present evidence of IR and if this could be linked with circulating OC levels.

**Methods:** Normal 0 21 false false false FR-CA X-NONE X-NONE /" style Definitions */ table.MsoNormalTable (mso-style-name: "Tableau Normal"; mso-tstyle-rowband-size:3; mso-tstyle-rowband-size:3; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""); mso-