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Chapter 4

Repeated measurements of uCTX-II, sCOMP, sPIIANP, uCTX-I, and hsCRP as biomarkers of progression or efficacy of intervention

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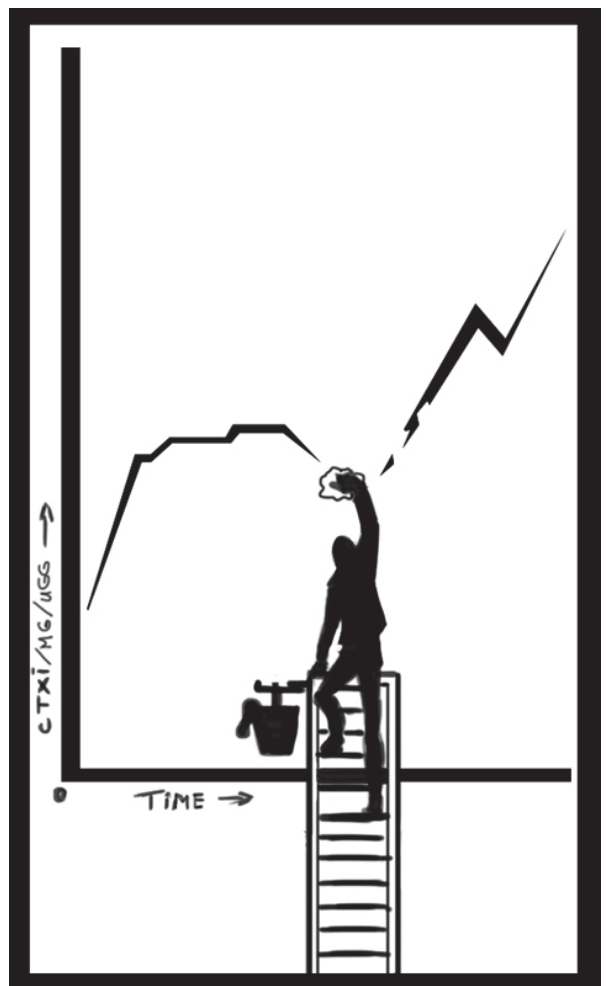[2] Department of Molecular Epidemiology, LUMC
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ABSTRACT

Objective

To investigate the association between repeated measurements of biomarkers: uCTX-II, sCOMP, sPIIANP, uCTX-I and hsCRP, and radiographic progression of osteoarthritis (OA).

Methods

One hundred and twenty-five patients with OA at multiple sites (mean age 59.6 years, 79% female) who participated in GARP (Genetics ARthrosis Progression) study were followed-up at 6-month, 1-year, 2-years, and 6-years. Time-integrated areas under the curve (AUCs) were selected to summarize longitudinal data. Radiographs of these patients were scored in two pairs: baseline and 2-years, baseline and 6-years, using the OARSI atlas for joint space narrowing (JSN) of knee, hip and hand joints. We calculated the risk ratios (RRs with (95% CI)) of OA progression (defined as JSN score changes above smallest detectable change) at 2- and 6-years for patients in the second and third AUC tertile relative to the first AUC tertile of biomarkers. Adjustments were made for age and sex.

Results

Patients in the highest AUC tertile of uCTX-II at 6, 12 and 24 months had a RRs of 2.9 (1.6 to 4.1), 1.8 (1.1 to 2.5) and 1.9 (1.1 to 2.7) to have OA progression at 2- years, respectively. Patients in the highest AUC tertile of uCTX-II at 6, 12 and 24 months had a RRs of 1.6 (1.1 to 2.0), 1.5 (0.9 to 1.9) and 1.8 (1.2 to 2.2) to have OA progression at 6-years, respectively. Other biomarkers were not associated with OA progression.

Conclusion

AUCs of uCTX-II are associated with progression of OA. The predictive power of uCTX-II levels at 0-6 months for OA progression at 2 years was highly promising and warrants further studies to investigate the value of this marker, that might also serve to evaluate the efficacy of intervention.

4.1. INTRODUCTION

Osteoarthritis (OA) is a slowly progressive disease. Due to this nature, an objective indicator (biomarker) of the OA disease process that could predict and measure the therapeutic response of drugs in OA is needed.[1,2] As proposed by the Osteoarthritis Biomarkers Network, a biomarker could be categorized into Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic (BIPED).[3]

Compared to radiograph, there are several possible advantages in using biomarkers in the studies on OA. Firstly, biomarkers could be more sensitive to change in the disease process. For example, it is not necessary to wait until cumulative effect of cartilage damage is seen on radiographs to get an information about the actual OA state. Secondly, biomarkers may provide more information about tissues involved in OA.[4] From imaging studies, it is now shown that OA is not merely a disorder characterized by cartilage loss [5] but also involve other tissues such as bone and synovium.[6,7]

Several biomarkers have been developed and studied for OA [8-10] and several remarks can be made on those studies. Firstly, published studies used mostly single-time measurement of the biomarker, while multiple measurements of biomarkers might be more informative. Secondly, most studies used knee and hip OA phenotypes separately, unaware of radiographic OA occurring in other sites such as the hand. Lastly, the studies were often performed in small study populations.

Therefore, we investigated the association between repeated measurements of uCTX-II, sCOMP, sPIIANP, uCTX-I, and hsCRP and the progression of OA at multiple sites over 2 and 6 years. These biomarkers have been selected to represent three components: cartilage, bone and synovium.[4] uCTX-II is a marker that was developed for measuring cartilage degradation, sCOMP for cartilage turnover, sPIIANP for collagen synthesis, uCTX-I for bone turnover and hsCRP for inflammation.

4.2. PATIENTS AND METHODS

4.2.1. Study design and patient population

Patients were participants of the Genetics, ARthrosis and Progression (GARP) study. GARP was a prospective cohort study that aimed at identifying determinants of OA susceptibility and progression. The recruitment criteria have been described elsewhere.[11] Briefly, 192 Caucasian sib-pairs (aged 40 to 70 years) were included with symptomatic OA at multiple joint sites in the hands or OA in two or more of the following joint sites: hand, spine, knee, or hip. Patients were recruited from the rheumatologic, orthopedic and general practice clinics around Leiden, The Netherlands. Patients with secondary OA, familial syndromes with a clear Mendelian inheritance, and a short life expectancy (<1 yr) were excluded.

Sib-pairs with at least one subject with knee or hip OA at baseline who were not in a radiological end stage (Kellgren and Lawrence score of 4, appendix C.1) were invited to attend 6-month, 1-year, 2-years, and 6-years follow up visit.[11] At each follow-up visits, 125 patients were seen. Demographic data and data on joint replacement surgery of these 125 patients were obtained during every visit. The GARP study was approved by the Medical Ethics Committee of the Leiden University Medical Center.

4.2.2. Radiographs

Standardized protocols were used to obtain the radiographs of the knees (posterior-anterior (PA); weight-bearing, non-fluoroscopic fixed-flexion protocol), hips (PA; weight-bearing) and hands (dorsal-volar) at baseline, at 2-years, and at 6-years. Baseline and radiographs at 2-years were analogue films and were digitized using a film digitizer at a resolution corresponding to a pixel size of 100 mu. Radiographs at 6-years follow-up were obtained digitally.

Two experienced readers scored radiographs in two pairs: baseline and 2-years, baseline and 6-years using the Osteoarthritis Research Society International (OARSI) atlas (appendix C.2).[12] The readers were blinded for patient characteristics. Joint space narrowing (JSN) was graded 0 to 3 in the tibiofemoral, hip and hand joints

(distal interphalangeal (DIP), proximal interphalangeal (PIP), first interphalangeal (IP-1), first carpometacarpal (CMC-1), metacarpophalangeal (MCP) and scaphotrapezotrapezoidal (STT) joints), leading to a sum score of JSN, ranging from 0 to 114. Intraclass correlation coefficients (ICC) for intrareader reproducibility based on random samples of 20 radiographs at 2- and 6-years follow-up were very good (at least 0.88 in the tibiofemoral knee joints, 1.00 in the hips and 0.92 in the hands). New knee or hip prosthesis on radiograph was scored as having increase in JSN score of 1.

4.2.3. Definition of progression

Progression was defined as difference between the sum of JSN's at follow-up and at baseline above the smallest detectable change (SDC). SDC reflects change above measurement error.[13] After calculating the SDC, increase in sum JSN score of ≥1 and ≥2 at 2- and 6-years respectively, was defined as progression.

4.2.4. Biochemical analysis

Serum and second void morning urine samples were collected from the study patients at baseline, at 6-months, at 1-year, 2-years and 6-years follow-up. All samples were stored within four hours at -80 °C until measurements of biomarkers were undertaken. Baseline biomarkers were measured by Synarc (Lyon, France) and the measurements at other time points were performed by TNO EELS (Leiden, The Netherlands).

CTX-II in the urine (uCTX-II) was measured using an enzyme linked immunosorbent assay (ELISA) based on a monoclonal antibody raised against the EKGPDP linear 6-amino acid epitope of the CII C-telopeptide (CartiLaps, Nordic Bioscience, Herlev, Denmark). Intra-assay and inter-assay variation (CV, %) was less than 9% and 12%, respectively. The ICC for uCTX-II measurements in two different laboratories was excellent (0.97) based on the re-measurement of 18 baseline samples. The concentration of uCTX-II (in ng/liter) was standardized to the total urine creatinine (mmol/liter), and the units for the corrected uCTX-II concentration are ng/mmol.

Serum COMP (sCOMP) was measured by a two-site immunoassay (COMP™ ELISA kit, AnaMar Medical, Lund, Sweden). Intra- and inter-assay CVs were below 7% and 8%, respectively. The ICC for COMP measurements in two different laboratories was excellent (0.97).

sPIIANP was measured using a polyclonal antibody specific for the type IIA of the N-propeptide of type II collagen.[4] Due to a very low ICC measurements in two laboratories, we only performed analysis on baseline and not on repeated data of sPIIANP.

uCTX-I was measured in the urine by the Crosslaps ELISA (Nordic Biosciences, Herlev, Denmark) that used a polyclonal antiserum raised against the β isomerized EKAH β DGGR sequence of the C-telopeptide of α1 chains of human type I collagen. Intra- and inter-assay CV were below 3% and 10 %, respectively. The ICC for uCTX-I measurements in two different laboratories was excellent (0.99).

High sensitivity CRP (hsCRP) was measured in the serum using ultrasensitive immunonephelometry method (N Latex CRP mono, Behringwerke, AG, Marburg, Germany) on a BNA Behring nephelometer. The intra- and inter-assay CVs were lower than 5%. The ICC for hsCRP measurements in two laboratories was 0.99.

4.2.5. Statistical analysis

To assess normality of their distributions and to visualize the course of biomarkers level within the group during the follow-up, we drew boxplots using GraphPad Prism (Graphpad Software Inc., La Jolla, USA).

Generalized Estimating Equations (GEE) with robust variance estimators to account for family effect was used to calculate the β- regression coefficients for the association between the baseline biomarkers levels (independent variable) and the increase in JSN scores in 2- and 6- years (outcome).

To incorporate measurements at multiple time-points, we calculated the area under the curve (AUC) baseline to 6-months follow-up (AUC 0-6), baseline to 1-year (AUC 0-12), baseline to 2-year (AUC 0-24), and baseline to 6-year (AUC 0-72) using the formula which has been used previously in rheumatology research.[14] For example, to calculate AUC uCTX-II 0-24:

AUC uCTX-II (ng/mmol creatinine)*month) =

((uCTX-II at baseline + uCTX-II at 6 months)/2)*6 +((uCTX-II at 6 months + uCTX-II at 1 year)/2)*6 + ((uCTX-II at 1 year + uCTX-II at 2 years)/2)*12.

Every AUC was calculated after excluding patients who received a joint prosthesis during that AUC follow-up. For example, a patient who received knee prosthesis after 11 months follow-up was excluded for the calculation of AUC 0-12. Consequently, this patient was also excluded for analyses with AUC 0-24 and 0-72. This was done because the replaced joint did not contribute to the amount of measured biomarkers.

To investigate the association between AUCs at different time points and OA progression, two types of statistical analyses were used. Firstly, mean difference (95% CI) in AUCs between patients with and without progression was estimated using GEE. Secondly, logistic regression analysis in GEE was used. In this analysis, patients were divided into their biomarkers AUC tertiles. Then, we calculated the odds ratio's (ORs with 95% CI) of radiographic OA progression for participants in the second and third AUC tertiles relative to the first tertile. All ORs were transformed to risk ratio (RRs with 95% CI) using the approximation formula of Zhang because ORs of common outcomes in a fixed cohort are not a good approximation of RRs.[15]

All analyses were performed on PASW Statistics 17 (SPSS Inc., Chicago, USA) and adjustment was made for age, sex, and BMI.

4.3. RESULTS

4.3.1. Study population

The characteristics of the 125 patients in the present study are shown in table 4.1. The mean age was 59.6 years, 79% were female and the mean BMI was 26.7 kg/m^2.

During 2- and 6-years follow-up, 45 and 67 patients respectively showed radiographic OA progression. No patients received joint prosthesis during 6-months follow-up. Between 6 and 12 months, between 12 months and 24 months, and between 24 months and 72 months, one, five and 16 patients, received joint prosthesis, respectively.

Table 4.1 Characteristics of the study sample (n=125).

Characteristics	
Age, mean (SD) years	59.6 (6.9)
Female sex, %	99 (79)
Body Mass Index, mean (SD), kg/m^2	26.7 (3.9)
Sites with osteoarthritis	
Knee	57 (45.6)
Hip	46 (36.8)
Hand	89 (71.2)
Baseline level, mean (SD); median (IQR)	
uCTX-II, ng/ mmol creatinine	266.2 (152.8); 229.7 (153.2 to 330.3)
sCOMP, U/L	11.5 (3.1); 11.3 (9.5 to 13.2)
sPIIANP, ng/ ml	219.5 (106.7); 182.7 (137.4 to 275.1)
uCTXI, μg/ mmol creatinine	178.1 (105.1); 154.8 (101.7 to 233.4)
hsCRP, mg/ L	3.3 (6.1); 1.7 (0.9 to 3.7)

4.3.2. The course of biomarkers level

The mean (SD) and median (IQR) of baseline levels of all biomarkers are presented in table 4.1. The course of biomarker levels over time is presented in figure 4.1.

4.3.3. Association between biomarkers levels at baseline and increase in JSN scores

At baseline, uCTX-II, sCOMP, and sPIIANP showed some correlation with age (respective Pearson's correlations 0.2 (p-value=0.03), 0.2 (p-value=0.05), and 0.2 (p-value=0.01). hsCRP and uCTX-I were not correlated with age. None of the baseline level of biomarkers differed across sexes. Although not significant, all baseline levels showed positive association with OA progression over 2 and/or 6 years, except for hsCRP over 6 years (table 4.2). None of the baseline biomarkers levels were associated with increasing JSN during 2- and 6-years follow-up.
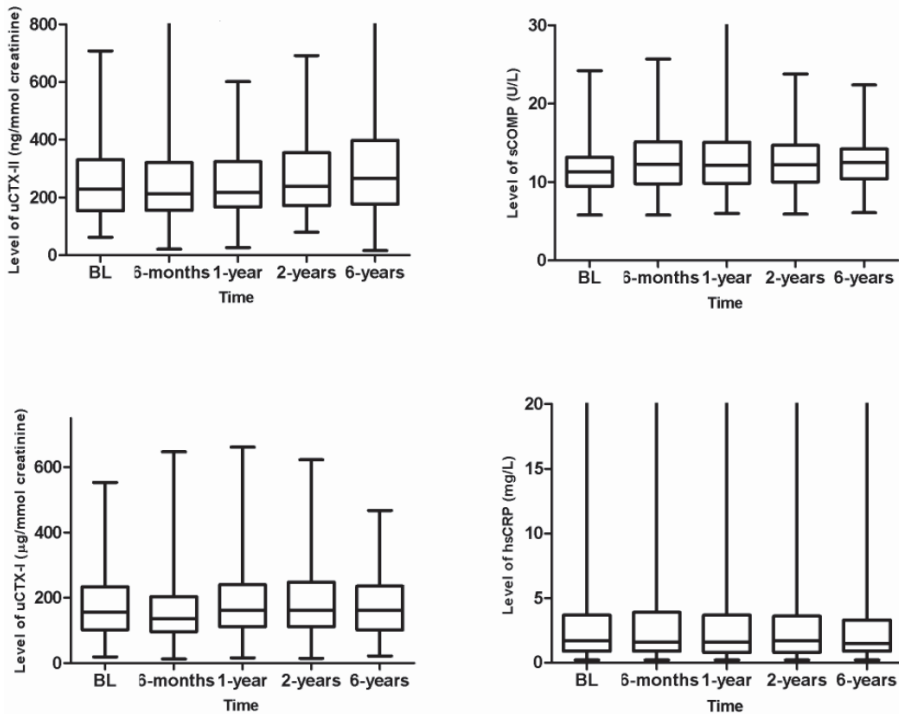
Figure 4.1 The course of the biomarkers level within the patient group during the follow-up presented using box-plots. The top and bottom of each box indicates the upper and the lower quartiles, and the thick black lines across the boxes represents the median of each group.

Table 4.2 Mean difference in baseline levels and Area Under the Curve's (AUC's) between patients with OA progression and patients without OA progression.

	2-years progression (45 patients with vs. 80 without progression)	6-years progression (67 patients with vs. 58 without progression)
uCTX-II		
baseline (ng/ mmol creat)	33.1 (-18.9 to 85.2)	32.9 (-24.6 to 90.4)
AUC 6 month	183.3 (-90.3 to 457.0)	118.2 (-184.0 to 420.3)
AUC 1 year	335.4 (-161.8 to 832.6)	145.3 (-409.5 to 700.0)
AUC 2 years	864.1 (32.6 to 1760.8)‡	272.9 (-691.6 to 1237.4)
AUC 6 years	n.a.	-867.6 (-6013.0 to 4277.9)

‡ statistically significant at p< 0.05

4.3.4. Association between AUC's of the biomarkers and 2-years OA progression.
AUCs (reflecting total change in biomarker level over time) were calculated over the follow-up time intervals in patients with and without progression over 2 years. Only AUC of uCTX-II was shown to be significantly higher (mean difference of 864.1 (95% CI 32.6 to 1760.8) in progressors of JSN over 2 years compared to non-progressors (table 4.2). The mean difference of other biomarkers that were not significant.

We explored the AUCs of uCTX-II (table 4.3). Patients with the highest AUC uCTX-II at consecutive time-intervals had a significantly increased risk to have 2-years progression as compared to the lowest AUC tertile uCTX-II (table 4.3). Especially patients with the highest AUC levels of uCTX-II in the first 6 months after baseline had a significant risk increase (RR 2.9 (1.6 to 4.1)) to have progression at 2-years.

Table 4.3 Associations between tertiles of Area Under the Curve's (AUC's) of biomarkers with 2- and 6-years progression of OA.

Biomarkers in tertiles	Association with 2-years OA progression			Association with 6-years OA progression		
	Number of patients		Relative Risk's (95% CI) [1]	Number of patients		Relative Risk's (95% CI) [1]
	+	-		+	-	
AUC uCTX-II ((ng/mmol creatinine) month)						
0-6 (n=125)						
1st	8	33	1 (reference)	16	25	1 (reference)
2nd	16	25	2.4 (1.2 to 3.6)‡	24	19	1.4 (0.8 to 1.9)
3rd	21	22	2.9 (1.6 to 4.1)‡	27	14	1.6 (1.1 to 2.0)‡
0-12 (n=124)						
1st	10	31	1 (reference)	18	23	1 (reference)
2nd	17	25	1.7 (0.9 to 2.5)	22	20	1.2 (0.7 to 1.7)
3rd	18	23	1.8 (1.1 to 2.5)‡	27	14	1.5 (0.9 to 1.9)
0-24 (n=117)						
1st	10	29	1 (reference)	15	24	1 (reference)
2nd	11	29	1.2 (0.5 to 2.1)	19	21	1.2 (0.7 to 1.8)
3rd	18	20	1.9 (1.2 to 2.7)‡	26	12	1.8 (1.2 to 2.2)‡
0-72 (n=100)						
1st			n.a.	14	20	1 (reference)
2nd				15	22	1.1 (0.6 to 1.6)
3rd				15	14	1.8 (0.8 to 1.8)

‡ statistically significant at p< 0.05. +: with progression, -: without progression

4.3.5. Association between AUC's of biomarkers and 6-years OA progression.

AUC of uCTX-II was not associated with 6-years progression (table 4.2). AUC's of other biomarkers were also not associated with 6-years progression.

Examining uCTX-II further, we found that patients with the highest AUC uCTX-II at consecutive time-intervals (up to AUC uCTX-II over 2-years) had a consistent increased risk to have progression after 6-years when compared with patient with the lowest AUC tertiles (table 4.3). For example patients in the highest uCTX-II tertiles of AUC 0-6 had an RRs (95% CI) of 1.6 (1.1 to 2.0) to have 6- years progression relative to patients in the lowest AUC tertile.

4.3.6. Association between AUC over 6 years (0-72) with 6-years OA progression

The AUCs over 6 years (0-72) of uCTX-II were not associated with 6-years OA progression (table 4.3).

4.4. DISCUSSION

The present study is the largest study investigating repeated measurements of biomarkers that might be involved in OA progression. While baseline levels of biomarkers are not informative for OA progression, multiple measurements of uCTX-II (summarized as AUCs at various time points) are shown to be associated with 2- and 6-years OA progression.

The published studies on multiple measurements of biomarkers mostly used knee OA as phenotype. Direct comparison is therefore difficult since we also take into account other joints (hands and hips) that might have OA but do not come to attention in the other studies. Differences between our results and results from other studies could be explained by the difference in the presence of OA in the other joints; other joints could contribute to the measured biomarker. In our study the presence of OA in the other joints is documented.

Our results showed the association between summary of multiple measurements of uCTX-II with OA progression and this is in line with several other studies. In a study of 62 knee OA patients (79% woman), it was shown that while baseline uCTX-II levels were not associated, an increase in uCTX-II over 3 months was associated with 1-year cartilage loss in the knee joints measured on MRI.[8] In another study in 84 patients with OA, Sharif, et.al. showed that patients with biomarkers level above the median of the 5-years mean of uCTX-II levels had a RR of 3.4 (95% CI 1.2 to 9.4) to have knee OA progression.[16] In the same study, patients in the highest quartile of the 5-years mean of sPIIANP levels had RR of 3.2 relative to patients in the lowest quartile, to have knee OA progression. Regarding sCOMP, our results differ with the results from a study in 115 knee OA patients.[17] In that study, the mean AUC sCOMP (summary of measurements at baseline, 6, 12, 18, 24, 30, 36, 42, 48 and 60 months) was higher in patients with progression (n=37, of which 22 had total knee replacement) than without knee OA progression (n=78) during 5-year follow-up. Concerning uCTX-I and hsCRP, data are only available from studies using single measurement. Our results support the notion that uCTX-I is not associated with OA progression.[8] Our study showing an indication of the association between CRP and 2-years OA progression is in line with several studies that showed the association between baseline hsCRP and incidence [18] and progression of OA.[19]

The consequence of our finding is that the AUC of uCTX-II could be tested in the clinical setting as a prognostic marker of OA progression since for example AUC uCTX-II of 6 months was shown to be associated with radiographic OA progression in mid- (2-years) and long- term (6 years). Another consequence is that uCTX-II could be used as a surrogate, or as an addition to radiograph to investigate the efficacy of intervention biomarkers. Potentially, it would lead to more sensitive detection of the effect of disease modifying anti osteoarthritic drugs, since the possible range of uCTX-II is broad. uCTX-II has indeed been used in several clinical studies. Garnero et. al. showed that uCTX-II decreased in knee OA patients who received risedronate and the level of decrease was related to the dose of risedronate.[20] Finally, our study adds to the knowledge on cartilage pathophysiology in OA by suggesting that OA is predominantly characterized by cartilage breakdown (as measured as uCTX-II)

and less associated with cartilage turnover (as measured as sPIIANP) or cartilage synthesis (as measured by sPIIANP). However the data are on the association with cartilage loss as seen on the radiograph, thus it is possible that JSN on radiograph do not reflect comprehensive cartilage defects in OA. It is also possible that other biomarkers such as uCTX-I and hsCRP are associated with other structure in the joint such as bone marrow lesion and synovium, structures that are not investigated in the present study.

Our study has several strengths. Firstly, we used a simple method to summarize the multiple biomarkers measurements instead of using complicated statistical method. Secondly, our study used a patient population. Practically, expensive prognostic tools in OA should concentrate on use in patient's rather than in the general population.[21] Thirdly, we assessed the presence of OA at multiple sites. Arguably, OA often presents at multiples sites, where only the site with the most severe pain attracts attention. Biomarkers measured in body fluid originate from every joint and not only from knee or hip alone. However, using OA at multiple sites as a phenotype has drawbacks too, such as the summarization of the JSN scores and how to deal with joint replacement during the follow-up.[17] In our study, having a joint prosthesis during follow-up was scored as increase in JSN score of 1. In a sensitivity analysis, where every patient who underwent a joint replacement during follow-up was defined as progression, no differences in effect sizes were seen (data are not shown).

In summary, AUCs uCTX-II were associated with the 2- and 6-years progression of OA. It is highly promising to use this biomarker as biomarker for prediction and to measure the efficacy of intervention.

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4