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; Mobasheri, A; Hansen, G; Karsdala, M; Bay-Jensen, A-C

Published in:
Osteoarthritis and Cartilage

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Table 1

	Baseline		6-month	
Variable	**CS/GH**	**CE**	**CS/GH**	**CE**
Adrenaline (pg/mL)	96 (82–119)	98 (76–114)	78 (61–96)*	80 (62–98)*
Noradrenaline (pg/mL)	1561 (1003-1916)	1540 (952–2643)	1344 (859–1856)	1558 (942–2212)
LPS (EU/mL)	0.9 (0.5–1.2)	0.7 (0.6–1.3)	1.2 (0.7–2.8)*	1.4 (0.6–2.1)*
IL-1beta	12.1 (10.2–15.2)	12.5 (10.7–15.4)	15.2 (13.3–15.9)	14.6 (12.5–15.5)
VAS (0–100) score	75 (66–87)	77 (70–82)	34 (24–50)*	39 (11–60)*
WOMAC score	371 (342–395)	356 (322–394)	153 (87–220)*	168 (89–284)*

* **$p < 0.05$ vs baseline values (Mann-Whitney-Wilcoxon Test)**

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BIOMARKERS VARIABILITY IN A CANINE HIP OSTEOARTHRITIS MODEL

S. Cardona Ramírez †, R.F. Silva Molano ‡, G.J. Castaño ‡. † *Univ. of Missouri/ Univ. de Caldas, Columbia, MO, USA;* ‡ *Univ. de Caldas, Manizales, Colombia*

Purpose: Animal models are considered to play a key role in the understanding and the developing of new research strategies regarding osteoarthritis (OA). Although recent preclinical animal models have shown several advantages, spontaneous osteoarthritis is more reliable because it can resemble human disease. Canine hip dysplasia is a common disease that affects dogs and results in chronic OA. Diagnosis of the disease is based on radiographic changes, which are presented at a late stage. Usually, the severity of the disease is not correlated with clinical signs. One important research area is the analysis of biomarkers. Since animal models of hip OA are not commonly used for biomarkers research, sources of variability are unknown. This work focuses on the effect of different variables that could affect plasmatic levels of type II collagen biomarkers (CIIC, PIICP) and non-collagen biomarkers (PGE2, KS).

Methods: Twelve dogs with clinical and radiographic hip dysplasia were included. Hip dysplasia severity was graded according to Norberg angle score (mild, moderate or severe, Fédération Cynologique Internationale - FCI). Lameness was graded according to intermittence and full or partial weight bearing. Osteoarthritis was graded according to osteophyte presence, sclerosis and femoral head and neck remodeling. All biomarkers were canine-specific ELISA assays and included Collagen type 2 Cleavage (CIIC), Procollagen II C-Terminal Propeptide (PIICP), Keratan sulfate (KS) and Prostaglandin E2 (PGE2). Blood samples were collected from the jugular vein in vacutainer tubes with EDTA on six different times (day 0, 15, 30, 60, 90 and 120). Samples were frozen at -80°C until analysis. For assessing possible associations between plasma biomarkers levels and other variables, a generalized mixed model (GLMM) was used. Sex, Age, Weight, osteoarthritis score, hip dysplasia grade and lameness score were used as fixed effects, and time was used as random variable. When normal distribution was not achieved, transformation to normality was performed. Significant P-value was considered <0.05.

Results: We identified age, sex, and weight as a source of variability in the four biomarkers ($p < 0.005$). Interestingly, OA score also affected biomarkers levels. This finding shows that if other confounding variables remain fixed, these biomarkers could be useful to measure the severity of the disease in this model. Additionally, collagen markers showed association with lameness score (PIICP) and hip dysplasia score (CIIC) ($p < 0.005$). Finally, KS had the highest variability being affected by all the variables evaluated, which make this marker not useful for specific purposes in this model.

Conclusions: To the author's knowledge, there are no reported studies regarding variability of these biomarkers in canine hip osteoarthritis. Furthermore, identifying confounding variables could lead future investigators to design more accurate strategies intended for hip osteoarthritis biomarkers research in this pre-clinical model. As a consequence, similar age, sex, and weight groups should be used in future research. Since this study evaluates plasma biomarkers in chronic disease, it can resemble in a more accurate way human hip osteoarthritis pathobiology, compared with common experimental animal models.

Collagen II Cleavage (CIIC)

VARIABLE	F	p-value
AGE	7.438	0.009
SEX	6.099	0.016
WEIGHT	11.665	0.000
DYSPLASIA GRADE	7.395	0.009
LAMENESS SCORE	0.375	0.771
OA SCORE	6.618	0.000

Procollagen II C-propeptide (PIICP)

VARIABLE	F	p-value
AGE	17.526	0.000
SEX	37.807	0.000
WEIGHT	9.658	0.000
DYSPLASIA GRADE	0.003	0.995
LAMENESS SCORE	3.065	0.350
OA SCORE	27.207	0.000

Keratan Sulfate (KS)

VARIABLE	F	p-value
AGE	14.841	0.000
SEX	7.930	0.007
WEIGHT	10.577	0.000
DYSPLASIA GRADE	10.048	0.002
LAMENESS SCORE	4.728	0.005
OA SCORE	12.796	0.000

Prostaglandin E2 (PGE2)

VARIABLE	F	p-value
AGE	7.253	0.009
SEX	14.950	0.000
WEIGHT	4.254	0.019
DYSPLASIA GRADE	3.581	0.063
LAMENESS SCORE	2.235	0.094
OA SCORE	5.705	0.001

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A NOVEL, HIGH SENSITIVITY MARKER OF CARTILAGE FORMATION WAS DEVELOPED AND TESTED IN A PHASE II CLINICAL TRIAL OF PTH

Y.Y. Luo †, Y. He †, I. Byrjalsen †, K. Henriksen †, N.S. Gudmann †, A. Mobasheri ‡, G. Hansen †, M. Karsdala †, A.C. Bay-Jensen †. † *Nordic BioSci. A/S, Herlev, Denmark;* ‡ *D-BOARD EU Consortium for Biomarker Discovery, Univ. of Surrey, Surrey, United Kingdom*

Purpose: Presently, measurement of cartilage formation rely on magnetic resonance imaging (MRI), which is a sensitive measure, but which need long follow-up time. Thus, there is an unmet need, in disease-modifying osteoarthritis drug (DMOAD) development, for an objective and non-invasive marker of cartilage formation, which can provide early indication of drug efficacy. The objective was to enable assessment of type IIB collagen synthesis in serum from human subjects. Such tool may be applicable as a non-invasive biomarker of cartilage repair and growth in the development of cartilage anabolic drugs. No cartilage anabolic DMOAD have to date been approved thus

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 translate to human OA.

Methods: A high sensitivity competitive electro-chemiluminescence immunoassay for detection of PIIBNP (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 rang 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
Summary of technical performance for two biomarkers assays

Parameters	hsPro-C2 Competition ECLIA	PINP Sandwiches ECLIA
Linear range of standard (ng/mL)	1.0 - 38.5	5–1200
LLOD (ng/ml)	0.13	<5
Intra-assay % CV	5.4	2.2
Inter-assay % CV	5.5	2.8
Spiking Recovery, % range	98	NA
Dilution Recovery, % range	99	NA

LLOD: lower limit of detection

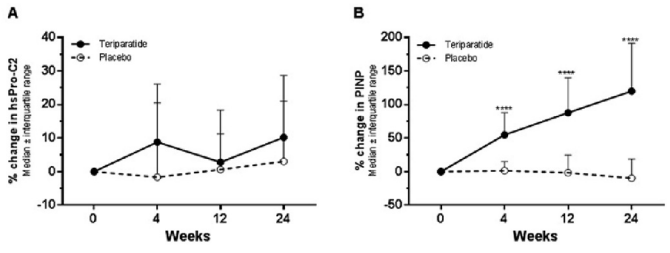

Figure 1Response as median percent change from baseline (± interquartile range) in hsPro-C2 (A) and PINP (B) in women receiving teriparatide (●) or placebo (O). Teriparatide was administered subcutaneously with 20 mg/day.

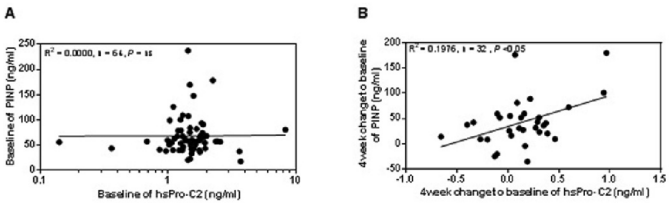

Figure 2. Correlation between the serum procollagen type IIB N-terminal propeptide (Pro-C2) change and serum procollagen type IN-terminal propeptide (PINP) change in patients receiving teriparatide (A, B) and placebo (A). Perso n's correlation coefficient (R^2).

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POST TRANSLATIONAL MODIFIED LUBRICIN PRESENT IN OSTEOARHTRITIS PATEINTS' 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 alteration and if they could be correlated with the pathological condition of an osteoarthritic joint.

Methods: Synovial fluid and plasma were collected form 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 protein was performed. This allowed us identify sandwich ELISA pair that could be used for screening of post translational modified lubricin in patient plasma. 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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CARBOXYLATED AND UNCARBOXYLATED OSTEOCALCIN LEVELS ARE NOT LINKED WITH INSULIN RESISTANCE IN OSTEOARTHRITIS PATIENTS

É. 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 TD2 individuals. In animal studies, a link between glycemia/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:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-