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Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage oligomeric protein and matrix metalloprotease degraded type II collagen and their role in prevalence, incidence and progression of osteoarthritis.

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\textsuperscript{*}denotes equal contribution

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Abstract

Objective: to evaluate the role of three cartilage-derived biomarkers on osteoarthritis (OA): urinary C-terminal telopeptide (uCTX-II), serum cartilage oligomeric protein (sCOMP), and serum MMP degraded type II collagen (sC2M).

Subjects and methods: Samples from 3582 individuals from the Rotterdam Study, the Genetics osteoArthritis and Progression (GARP), the Chingford Study and the TwinsUK cohort were assayed using enzyme linked immune sorbent assays. Log10 of concentration levels were correlated with risk of hip, hand and knee OA, hip and knee OA severity and incidence, and progression of knee OA, adjusting for age, gender and body mass index. Results were meta-analyzed to assess overall significance.

Results: After adjusting for covariates, sCOMP was associated with knee OA and hip and knee OA incidence. Furthermore, sC2M was associated with knee OA incidence and progression. After adjustment for multiple tests (Bonferroni p<0.002) only the association between sCOMP and knee OA remained significant (OR=3.26 (95%CI 1.63-10.1) p=0.0008 for each SD increase in biomarker levels). Levels of uCTX-II were significantly associated with risk of hand, hip and knee OA, progression and incidence of knee OA. A receiver operating characteristics analysis showed a consistent improvement in prediction of knee OA progression from an average area under the curve is 0.646 for age, sex and BMI alone to an AUC=0.668 including uCTX-II for prediction.

Conclusions: uCTX-II is the most informative biochemical marker for prediction of OA. Both sCOMP and C2M showed some association with OA, thus indicating that they are descriptive of disease activity. {244 words}
Introduction

Osteoarthritis (OA) is the most common joint disorder, and its prevalence is expected to climb as the age of the population increases [http://www.who.int/chp/topics/rheumatic/en/]. A hindrance to developing disease modifying drugs has been the inability to identify individuals who will rapidly progress towards severe disease and there is a need for biomarkers that enable early diagnosis of OA. The use of cartilage-derived biomarkers to examine direct processes of cartilage degradation and synthesis are increasingly being used to examine clinically relevant aspects of OA. The degradation of cartilage can in some part be attributed to an elevated proteolytic activity, which results in the release of protein specific tissue fragments, neo-epitopes, that have been investigated as biomarkers of joint disease (1). A validated example of neo-epitope biomarkers are those released following cleavage of type II collagen by MMPs, which reflect cartilage degradation. Among these biomarkers are serum MMP degraded type II collagen (C2M) (2) and urinary C-terminal telopeptide (uCTX-II) (3). Circulating levels of serum cartilage oligomeric protein (COMP), a marker of cartilage metabolism, have also been reported to be associated with disease progression (4-6). Hunter and co-workers found that baseline serum COMP was an independent predictor of cartilage loss by MRI in 137 patients followed up for 30 months (4). A series of type II collagen and aggrecan-derived biomarkers were evaluated in this study; however, only COMP showed a significant association to cartilage with an adjusted OR of 6.35 (range 1.4–29.7) for each SD increase in biomarker levels. C2M is also thought to reflect cartilage degradation (7) and it was recently reported that serum levels were highly elevated in OA, Reumatoid Arthritis (RA) as well as Ankylosis Spondylitis (AS) and that the diagnostic utility of that biomarker was high (AUC 0.87, p<0.0001) (8). However more data is needed to fully understand the information which C2M as a biomarker provide.

Of the three markers mentioned above, uCTX-II is the one best validated so far. uCTX-II was originally described by Christgau et al. (9) and has since been evaluated in more than 50 clinical studies. A large number of studies have associated this index with long-term structural progression of joint damage in both RA and OA. The first large-scale, longitudinal study of this marker was reported in 2004 based on a follow-up of 1235 subjects originating from the Rotterdam (10) prospective study of men and women (>55 years of age). Those individuals who had baseline CTX-II levels in the highest quartile, had a 6-fold (95% CI range; 1.2–31) increased risk for radiographic progression (JSN<2 mm) of knee OA. A similar odds ratio (OR) for hip OA
progression was 8.4 (95% CI range; 1.0–73) for those having JSN of less 1.5mm. The data support the use of a single biochemical marker for identification of patients at high risk for rapid progression of joint destruction.

One problem that has affected biomarker studies has been the use of small sample sizes and the use of case-control designs with cases being recruited from secondary care settings. Moreover different biomarker studies have used different outcome measures and/or focused on different joints. These factors make it difficult to evaluate the value of cartilage based biomarkers for clinical use.

In this study, as part of the TREAT-OA consortium, we have combined data from three large population-based cohorts and a familial study of OA with hand, knee and hip X-rays, three of them with longitudinal data. The total sample size of over 3,582 individuals measured for three separate cartilage based biomarkers enables to assess the role of biomarkers on prevalence, incidence and progression of OA and to assess the prognostic value of these biomarkers.

**Subjects and methods**

**Serum COMP, C2M and urinary CTX-II measurements**

Serum COMP (COMP®, AnaMar, Göteborg, Sweden) and urinary CTX-II (CartiLaps®, IDS, Paris, France) were measured according to manufacture in the Rotterdam, GARP, Twins-UK and Chingford studies. Serum C2M was measured in the studies using a novel assay developed by Nordic Bioscience as described by Bay-Jensen et al. (11). All three assays were measured using enzyme-linked immunosorbent assays based on a monoclonal antibody. Intra- and inter-assay CV (%) were below 10%.

**The cohorts**

*Chingford Study*: This study is a prospective population-based longitudinal cohort, which includes women derived from the age/sex register of a large general practice in North London. The study design and rationale have been described elsewhere in detail (12). The Guy’s St. Thomas’ Trust and the Waltham Forest Trust ethics committees approved the study protocol. After study procedures were explained to participants, written informed consent was given by
each participant. OA was classified radiologically using standard X-rays of the pelvis, thoracolumbar spine, hands and weight-bearing knees. (13)

TwinsUK: The study participants were white monozygotic and dizygotic twin pairs from the TwinsUK adult twin registry, a group used to study the heritability and genetics of age-related diseases(14). These unselected twins were recruited from the general population through national media campaigns in the United Kingdom. Ethics approval was obtained from the Guy’s and St. Thomas’ Hospital Ethics Committee. Written informed consent was obtained from every participant. The radiographs were taken between 1995 and 2000.(14) Anteroposterior extended-view weight-bearing radiographs of both knees were obtained at baseline and follow-up using the same protocol and a tube-to-film distance of 100 cm.(15) Pelvic radiographs with the subject in the supine anteroposterior position, with a standard tube-to-film distance of 100 cm and the feet positioned in 15 degrees of internal rotation were obtained.(14) Radiographs of both hands were taken with a standard posteroanterior view.

Genetics OsteoArthritis and Progression (GARP) Study: The GARP study from Leiden, the Netherlands, consists of 192 sibling pairs concordant for clinical and radiographically (K/L score) confirmed OA at two or more joint sites among hand, spine (cervical or lumbar), knee or hip.(16) Written informed consent was obtained from each subject as approved by the ethical committees of the Leiden University Medical Center. At conventional radiographs of the hands (dorso-volar), knees (posterior-anterior (PA) in weight bearing semiflexed and lateral), hips (PA), lumbar (PA and lateral), and cervical spine (anterior-posterior, lateral, and transbuccal) were obtained from all participants. They were taken in a standard manner with a fixed film focus distance and a fixed joint position. Radiographs of the knees were taken using the fixed flexion radiography.(16) Baseline were analogue films and were digitized using a film digitizer at a resolution corresponding to a pixel size of 100 μm. Radiographs at 6-years follow-up were obtained digitally. Radiographs were scored in pairs (baseline-six year) blinded for patient characteristics by consensus opinion of two experienced readers using the OARSI atlas.[11] Osteophytes and joint space narrowing (JSN) were each graded 0-3 in the tibiofemoral knee and hip joints. Intraclass correlation coefficients (ICC) for intrarreader reproducibility based on random samples of 20 radiographs were very good (at least 0.88 in the tibiofemoral knee joints.
and 1.00 in the hips). New knee or hip prosthesis on radiograph was scored as having increase in
JSN score of 1. Progression was defined as described in detail previously ([12]).

**Rotterdam Study.** The Rotterdam Study is a population-based prospective cohort study ongoing
since 1990 to study determinants of chronic disabling diseases ([18]). The Rotterdam Study consists
of three sub-populations. The Rotterdam Study I (RSI) is the first cohort of 7,983 persons living
in the Ommoord district of Rotterdam in the Netherlands. All subjects were aged 55 years and
older and recruitment started in 1990. The Rotterdam Study II (RSII) started in 1999 when 3,011
participants moved into the study since they became 55 years of age or moved into the study
district. A further extension, the Rotterdam Study III (RSIII), was initiated in 2006 and to date
3,829 participants, aged 45-54 years, are included in this study. The medical ethics committee of
Erasmus University Medical School approved the study and written informed consent was
obtained from each participant. Weight bearing anteroposterior radiographs of the knee and hip
were obtained at 70 kV, a focus of 1.8, and a focus to film distance of 120 cm, applying a Fuji
High Resolution G 35x43 cm film ([10]). Standard anteroposterior radiographs of both hands were
taken ([20]). For the current study, data was available for 1311 individuals of RS-II, with a mean
follow-up time of 4.1 (SD 0.58) years.

**Standardisation of the radiographic OA traits**
Standardisation of radiographic assessment for all four cohorts has been previously described
([21]).

Eight different OA outcomes were considered for the present study:

- Knee OA prevalence: Knee ($K/L \leq 1$) vs. Knee ($K/L \geq 2$)
- Knee OA severity: Knee ($K/L=2$) vs. Knee ($K/L \geq 3$)
- Hip OA prevalence: Hip ($K/L \leq 1$) vs. Hip ($K/L \geq 2$)
- Hip OA severity: Hip ($K/L=2$) vs. Hip ($K/L \geq 3$)
- Hand OA: presence of either both finger or thumb OA in both hands
- Incident knee OA: knee $K/L < 2$ at baseline and $K/L \geq 2$ at follow-up, non-incident $K/L < 2$ at both baseline and follow-up
- Incident hip OA = hip $K/L < 2$ at baseline and $K/L \geq 2$ at follow-up vs. non incident = $K/L < 2$ at both baseline and follow-up
• Knee OA progression any K/L at baseline (including K/L <2), progressor if K/L at follow-up was higher than at baseline, non-progressor otherwise.

Statistical methods

All concentration values were log10-transformed. Logistic regression analyses were carried out on all traits without adjustment (univariate) for each of the traits. In addition, the regression adjustments were made for age, sex and body mass index (BMI) (multivariate analysis). Summary statistics (the regression coefficient beta and its standard error) for each cohort for each outcome for all three biomarker measurements were then meta-analyzed using fixed effect models. For selected traits, that showed a significant association overall random effect, meta-analysis was also performed. The “meta” package for R was used (http://cran.r-project.org/web/packages/meta/index.html)

Adjustment for multiple comparisons: a Bonferroni cut-off of p<0.002 (0.05/ (3 biomarkers x 8 traits)) was used.

Receiver operating characteristic (ROC) analysis: In order to assess the discriminating power of the biochemical markers studied we generated ROC curves using knee incidence and knee progression as outcomes in each of the four cohorts.

Results

Study participant characteristics

The descriptive characteristics of study participants are presented in Table 1 along with the number of cases for the outcomes investigated. The mean and standard deviation (SD) for the three biochemical markers are also shown for each cohort.

Association between the biomarkers and OA traits

In the univariate analysis of the 8 traits very strong associations were found between sCOMP and uCTX-II with most of the traits studied with the exception of hip severity (Table 2). After adjustment for covariates sCOMP remained significantly (p<0.002) associated only with risk of knee OA whereas uCTX-II remained associated with risk of hand OA, of hip OA, of knee OA,
with progression and severity of knee OA (Table 2). None of the traits was associated at the Bonferroni level with C2M levels before or after adjustment for covariates (Table 2).

We investigated inter-study heterogeneity and found that although significant heterogeneity exists between studies for some of these traits they remain statistically significant even when random effects models are used (Figures 1 and 2), with the exception of risk of hip OA (Figure 2B).

As one of the unmet medical needs concerning OA is being able to identify which patients will progress to severe OA we decided to focus on progression of knee OA to perform a receiver operating characteristics (ROC) analysis. Three models were fitted with knee OA progression as the outcome, model 1 consisted only of anthropometric age, gender and BMI, model 2 included only uCTX-II and model 3 included age, gender, BMI, and uCTX-II (Table 3). In none of the cohorts studied are the anthropometric traits alone or uCTX-II alone sufficient to reach a clinical usefulness (AUC <0.7). In all the four cohorts the addition of uCTX-II improves prediction of radiographic knee OA progression.

One of the problems that has hindered the evaluation of markers of OA has been the lack of a threshold level set for the marker to classify patients as having or not having a positive or negative result. A survival regression analysis taking the uCTX-II levels as the time-dependent variable on OA progression was performed within the Rotterdam Study (adjusted for age, gender, BMI). Based on these data it appeared that a threshold of log_{10} uCTX-II should be somewhere between 2.5 and 2.8 should be used for radiographic progression (not shown). We tested cut-offs of log_{10} uCTX-II uCT2.55, 2.60, 2.65, 2.70 and 2.80 (including age, gender and BMI as covariates) in all four cohorts. Results were meta-analysed across all cohorts resulting in OR\(_{(\text{cut-off}=2.55)}\) =1.61 (95%CI 1.25-2.09 p=0.00026), OR\(_{(\text{cut-off}=2.60)}\) =1.78 (95%CI 1.35-2.36 p=5x10^{-5}), OR\(_{(\text{cut-off}=2.65)}\) =1.66 (95%CI 1.21-2.26 p=0.0015), OR\(_{(\text{cut-off}=2.70)}\) =1.75 (95%CI 1.23-2.50 p=0.002), and OR\(_{(\text{cut-off}=2.80)}\) =1.61 (95%CI 1.04-2.51 p=0.034). Having identified a log_{10} uCTXII cut-off of 2.60 as the best for progression of knee OA, ROC analyses were performed using this cut-off alone (Model 4, Table 3) and with age, gender and BMI (Model 5, Table 3). The performance alone of this binary cut-off in a ROC analysis is much inferior to the actual uCTX-II levels, but in combination to age, BMI and gender results are similar to the quantitative variable (Table 3).
Discussion

In this study we have analysed the largest sample to date with regards to biochemical markers of cartilage degradation. We confirm the value of uCTX-II as a degradation product that correlates with a vast number of OA traits, namely hip knee and hand OA in addition to knee OA progression and radiographic severity of knee OA. On the other hand, although COMP and C2M are strongly correlated with some of the OA traits studied here, the associations become very weak or non-significant when adjustments for age, gender and BMI are performed.

The analyses carried out also highlight the challenges of using biochemical markers for prognostic uses. It is commonly accepted that a clinically useful diagnostic should have an AUC of 0.7 or higher and a high accuracy diagnostic of 0.9 or higher. The results from the four study cohorts show that cartilage degradation markers do not achieve such predictive values yet neither alone or in combination with anthropometric characteristics. On the other hand, they form the basis onto which it may be possible to add other biochemical or imaging or molecular markers that may improve prediction.

An observation worth of note is that uCTX-II, but not C2M was strongly associated with the OA traits, although both are products of type II collagen degradation (2-3). Recent studies have shown that there may be a difference in origin of the two type II collagen markers. Immunolocalization studies have shown that CTX-II is highly associated with calcified cartilage remodelling as well as fibrillation of the cartilage (22), whereas C2M seems to be present in the core of the articular cartilage.(2) This is supported by studies showing a strong association between uCTX-II and clinical features such as joint space narrowing, pain, osteophyte formation and subchondral bone remodelling.(23) A recent cluster analysis has shown a relationship between uCTX-II and bone resorption markers further supporting a strong association of uCTX-II with calcified cartilage. Interesting we did see a weak association of C2M with knee OA incidence and progression to a similar degree as CTX-II, which may indicate connection to knee OA. C2M is yet another novel marker which needs to be further validated in studies with cartilage measures. Another observation worth of note is that our results confirm a role for sCOMP as a biomarker for knee OA but not hip OA as has been recently reported (24).

The current study has some limitations that must be taken into account. The results from C2M combine data from serum (Rotterdam, TwinsUK and Chingford) and from urine (GARP). Thus
although the combined data are not significant the heterogeneity introduced by the two types of biospecimen may have reduced our statistical power to detect strong associations with OA. More importantly, two of the population based cohorts consist only of females, and the GARP study is also biased towards a large proportion of females. Other limitations include the fact our study is based on K/L grades which conflate osteophyte and joint space narrowing and some studies have shown that biomarker associations can be different with respect to these related but different features (25). Finally the study has not investigated other joints, e.g. facet joints in the spine, nor have we carried out a number of sub-analyses which may reveal stronger or weaker biomarker associations. Specifically, although all analyses are adjusted for BMI, we have not explored the role of biomarkers within the obese population.

Nonetheless, the current study is the largest by far in its kind and shows that biomarkers can be valuable prognostic tools for progression of knee OA. However these data also highlight is a need for more biomarkers to achieve sufficient value to predict OA progression in the clinic.

Author contributions: All authors contributed to the study design, data interpretation and were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be published. AMV, IM, JBvM and ACBJ analyzed and interpreted the data. EC carried out biomarker assays. AMV and ACJB wrote the manuscript.

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Conflict of interest
ACBJ and MK are full time employees at, and MK holds stocks in Nordic Bioscience. Nordic Bioscience is a privately-owned; small-medium size enterprise (SME) partly focused the development of biomarkers for rheumatic and fibrotic diseases. EC is a full-time employee at Synarc Laboratories, a company doing fee for service measurement of biomarkers from different suppliers. None of the authors received fees, bonuses or other benefits for the work described in the manuscript.
Reference List


### Tables

**Table 1.** Descriptive statistics of study cohorts

<table>
<thead>
<tr>
<th>Study cohort</th>
<th>GARP</th>
<th>Rotterdam</th>
<th>Chingford</th>
<th>TwinsUK</th>
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</thead>
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<td>sample size</td>
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<td>1403</td>
<td>753</td>
<td>1034</td>
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<tr>
<td>age (SD)</td>
<td>59.9 (7.86)</td>
<td>63.2 (6.57)</td>
<td>53.9 (5.91)</td>
<td>55.7 (7.57)</td>
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<tr>
<td>BMI (SD)</td>
<td>27.0 (4.78)</td>
<td>27.1 (3.88)</td>
<td>25.3 (3.88)</td>
<td>24.6 (4.16)</td>
</tr>
<tr>
<td>%-females</td>
<td>80%</td>
<td>56%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>knee OA (controls/all cases/ KL≥3)</td>
<td>238/147/59</td>
<td>1084/262/55</td>
<td>522/231/86</td>
<td>661/280/66</td>
</tr>
<tr>
<td>hip OA (controls/all cases/ KL≥3))</td>
<td>287/78/17</td>
<td>1237/131/43</td>
<td>634/91/21</td>
<td>593/66/14</td>
</tr>
<tr>
<td>hand OA (controls/cases)</td>
<td>282/110</td>
<td>1131/218</td>
<td>391/260</td>
<td>593/238</td>
</tr>
<tr>
<td>Knee OA non progressors/ progressors</td>
<td>316/76</td>
<td>1150/122</td>
<td>528/222</td>
<td>232/82</td>
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<td>Knee OA non incident/incident</td>
<td>N/A</td>
<td>1017/91</td>
<td>510/178</td>
<td>188/61</td>
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<td>Hip OA non incident/incident</td>
<td>N/A</td>
<td>1245/42</td>
<td>666/58</td>
<td>N/A</td>
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<tr>
<td>log10 CTX-II (SD)</td>
<td>2.31 (0.26)</td>
<td>2.31 (0.23)</td>
<td>2.28 (0.25)</td>
<td>2.29 (0.24)</td>
</tr>
<tr>
<td>log10 COMP (SD)</td>
<td>1.05 (0.12)</td>
<td>1.03 (0.10)</td>
<td>0.98 (0.15)</td>
<td>1.02 (0.13)</td>
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<tr>
<td>log10 C2M (SD)</td>
<td>1.67 (0.34)</td>
<td>-0.49 (0.17)</td>
<td>-0.56 (0.23)</td>
<td>-0.45 (0.13)</td>
</tr>
</tbody>
</table>
Table 2. Fixed effect meta-analysis results. Odds ratios (OR) refer to a log10 unit for each one of the biochemical markers. Multivariate analyses are adjusted for age, gender and body mass index. Bonferroni p-value corresponds to p=0.002, values under this threshold are highlighted in bold.

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>biomarker</th>
<th>Univariate</th>
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<th>Multivariate</th>
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<tr>
<td></td>
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<td>OR</td>
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<td>p-value</td>
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<td>C2M</td>
<td>1.50</td>
<td>(1.02 - 2.30)</td>
<td>0.1914</td>
<td>1.19</td>
<td>(0.78 - 2.13)</td>
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<td></td>
<td>COMP</td>
<td>7.29</td>
<td>(3.8 - 49.11)</td>
<td>0.0213</td>
<td>1.86</td>
<td>(0.91 - 3.68)</td>
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<tr>
<td></td>
<td>CTX-II</td>
<td>11.20</td>
<td>(7.78 - 113.91)</td>
<td>1.1E-09</td>
<td>5.01</td>
<td>(3.36 - 23.5)</td>
</tr>
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<td>hip OA incidence</td>
<td>C2M</td>
<td>1.28</td>
<td>(0.62 - 2.7)</td>
<td>0.50</td>
<td>1.69</td>
<td>(0.69 - 3.59)</td>
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<tr>
<td></td>
<td>COMP</td>
<td>8.26</td>
<td>(1.16 - 64.9)</td>
<td>0.0351</td>
<td>7.25</td>
<td>(0.97 - 51.3)</td>
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<td>CTX-II</td>
<td>5.75</td>
<td>(2.38 - 30.8)</td>
<td>1.0E-04</td>
<td>4.89</td>
<td>(1.7 - 22.5)</td>
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<td>C2M</td>
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<td>(0.76 - 2.4)</td>
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<td>1.12</td>
<td>(0.63 - 2.51)</td>
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<td>6.87</td>
<td>(2.55 - 43.6)</td>
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<td>(0.96 - 7.36)</td>
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<td></td>
<td>CTX-II</td>
<td>6.78</td>
<td>(3.97 - 42.6)</td>
<td>2.3E-12</td>
<td>4.33</td>
<td>(2.38 - 17.6)</td>
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<td>hip OA severity</td>
<td>C2M</td>
<td>1.42</td>
<td>(0.43 - 3.47)</td>
<td>0.56</td>
<td>1.41</td>
<td>(0.41 - 3.52)</td>
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<td>COMP</td>
<td>2.60</td>
<td>(0.26 - 9.86)</td>
<td>0.41</td>
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<td>(0.18 - 8.41)</td>
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<td>CTX-II</td>
<td>4.82</td>
<td>(1.42 - 22.0)</td>
<td>0.0114</td>
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<td>(1.14 - 20.4)</td>
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<tr>
<td>knee OA incidence</td>
<td>C2M</td>
<td>1.74</td>
<td>(1.02 - 3.09)</td>
<td>0.0423</td>
<td>1.95</td>
<td>(1.16 - 3.75)</td>
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<tr>
<td></td>
<td>COMP</td>
<td>9.92</td>
<td>(3.12 - 89.7)</td>
<td>1.0E-04</td>
<td>5.85</td>
<td>(1.74 - 32.0)</td>
</tr>
<tr>
<td></td>
<td>CTX-II</td>
<td>2.29</td>
<td>(1.55 - 5.07)</td>
<td>3.5E-05</td>
<td>1.74</td>
<td>(1.14 - 3.0)</td>
</tr>
<tr>
<td>Knee OA progression</td>
<td>C2M</td>
<td>1.94</td>
<td>(1.23 - 3.69)</td>
<td>0.0042</td>
<td>1.69</td>
<td>(1.05 - 2.87)</td>
</tr>
<tr>
<td></td>
<td>COMP</td>
<td>11.0</td>
<td>(3.99 - 109.)</td>
<td>3.5E-06</td>
<td>4.07</td>
<td>(1.37 - 15.8)</td>
</tr>
<tr>
<td></td>
<td>CTX-II</td>
<td>3.15</td>
<td>(2.23 - 9.5)</td>
<td>8.0E-11</td>
<td>2.73</td>
<td>(1.77 - 7.18)</td>
</tr>
<tr>
<td>knee OA risk</td>
<td>C2M</td>
<td>1.54</td>
<td>(1.04 - 2.41)</td>
<td>0.0307</td>
<td>1.29</td>
<td>(0.85 - 2.07)</td>
</tr>
<tr>
<td></td>
<td>COMP</td>
<td>12.1</td>
<td>(6.36 - 133.)</td>
<td>3.6E-14</td>
<td>3.26</td>
<td>(1.63 - 10.1)</td>
</tr>
<tr>
<td></td>
<td>CTX-II</td>
<td>10.0</td>
<td>(7.01 - 91.4)</td>
<td>7.2E-37</td>
<td>5.72</td>
<td>(3.88 - 30.5)</td>
</tr>
<tr>
<td>knee OA severity</td>
<td>C2M</td>
<td>0.93</td>
<td>(0.52 - 1.97)</td>
<td>0.82</td>
<td>0.75</td>
<td>(0.4 - 0.82)</td>
</tr>
<tr>
<td></td>
<td>COMP</td>
<td>5.96</td>
<td>(2.53 - 33.1)</td>
<td>4.5E-05</td>
<td>1.45</td>
<td>(0.57 - 3.2)</td>
</tr>
<tr>
<td></td>
<td>CTX-II</td>
<td>9.23</td>
<td>(5.61 - 77.8)</td>
<td>1.4E-18</td>
<td>5.72</td>
<td>(3.33 - 30.5)</td>
</tr>
</tbody>
</table>
Table 3. Receiver Operating Characteristics (ROC) analysis in four study cohorts for the use of uCTX-II levels in predicting progression of knee OA. The area under the curve (AUC) for three models is shown for all four study cohorts. Model 1 includes only age, gender and BMI, Model 2 includes only uCTX-II and Model 3 includes age, gender, BMI and uCTX-II, Model 4 includes uCTX2 alone as a binary variable using a cut-off of log10 uCTX2<2.6 (0) or ≥2.6, Model 5 includes age, gender, BMI and uCTXII as a binary variable.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>AUC difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>Mod3–Mod1</td>
<td>Mod5–Mod1</td>
</tr>
<tr>
<td>GARP</td>
<td>0.636 (0.56 - 0.71)</td>
<td>0.628 (0.56 - 0.7)</td>
<td>0.672 (0.60 - 0.74)</td>
<td>0.037</td>
<td>0.525 (0.45-0.60)</td>
<td>0.037</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>0.696 (0.65 - 0.75)</td>
<td>0.607 (0.55 - 0.67)</td>
<td>0.706 (0.66 - 0.76)</td>
<td>0.010</td>
<td>0.560 (0.50-0.62)</td>
<td>0.007</td>
</tr>
<tr>
<td>Chingford</td>
<td>0.657 (0.62 - 0.7)</td>
<td>0.601 (0.56 - 0.65)</td>
<td>0.669 (0.63 - 0.71)</td>
<td>0.012</td>
<td>0.570 (0.54-0.60)</td>
<td>0.009</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>0.599 (0.53 - 0.67)</td>
<td>0.615 (0.55 - 0.69)</td>
<td>0.626 (0.56 - 0.7)</td>
<td>0.027</td>
<td>0.533 (0.49-0.57)</td>
<td>0.026</td>
</tr>
<tr>
<td>average</td>
<td>0.647</td>
<td>0.613</td>
<td>0.668</td>
<td>0.021</td>
<td>0.547</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Forest plot of study-specific estimates and summary odds ratio (OR) estimates and 95% confidence intervals (95% CIs) for the association between (A) serum COMP and prevalence of knee OA (B) urinary CTX2 and prevalence of knee OA. fe summary = fixed effects summary statistic, re summary = random effects summary statistic. The inter-study heterogeneity $I^2$ is shown along with the corresponding significance level for each fixed effects estimate. The statistical significance for fixed and random effects summary statistics is also shown.

Figure 2. Forest plot of study-specific estimates and fixed-effects summary odds ratio (OR) estimates and 95% confidence intervals (95% CIs) for the association between urinary CTXII and (A) prevalence of hand OA (B) prevalence of hip OA (C) radiographic severity of knee OA (D) progression of knee OA. fe summary = fixed effects summary statistic, re summary = random effects summary statistic. The inter-study heterogeneity $I^2$ is shown along with the corresponding significance level for each fixed effects estimate. The statistical significance for fixed and random effects summary statistics is also shown.
A  COMP and risk of knee OA

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chingford</td>
<td>4.10 (1.29, 12.99)</td>
</tr>
<tr>
<td>GARP</td>
<td>2.94 (0.44, 19.64)</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>10.24 (2.02, 51.78)</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>1.41 (0.42, 4.69)</td>
</tr>
</tbody>
</table>

Fe summary
$\chi^2=24\%$ p<0.26

B  CTX-II and risk of knee OA

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chingford</td>
<td>5.12 (2.41, 10.87)</td>
</tr>
<tr>
<td>GARP</td>
<td>4.22 (2.10, 8.50)</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>8.81 (4.05, 19.15)</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>1.87 (1.38, 2.53)</td>
</tr>
</tbody>
</table>

Fe summary
$\chi^2=84\%$ p<0.0003

Re summary
p= $7 \times 10^{-19}$

p= 0.0002
**CTX-II and severity of knee OA**

- **Chingford**
  - Odds ratio: 4.98 (2.61, 9.47)
  - p = 61% p < 0.053

- **TwinsUK**
  - Odds ratio: 4.85 (1.90, 12.36)
  - p = 83% p < 0.0006

- **Rotterdam**
  - Odds ratio: 5.01 (3.36, 7.46)
  - p = 5.1 x 10^-6

- **GARP**
  - Odds ratio: 5.21 (1.22, 22.20)
  - p = 0.0018

**CTX-II and progression of knee OA**

- **Chingford**
  - Odds ratio: 3.28 (1.56, 6.91)
  - p = 62% p < 0.05

- **TwinsUK**
  - Odds ratio: 2.75 (1.77, 4.22)
  - p = 1.6 x 10^-6

- **Rotterdam**
  - Odds ratio: 4.41 (1.28, 15.19)
  - p = 2.6 x 10^-10

- **GARP**
  - Odds ratio: 6.99 (2.50, 19.51)
  - p = 2.2 x 10^-15

- **Chingford**
  - Odds ratio: 1.46 (0.77, 2.75)
  - p = 4.7 x 10^-7