Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis (OA) in hip, knee, hand and facet joints in subject with familial OA at multiple sites. The GARP study

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Abbreviations:
OA, Osteoarthritis
ROA, Radiographic signs of osteoarthritis
CII, collagen type II
CIL, confidence interval limits
CTX-II, C-terminal cross linking telopeptide of type II collagen
DD, discus degeneration
DIP, distal interphalangeal
PIP, proximal interphalangeal
MCP, metacarpo-phalangeal
PA, posterior Anterior
OR, odds ratio
UCTX-II, urinary levels of type II collagen C-telopeptide
GARP, Genetics osteoArthritis and Progression
ACR, American College of Rheumatology
Abstract

Objective: To assess the relationship between the urinary levels of type II collagen C-telopeptide (UCTX-II) and radiographic signs of osteoarthritis (ROA) in the GARP (Genetics osteoARthritis and Progression) study.

Methods: UCTX-II levels were measured in the GARP study consisting of sibling pairs predominantly with symptomatic OA at multiple sites. ROA was assessed in the knees, hips, hands, vertebral facet joints and spinal disc degeneration (DD) by the Kellgren and Lawrence score. A proportionate score was made for each joint location based on the number of joints with ROA. Summed, the total ROA score represents a measure of cartilage abnormalities within each patient. By using linear mixed models the total ROA score and the joint-site specific ROA scores were associated to the UCTX-II level.

Results: In 302 subjects the mean (sd) and median (range) level of UCTX-II was 265 (168) and 219 (1346) ng/mmol creatine, respectively. We found significant association between the total ROA score and UCTX-II levels. Subsequent multivariate analysis showed that the joint site-specific ROA score of all joint sites, except for spinal DD, independently of each other contributed to this association.

Conclusion: The total ROA score of GARP patients representing cartilage abnormalities at the most prevalent ROA joint locations showed a remarkable sensitive association with UCTX-II levels. Furthermore, it is shown that the specific ROA score of the hip, hand, facet and knee joints additively and independently explain this association. Even within patients with OA at multiple sites, UCTX-II may be a sensitive quantitative marker of ROA.
Osteoarthritis (OA) is a joint disease characterized by degeneration of articular cartilage and bone remodeling leading to pain and joint stiffness. The extent of OA within each joint is usually assessed by radiographic characteristics and expressed as the Kellgren score (1). However, a limitation of using radiographs to detect cartilage destruction is the sensitivity; significant cartilage degradation must have occurred before it is visible on an autoradiograph (2). Sensitive biochemical markers have been developed with the aim of detecting the overall cartilage degradation with more reliability and sensitivity preferably in an early stage of OA (3;4). Such markers may be useful for the early identification of patients with OA and/or for assessing therapeutic response during treatment (5).

One of the primary disease processes of OA is degradation of the type II collagen (CII) (6) which is most abundant and highly specific for cartilage tissue (7). In addition to the cartilage of synovial joints, collagen type II is also present in the nucleus pulposus and annulus fibrosus of the spinal discs (8). Measuring CII degradation fragments may, thus, be a specific marker of cartilage degradation occurring at both synovial joints and spinal discs and may be more sensitive as compared to radiographic characteristics (9-11). Assays have been developed for several years that allow the CII degradation products to be detected (3;4;12). More recently, immunoassays detecting C-terminal cross linking telopeptide of type II collagen (CTX-II) have been developed, and increased levels of CTX-II have been reported in synovial fluid soon after knee injury (13). In the urine elevated levels were associated with more rapidly progression of OA and rheumatoid arthritis (12;14;15). In OA, two recent cross sectional studies showed association of the UCTX-II level and the presence of OA and spinal DD. In a study of Reijman et al. (16) it was shown that subjects within the highest quartile of UCTX-II levels as compared to the lowest quartile have a four times increased risk of having knee or hip ROA and an increased OA progression risk. Garnero et al. (17) showed that, in addition to knee and hand OA, UCTX-II levels were independently influenced by lumbar spine DD. None of these studies have data available on all major joints with OA, their results could, therefore, easily be confounded by cartilage degradation at other major joint locations for which radiographic data were lacking. The aim of the current study was to investigate whether, in the subjects of GARP, cartilage degradation occurring at each prevalent OA joint location (knee, hip, hand, spinal facet joints and DD) represented by a total ROA score was associated to the UCTX-II level. By using multivariate analysis and a joint site specific ROA score the independent contribution of each joint location to the UCTX-II level was investigated.

Patients and methods

The GARP study (Genetics, osteoARthritis and Progression)

The ongoing GARP study, which consists of Caucasian sib pairs of Dutch ancestry affected predominantly with symptomatic OA at multiple sites, is primarily aimed at the identification of genetic determinants of OA susceptibility and progression. Details of the ascertainment has been described elsewhere (18). Symptomatic OA of a joint within the study was defined as having symptoms of OA in addition to radiographic signs. Informed consent was obtained from all patients. Probands (aged 40-70 year) and their siblings were included in the GARP study with OA at multiple joint sites of the hand, or with symptomatic OA in two or more of the following joint sites: hand, spine (cervical or lumbar), knee or hip (18). Subjects with symptomatic OA in just one joint site were required to have structural abnormalities
in at least one other joint site defined by the presence of ROA in either hip, knee, hand or spine or the presence of two or more Heberden nodes, Bouchard nodes or squaring of at least one CMC joint on physical examination.

Conventional radiographs of the hands (dorso-volair), knees (Posterior-Anterior in weight bearing / semi flexed and lateral), hips (PA), lumbar (PA and lateral) and cervical spine (Anterior-Posterior, lateral and transbuccal) were obtained of all participants. This was performed in a standard manner with a fixed film-focus distance and a fixed joint position. Radiographic characteristics of OA were defined according to Kellgren and Lawrence(1). Definite ROA at a particular joint site was defined as a Kellgren score of two or higher. Symptoms of OA were defined as pain in the particular joint on most days of the prior month which is in accordance with the American College of Rheumatology (ACR) recommendations(19-21). Radiographic and symptomatic OA scoring has been described in detail elsewhere(18). In the GARP study N = 7 and N = 1 subjects had undergone, respectively, uni- and bi-lateral knee replacements and N = 23 and N = 15 for respectively, uni- and bi-lateral hip replacement. Based on these selection criteria, in total 191 sibling pairs were included. In the current paper we defined a proportionate score for each joint location based on the number of joints with ROA. Since no collagen type II breakdown products (UCTX-II) are expected from arthroplastic joints, these were considered as Kellgren score = 0 in the analyses performed in this paper. The specific ROA score for the hips and knees (0-2) represented no, uni- or bi-lateral ROA involvement of the joints. For hand, facet ROA and spinal DD first a sum score was made based on the number of joint sites with Kellgren score ≥2. The sum score of the hand ranged from 0-20 consisting of DIP 2-5, PIP 2-5, IP 1 and CMC 1. For spinal facet joints the sum score ranged from 0-11 and was based on the cervical joints (0-6, e.g. C1/C2-C6/C7) and lumbar joints (0-5, e.g. L1/L2-L5/S1). For DD of the spine the sum score ranged from 0-11 and was based on degeneration in the cervical discs (0-6, e.g. C1/C2-C6/C7) and lumber discs (0-5, e.g. L1/L2-L5/S1). These sum scores were subsequently compressed to a score from 0-2 in order to be proportional to the ROA score of hip and knee. The hand ROA score (0-2) represents subjects with, respectively, 0-2, 3-6, and ≥7 hand joints affected. For facet joints the ROA score (0-2) represents subjects with, respectively, 0-3, 4-6 and ≥7 facet joints affected. For spinal DD score (0-2) represents subjects with DD at respectively 0-2, 3-5 and ≥6 levels. The total ROA score (0-10) was made by the sum of the joint-site specific ROA scores that may represent a score proportional to cartilage abnormalities at each joint locations. For 360 subjects of GARP, ROA score data were available on all 5 joint locations evaluated.

The GARP study consists of 382 subjects (312 women and 70 men). Of the women 260 (83 %) were postmenopausal, including 8 women who currently used hormone replacement therapy (HTR). Since menopause and HTR have recently been shown to influence urinary CTX-II levels (22) untreated postmenopausal women were selected (N = 252). The present study was performed in 234 untreated postmenopausal women, and 68 men for whom we had UCTX-II levels available.

Biochemical analysis

For each participant of the GARP study we have collected non-fasted second void morning urine samples which were stored within 4 hours at ~80°C until measurement of urinary CTX-II was performed. Urinary CTX-II was measured by Synarc (Lyon, France) using an enzyme-linked immunosorbent assay 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- and interassay 
variation was lower than 9% and 11%, respectively.

**Statistical analysis**

In order to assess the contribution of the joint site specific ROA scores and the total 
ROA score to the UCTX-II level as outcome, a mixed model regression analyses was 
performed using SPSS version 11 (SPSS, Chicago, IL) with the UCTX-II level as 
dependent variable and as co-variable either the total ROA score as a quantitative 
measure or the specific ROA scores as defined above in knee (0-2), hip (0-2), hand (0- 
2), facet (0-2) and spinal DD (0-2). In the mixed model analysis we used family 
identity numbers (representing family relations) as random effect variables in order to 
model the familial dependencies that might occur for the UCTX-II level. Results of 
the mixed model analyses are expressed as estimates (β) that represent the association 
between increasing ROA grades and UCTX-II level.

In order to assess the risk of ROA given the UCTX-II level a logistic and ordered 
logit regression analysis was performed with the ROA scores as dependent variable 
and as categorical variable the UCTX-II levels into quartiles. For the logistic 
regression analysis the dependent joint site specific ROA scores of knee, hip, hand, 
facet and spinal DD were dichotomized by pooling ROA score grade 1 and 2, whereas 
the total ROA score for the ordered logit regression analysis was categorized into 4 
groups. Total ROA score grade 0-2 (N = 99) as group 1, grade 3 (N = 64) as group 2, 
grade 4-5 (N = 97) as group 3 and grade 6-10 (N = 45) as group 4. In these analyses 
standard errors were estimated from the variance between sibling pairs (robust 
standard errors) (23) using StataSE8 software (Stata Corporation, USA) to adjust for 
the familial dependencies that might occur for the UCTX-II level.

The outcome of all analyses are presented with the 95% confidence interval limits 
(CIL) and adjusted for age (years), body mass index (BMI in kg/m²) and sex because 
they have previously been shown to be associated with both OA and CTX-II 
levels(22). Familial aggregation of the UCTX-II level was estimated by comparing 
twice the between sibling variance divided by the total variance. Because UCTX-II 
levels were not normally distributed, data were logarithmically transformed in the 
analyses.
Table 1 Characteristics and frequencies of ROA among sibling pairs of the GARP study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GARP study</th>
<th>Hip</th>
<th>Knee</th>
<th>Hand</th>
<th>Facet</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROA score 0 (freq)</td>
<td>382 (1.0)</td>
<td>115 (0.30)</td>
<td>150 (0.40)</td>
<td>301 (0.79)</td>
<td>379 (0.99)</td>
<td>344 (0.99)</td>
</tr>
<tr>
<td>ROA score 1 (freq)</td>
<td>293 (0.77)</td>
<td>236 (0.62)</td>
<td>162 (0.45)</td>
<td>139 (0.39)</td>
<td>118 (0.33)</td>
<td></td>
</tr>
<tr>
<td>ROA score 2 (freq)</td>
<td>60 (0.16)</td>
<td>89 (0.23)</td>
<td>106 (0.29)</td>
<td>163 (0.45)</td>
<td>170 (0.47)</td>
<td></td>
</tr>
<tr>
<td>Age (SD)</td>
<td>60.3 (7.5)</td>
<td>62.5 (7.7)</td>
<td>61.8 (7.3)</td>
<td>61.3 (7.4)</td>
<td>60.3 (7.5)</td>
<td>60.9 (7.4)</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>27.0 (4.7)</td>
<td>26.5 (3.9)</td>
<td>28.1 (5.1)*</td>
<td>27.0 (4.5)</td>
<td>27.0 (4.7)</td>
<td>27.2 (4.7)</td>
</tr>
<tr>
<td>Women (freq)</td>
<td>312 (0.82)</td>
<td>83 (0.27)</td>
<td>122 (0.39)</td>
<td>244 (0.78)</td>
<td>309 (0.99)</td>
<td>279 (0.89)</td>
</tr>
<tr>
<td>Postmenopausal no HTR</td>
<td>252 (0.81)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Numbers represent patients within GARP with ROA in at least one site at the specific joint location including subjects with uni and/or bilateral joint replacement (N=38 for Hip and N = 8 for Knee). 2Proportional ROA scores were made based on the number of joint sites with ROA at each location. ROA scores of joint replacements were scored as ROA absent. *P-value < 0.05, DD = spinal disc degeneration.
Results
Table 1 shows the characteristics from the GARP study. In the GARP study, 82 percent of included patients were female and especially DD and facet ROA of the spine were very prevalent. Sibling pairs were selected for the concomitant presence of OA at hip, knee, hand or spine (see Materials and Methods), which is reflected by the numbers of individuals with an ROA score of > 0 at different combinations of joints (Table 2).

In 302 subjects (postmenopausal women (N = 234) and men (N = 68)) the mean (sd) and median (range) UCTX-II level was, respectively 265 (168) and 219 (1346) ng/mmol creatine, respectively. Although the respective mean (sd) and median (range) level among females is higher 271 (175) and 219 (1335) ng/mm mol creatine as compared to males 242 (137) and 216 (657) this difference did not reach statistical significance (Mann-Whitney P = 0.34). As shown in Figure 1 positive relation of raw UCTX-II levels was observed for subjects with an increasing total ROA score (ranging from 0-10).

<table>
<thead>
<tr>
<th>GARP study</th>
<th>Knee</th>
<th>Hand</th>
<th>Facet</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROA scores</td>
<td>360 (0.94)¹</td>
<td>38 (0.13)</td>
<td>57 (0.19)</td>
<td>65 (0.23)</td>
</tr>
<tr>
<td>Hip</td>
<td>77 (0.25)</td>
<td>88 (0.29)</td>
<td>96 (0.34)</td>
<td></td>
</tr>
<tr>
<td>Knee</td>
<td>122 (0.40)</td>
<td>130 (0.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand</td>
<td>147 (0.51)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹In total of 360 out of 382 individuals ROA scores were available at all 5 sites. DD = spinal disc degeneration.

In order to investigate the extent and significance of the positive association between the total ROA score and the UCTX-II level measured, a mixed model was fitted. Using logarithmically transformed UCTX-II levels as dependent and the total ROA score as independent variable a significant positive association was observed with an estimate 0.06, 95% with confidence interval limits (CIL) 0.04 – 0.07: P-value = 0.0001 (Table 3). The heritability estimate of the UCTX-II level was 0.33 and not significant, indicating a minor familial component influencing the UCTX-II level in this study group.

In order to investigate which joints contributed most to this association, multivariate mixed model analysis was subsequently performed with the joint-site specific ROA scores, knee (0-2), hip (0-2), hand (0-2), facet (0-2) and spinal DD (0-2).

As shown in Table 3, for each of the joint groups except for intervertebral DD a significant independent contribution to increased UCTX-II levels could be detected. For hip ROA the highest estimate (0.11, 95%CIL 0.07-0.15) was observed. Age and BMI did not influence the UCTX-II level, however, for women a significant (P = 0.03) a higher UCTX-II level was observed independent on the presence of ROA. The relative contribution, however, of the joint location to the UCTX-II level in men and women was similar.

Table 3. Mixed model analysis of UCTX-II and the total ROA score (grade 0-10) and multivariate to ROA scores (grade 0-2) of hip, knee, hand, facet and spinal DD.

<table>
<thead>
<tr>
<th>ROA scores</th>
<th>Median UCTX-II¹</th>
<th>Estimate⁴</th>
<th>P-value</th>
</tr>
</thead>
</table>

¹In order to investigate the extent and significance of the positive association between the total ROA score and the UCTX-II level measured, a mixed model was fitted.
<table>
<thead>
<tr>
<th>Joint</th>
<th>Grade</th>
<th>Sample Size (N)</th>
<th>UCTX-II (mean ± SE)</th>
<th>(95% CIL)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0-10</td>
<td>301</td>
<td>219 (1346)</td>
<td>0.06 (0.04–0.07)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hip grade 0</td>
<td></td>
<td>228</td>
<td>201 (1346)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip grade 1</td>
<td></td>
<td>48</td>
<td>288 (909)</td>
<td>0.11 (0.07–0.15)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hip grade 2</td>
<td></td>
<td>26</td>
<td>325 (639)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee grade 0</td>
<td></td>
<td>183</td>
<td>210 (957)</td>
<td>0.05 (0.01–0.08)</td>
<td>0.011</td>
</tr>
<tr>
<td>Knee grade 1</td>
<td></td>
<td>70</td>
<td>233 (483)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee grade 2</td>
<td></td>
<td>48</td>
<td>275 (878)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand grade 0</td>
<td></td>
<td>129</td>
<td>188 (742)</td>
<td>0.05 (0.02–0.09)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hand grade 1</td>
<td></td>
<td>90</td>
<td>226 (1329)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand grade 2</td>
<td></td>
<td>48</td>
<td>290 (888)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facet grade 0</td>
<td></td>
<td>109</td>
<td>182 (540)</td>
<td>0.07 (0.03–0.11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Facet grade 1</td>
<td></td>
<td>139</td>
<td>245 (742)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facet grade 2</td>
<td></td>
<td>54</td>
<td>269 (1302)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD grade 0</td>
<td></td>
<td>91</td>
<td>188 (652)</td>
<td>0.03 (-0.01–0.06)</td>
<td>0.199</td>
</tr>
<tr>
<td>DD grade 1</td>
<td></td>
<td>144</td>
<td>219 (957)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD grade 2</td>
<td></td>
<td>67</td>
<td>265 (1329)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 mean UCTX-II expressed in ng/mmol creatine. 2 Data was analyzed using mixed model regression analyses with UCTX-II levels logarithmically transformed as dependent variable and as co-variables age, sex, BMI and the presence of ROA scores. Family identity numbers were added as random effect variable to adjust for possible familial dependencies of the UCTX-II levels between siblings. DD = spinal disc degeneration. CIL = confidence interval limits

ROA risk associated to UCTX-II levels in GARP

In order to assess the ROA risk associated to the UCTX-II level among subjects of the GARP study, relative risks expressed as the odds ratio (OR) were calculated for the presence of ROA at a specific joint site or for the total ROA score for subjects that reside within different UCTX-II quartiles. Table 4 shows that subjects within the highest UCTX-II quartiles have the increased risks to have both ROA at a specific joint site and higher grades of the total ROA score. Subjects within the highest UCTX-II quartile as compared to the lowest show a high and significant OR, for hip ROA (OR = 7.7 (95% CIL 3.0–19.7). Furthermore, it is shown that subjects within this quartile also have substantial risk to have ROA at other joint locations (OR = 7.7 (95% CIL, 4.0-14.8). For spinal DD we did not find such associations.
Table 4 Logistic and ordered logit regression analysis to assess the risk of having ROA in relation to quartiles of UCTX-II level

<table>
<thead>
<tr>
<th>UCTX-II quartiles ng/mmol Creatine</th>
<th>Adjusted OR (95% CIL)¹</th>
<th>Hip²</th>
<th>Hand²</th>
<th>Facet²</th>
<th>Knee²</th>
<th>Total ROA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.3-147.4</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>147.4-218.9</td>
<td>2.3 (0.9-5.4)</td>
<td>1.3  (0.6-2.6)</td>
<td>1.2 (0.6-2.4)</td>
<td>1.2 (0.5-2.5)</td>
<td>1.5 (0.9-2.8)</td>
<td></td>
</tr>
<tr>
<td>218.9-328.1</td>
<td>5.6 (2.2-14.2)</td>
<td>2.4  (1.1-5.1)</td>
<td>2.1 (1.0-4.3)</td>
<td>1.5 (0.7-3.3)</td>
<td>4.3 (2.3-8.2)</td>
<td></td>
</tr>
<tr>
<td>328.1-1390.5</td>
<td>7.7 (3.0-19.7)</td>
<td>2.7  (1.3-5.8)</td>
<td>4.3 (1.9-9.7)</td>
<td>2.6 (1.2-5.7)</td>
<td>7.7 (4.0-14.8)</td>
<td></td>
</tr>
</tbody>
</table>

¹Analysis were adjusted for age, BMI, gender and the presence of ROA in the joint sites that were not the dependent variable. ²The joint site-specific ROA scores were dichotomized and used as dependent variable in the logistic regression analysis. ³The total ROA score was divided into 4 categories and used as dependent variable in an ordered logit regression analysis. DD = discus degeneration of the spine. CIL = confidence interval limits.
Discussion
Primary OA is a common disorder for which a wide variety of phenotypic definitions have been described. These definitions differ on the basis of either radiographic or symptomatic features of OA or by the distinction between the specific joint locations affected. In the GARP patients a clear relationship exists (Table 1) between the presence of OA at articular joints in combination with spinal DD. Since the nucleus pulposis and annulus fibrosus of spinal discs does contain collagen type II, the presence of DD should be considered when measuring a collagen type II degradation marker. The current study is the first to investigate the relationship between cartilage degradation, measured as UCTX-II level, and the concomitantly occurrence of ROA at all prevalent joint locations i.e. hip, knee, hand, spinal facet joints in addition to intervertebral disc abnormalities (Table 2). A mean and median UCTX-II level among subjects of the GARP study was measured of respectively, 265 and 219 ng/mmol creatine. As expected for a study group selected for the presence of OA at multiple joint sites, this level appears higher as compared to the median level observed in a recent studies of Reijman et al. (16) (median level 177 ng/mmol) which investigated the relationship of UCTX-II levels and OA within a population based sample and Garnero et al. (17) mean UCTX-II levels ranged from 166 to 299 ng/mmol for respectively subjects without and with the presence of intervertebral DD, knee ROA and clinical hand OA. Even within a group of subjects with symptomatic OA at multiple joint sites (the GARP study) a highly significant association was observed between UCTX-II levels and the total ROA score with estimate 0.06, 95% CIL 0.04 – 0.07, indicating a significant and sensitive increase in UCTX-II level with increasing scores of ROA occurring in the body. Subsequently, it was shown that ROA scores of the hip, knee, hand and facet joints independently contributed to the UCTX-II levels, whereas spinal DD did not. For hip ROA the highest estimate (0.11, 95% CIL 0.07-0.15) was observed indicating that the presence of ROA at one single joint site contributes significant to the variation in the UCTX-II level. The fact that we did not observe an independent effect for spinal DD may either indicate that DD has a different pathophysiological process and, as such, does not contribute to the UCTX-II level or that we were not able to detect its contribution due to the high concomitant occurrence with ROA at the other joint locations (Table 2). The latter explanation, however, does account for facet ROA scores, with similar co-occurrence of ROA at other joint locations as DD.

In order to explore the usefulness of the UCTX-II level as biomarker for OA it was shown that subjects within the highest UCTX-II quartile had a substantial risk to have ROA of the hip (OR = 7.7, 95% CIL 3.0–19.7) in addition to a high risk of OA at multiple joint sites simultaneously (OR = 7.7, 95% CIL 4.0-14.8). These data indicate that, when using the UCTX-II assay, subjects within the highest UCTX-II quartile (levels above 328 ng/mmol Creatine) may need assessment of ROA at the hip joint in addition to other joint locations. The strength of the current study is that it is a large, well-characterized sample of subjects with OA at multiple joint sites simultaneously. As a result cartilage degradation, measured as UCTX-II level, could be associated to a proportionate ROA score representing degenerative disease at i.e. hip, knee, hand, spinal facet joints in addition to intervertebral disc abnormalities. Together these locations represent the major skeletal sites for which degenerative disease is prevalent. The results of our study did, therefore, not seem to be confounded by cartilage degradation at joint locations for which radiographic data were lacking. Although our UCTX-II measurement appeared sensitive among subjects with OA at
multiple joint sites simultaneously, the absence of radiographic data for example of shoulders and diarthroidal joints may have caused some bias. Furthermore, the women of our study consist mainly of postmenopausal women. Although the total ROA score showed significantly contribute to the UCTX-II level, we were not able to assess a robust estimation of the contribution of the joint site-specific ROA scores to the UCTX-II level. Some of our findings may, therefore not apply to younger women or postmenopausal women receiving HTR.

Our results contradict the recently shown association of UCTX-II levels and DD of especially the lumbar spine in the study of Garnero et al. (17). Although the scoring method was similar between the studies, we did not analyze cervical and lumbar spine DD separately. It could therefore be argued that the study of Garnero et al. (17) represents a different subset of spinal abnormalities or that the sensitivity to detect the contribution from DD is different between the studies. However, separating DD of cervical and lumbar discs or changing the DD ROA score to different proportions in our study did not alter the outcome (results not shown). It could also been that the differences between the studies may be due to the fact the study of Garnero et al. (17) may have been somewhat confounded by the absence of OA data at the hip. Furthermore, in a recent study of Reijman et al. (16) it was shown that subjects in the highest quartiles of UCTX-II levels had a high increased risk to have OA at the knee and/or hip joint. However, in this study radiographic data on hand, facet OA and intervertebral DD of the spine was not taken into account. In view of the prevalence of hand and facet ROA, its correlation with knee ROA and their relative strong effects on UCTX-II levels as compared to knee ROA (Table 3) it may be that the risk for, specifically, knee ROA in this study was somewhat overestimated.

Based on the results of the present study, we conclude that UCTX-II levels are markedly associated to overall cartilage degradation occurring in hip, hand, facet and knee joints. For spinal DD we were not able to detect an independent effect. Further research is necessary to establish its association with progression of OA.

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Ethics approval Any necessary ethical approval of the GARP study was secured by the committee medical ethics (CME) of the Leiden University Medical Center, Leiden, The Netherlands.
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Figure legends
Figure 1: Box plot showing UCTX-II level in ng/mmol creatine (not logarithmically transformed) for the increasing total ROA score (0-10). The box length represent the interquartile range (boundaries for 50% of the outcomes) with the thick horizontal line the median UCTX-II level. The vertical line represents the 5th and 95th percentile. Circles are outliers (1.5-3 box lengths from the upper edge of the box) and stars are extremes (more then 3 box lengths from the upper edge of the box).

References


