Cartilage collagen neoepitope C2C in urine as an integrative diagnostic marker for early knee osteoarthritis

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SUMMARY

Objective: To investigate the suitability of urinary collagen type-II C-terminal cleavage neoepitope (uC2C) as a marker for early knee osteoarthritis (kOA).

Design: We examined 302 Estonian subjects (mean age, 49 years): 186 subjects with and 20 control subjects without knee symptoms, and 96 patients treated by arthroscopy. For the latter, cartilage lesions were characterized using Société Française d’Arthroscopie (SFA) scores. Standardized radiographs of bilateral tibiofemoral (TF) and patellofemoral (PF) joints were assessed for osteoarthritis (OA) features. Osteophytes (Ophs) and joint space narrowing (JSN) were graded separately. uC2C was measured by the uC2C-HUSA assay. Logistic and linear regression models were used for data analysis.

Results: Of the kOA cases, 50% were isolated (TF or PF) grade 1; 10% were grade 2. JSN with Ophs was more frequent in females than in males (52% vs. 34%, p = 0.01). Increased uC2C level was associated with gradual increase in the risk of kOA grade of severity (odds ratio = 2.14–3.71) including grade 1 vs. 0. TF-OA and PF-OA equally predicted uC2C concentration (R² = 0.33–0.35). uC2C prediction was better for females than for males (R² = 0.42 vs. 0.22 by TF-OA). The best predictive model for uC2C level (R² = 0.75) included three OA features: macroscopic cartilage lesions, TF Ophs, and PF-JSN.

Conclusions: uC2C as an integrative marker of kOA is associated with cartilage degradation and Oph formation in the PF- and TF-joints. Increased uC2C concentration could be used as an early diagnostic marker for kOA in clinical studies.

1. Introduction

Osteoarthritis (OA) is the most common joint disorder, causing disability in 22% of persons aged > 50 years and in 39% of those aged > 85 years [1]. The disease manifests first as molecular derangement, followed by anatomical and/or physiological derangement characterized by cartilage degradation, bone remodeling, osteophyte (Oph) formation, joint inflammation, and loss of normal joint function that can culminate in illness [2]. Joint replacement is the traditional treatment for symptomatic end-stage OA [3], but assessment and treatment should be shifted to the early phase of the disease to minimize, arrest, or even avoid derangement. The diagnostic potential of various biomarkers, including those related to collagen and aggrecan metabolism, non-collagenous proteins, and processes such as inflammation, has been investigated. Various markers of type II collagen (Col2) degradation have been found to be associated differently with increased risk of knee osteoarthritis (kOA) of different stages [4]. A 45-mer peptide fragment of Col2, produced mainly by the activity of matrix metallopeptidase (MMP)-13, is the most abundant neoepitope peptide in urine [5]. Recently, a new sandwich assay for human urinary collagen type II C-terminal cleavage neoepitope (uC2C) was developed and its potential for the diagnosis and prediction of knee OA worsening was investigated [6,7]. Highly significant correlations of increased uC2C concentrations with knee complaints and decreased lower-limb functional ability have been detected.
However, the few studies of \( \text{uC2C} \) that have been conducted have focused mainly on the tibiofemoral (TF) joint in patients with knee pain. We propose that another knee compartment, the patellofemoral (PF) joint, may be the source of \( \text{uC2C} \). Furthermore, the association of \( \text{uC2C} \) with the distinct pathogenetic processes of cartilage degeneration expressed as joint space narrowing (JSN), and Oph formation has not been evaluated. In addition, well-known sex differences in OA are rarely considered in investigations of the pathogenesis and treatment of the disease, and many previous studies have been based on the Kellgren and Lawrence (KL) grading of radiographic OA, which overlooks early OA grades and does not involve separate grading of JSN and Ophs. Thus, we aimed in this study to determine: 1) whether the \( \text{uC2C} \) concentration can be used to distinguish subjects with early-grade kOA from those with radiographically apparent (KL grade \( \geq 2 \)) kOA and healthy subjects, 2) which processes in the knee (i.e., Oph formation and/or JSN) are associated with elevated \( \text{uC2C} \) levels, 3) whether \( \text{uC2Cs} \) are associated with processes occurring in the TF and/or PF joint, and 4) whether the \( \text{uC2C} \) level differs between cases of bilateral and unilateral kOA.

2. Method

2.1. Subjects

In this cross-sectional study, a total of 302 subjects aged 35–64 years, divided into two groups, were investigated. The first group contained samples of 206 participants from the South-Estonian population-based study (aged 36–62 years, mean age 49.4 years), recruited by general practitioners, and described previously in detail [9]. The second group, the 96 subjects of the arthroscopy cohort (aged 35–64 years, mean age 48.3 years) were recruited by orthopaedic practice and treated by arthroscopic surgery, mainly due to degenerative meniscal lesions. Demographic and clinical data were collected for all subjects. Subjects’ knee symptoms were evaluated using the Knee injury and Osteoarthritis Outcome Score (KOOS) [10]. Twenty of the study subjects without knee complaints formed the control group. Subjects with radiographic evidence of rheumatoid arthritis or other inflammatory arthropathies in the knees were excluded. Fifty-five subjects from 68 cases (80%) in the arthroscopy cohort had degeneration of the meniscus. These data were not available for 28 subjects of the cohort. 29 of 55 cases had a combination of degeneration and knee trauma (11 subjects had trauma ≤ 1 year ago trauma and 18 had trauma ≥ 1 year ago). In addition, seventeen subjects from 68 cases in the arthroscopy cohort had isolated knee trauma (12 cases trauma ≤ 1 year ago and 5 cases > 1 year ago).

The Research Ethics Committee of the University of Tartu approved the study, which was conducted according to the precepts of the Declaration of Helsinki. The subjects provided written informed consent to study participation.

2.2. Radiographic evaluation

Standardized anteroposterior radiographs of TF joints and radiographs of PF joints with the knees at 60° flexion were obtained for all study subjects. Details of the radiographic evaluation have been published elsewhere [11]. Briefly, two radiologists independently graded JSN and Oph formation separately in the two joints on the radiographs using the Nottingham system (grades 0–3) [12]. Cases were determined to be unilateral when only one knee was involved, irrespective of knee compartment involvement. For bilateral cases, the knee with more severe OA served as the study knee. The highest grade of OA changes among all of each subject’s knee compartments was expressed as the radiographic global grade of knee osteoarthritis (gOA). gOA grade 1 was defined as early-grade kOA.

2.3. Articular cartilage evaluation

For a small number of subjects in the arthroscopy group \( [n = 14 \text{ (5 males and 9 females)}] \), orthopaedic surgeons performed direct visual assessment and reporting of knee articular cartilage status (lesions’ location, depth, and extent) [13]. The surgeons’ findings were used to calculate Société Française d’Arthrose (SFA) scores (continuous scale, 0–100) for the medial TF compartments [13,14]. Grades 0–IV of the extent (%) correspond to the mean extent of the respective grades on the two articular surfaces of the medial TF compartment, medial femoral condyle, and tibial plateau.

2.4. Biomarker measurement

The study subjects were instructed to collect urine from the second morning void. Urine samples were stored at –80 °C on the day of collection. Urinary levels of collagenase-generated \( \text{C2C} \) neoeptope fragments of human Col2 were measured by the IBEX \( \text{C2C} \) human urine sandwich assay (IB–\( \text{C2C-HUSA} \)) in IBEX Pharmaceuticals Inc., Montreal, Quebec, Canada). The assay details and performance characteristics have been described by Poole et al. [6] and https://www.ibex.ca/product-catalog/. All samples were tested in duplicate, and each measured \( \text{C2C} \) concentration was corrected with the creatinine concentration in the same urine sample, determined by using the QuantiChrom™ Creatinine Assay kit (DICT-500; BioAssay Systems, Hayward, USA).

2.5. Statistical analysis

The data were analyzed using the R software (version 3.4.3; Free Software Foundation, Boston, MA, USA; http://www.r-project.org). Subjects’ demographic data and body mass indices (BMIs) were summarized as means with standard deviations and compared using analysis of variance. The percentages of subjects in different gOA grade groups were compared using the chi-squared test. As \( \text{uC2C} \) concentration data were not distributed normally, medians and 25th and 75th percentiles were calculated and they were analyzed using the Kruskal–Wallis rank-sum test and Mann–Whitney U test. \( P \) values < 0.05 were considered to be significant.

To assess the association of changes in \( \text{uC2C} \) concentrations with gOA grades, forward method for multiple logistic regression with the calculation of odds ratios (ORs) and 95% confidence intervals was used for each of the following comparisons: gOA grade 1 versus grade 0; grade 2 versus grade 1; grade 3 versus grade 2; grade 2 versus grade 0; grade 3 versus grade 0; grade 3 versus grade 1. As several confounders may influence the course of kOA, each model was adjusted for age, sex, and BMI. The discriminative ability of \( \text{uC2C} \) was assessed using the \( c \) statistic (area under the curve (AUC)) and receiver-operating characteristic (ROC) curves were generated for each of the comparisons. The forward method for multiple linear regression were used to determine the ability of OA features (gOA, TFOA, FFOA, TFOph, TFJSN, PFph, PFJSN, and the SFA score alone and in combinations) and clinical/demographic data (BMI, age, gender) to predict \( \text{uC2C} \) concentration.

3. Results

3.1. Characteristics of the study group

The subjects of the study group were middle-aged (aged 35–64 years, mean age, 49.1 ± 6.5 years) and overweight (mean BMI, 28.4 ± 5.2 kg/m²; Table 1). More than half (60.6%) of the subjects were women. Twenty of the study subjects had no knee complaints and formed the control group. The mean age and BMI of the subjects of the control group
[gOA grade 0, no knee symptom, KOOS ≥ 85; 45% female] were 48.4 ± 5.8 years and 25.9 ± 4.6 kg/m², respectively. The mean age and BMI of the subjects of the control group did not differ significantly from those of other subjects with gOA grade 0.

The distribution of the subjects based on radiographic evaluation is shown in Fig. 1. The gOA severity groups contained different numbers of subjects, with gOA grade 1 being the largest group. This group contained only cases of combined TFOA and PFOA. Cases of JSN cases such as those were much smaller in the grade 2 group. The grade 3 group contained only cases of combined TFOA and PFOA. Cases of JSN cases showed in Fig. 1. The gOA severity groups contained different numbers of subjects, with gOA grade 0.

The prevalence of the SFA scores and radiographic features in the arthroscopy group is shown in Supplementary Tables 1 and 2. A higher SFA score value was associated with a more severe grade of gOA, (Supplementary Figure). Four cases with no radiographic change presented macroscopic cartilage lesions.

### 3.2. Associations between uC2C concentration and gOA

Median uC2C level was the lowest in the gOA grade 0 group and increased gradually until grade 3 (Fig. 2A, Supplementary Table 3). Similar gradual increases of uC2C level were seen for cases of TFOA and PFOA separately (Fig. 2B and C). The uC2C level did not differ between unilateral and bilateral kOA cases.

The increase of uC2C level was associated with a gradual increase in the risk of a more severe grade of kOA (adjusted OR per grade change = 2.14–3.7; Table 2). Similar to the unadjusted data, an increase in uC2C level significantly predicted gOA grade 1 (grade 1 vs. 0: OR = 2.14, p = 0.01). BMI had a significant predictive value only for grade 2 vs. 1 and grade 3 vs. 2, and not for grade 1 vs. 0. Overall, the adjusted uC2C models predicted the presence of radiographic gOA excellently (grade 3 vs. 0, AUC = 0.934; grade 2 vs. 0, AUC = 0.861).

### 3.3. Associations of uC2C level with TFOA and PFOA

The uC2C level was significantly higher in isolated TFOA cases than in cases with no radiographic change (Fig. 3A); no such difference was found for cases of isolated PFOA. This level was also significantly higher in cases of combined TFOA and PFOA than in cases of isolated TFOA.

The gOA grade, TFOA, and PFOA equally predicted uC2C concentration in the whole study group (adjusted $R^2 = 0.33–0.36$; Table 3). uC2C prediction by radiographic features, especially TFOA, was better in females than in males (adjusted $R^2 = 0.42$). The combined use of TFOA and PFOA did not increase the predictive power, but age, sex and BMI should be taken into consideration as significant confounders.
statistically significant (joint). Both the presence of Ophs and cartilage damage were associated with macroscopic cartilage lesion and Ophs in the TF joint with JSN in the PF joint. Box-whiskers plot with 5th-95th percentiles, p-value of Mann-Whitney U test. TFOA grade groups' numbers (n) were irrespective of PFOA grades, PFOA grade groups' numbers were irrespective of TFOA grades.

Table 2
Adjusted glm-models of uC2C for subjects with different gOA grades, multiple logistic regression analysis.

| gOA        | OR (CI 95%) | P-value | AUC |
|------------|-------------|---------|-----|
| Grade 1 vs 0 | 2.14 (1.2-3.8) | 0.01*** | 0.660 |
| Grade 2 vs 0 | 3.83 (1.8-8.3)** | 0.0007*** | 0.861 |
| Grade 3 vs 0 | 83.3 (11-631) | 0.00002*** | 0.934 |
| Grade 2 vs 1 | 2.84 (1.6-5.2)** | 0.0007** | 0.790 |
| Grade 3 vs 2 | 3.70 (1.4-9.8)** | 0.008** | 0.744 |

OR – increase in the odds of being in an OA class for a one-unit increase of log 2 of uC2C concentration (ng/mmol); AUC – area under the curve; *adjusted for age, sex and body mass index (BMI); **significant P-value for BMI; ***significant P-value after Bonferroni correction for multiple (three) comparisons.

3.4. Associations of uC2C level with Ophs, JSN, and the SFA score

The presence of Ophs with no JSN was associated with an increase in uC2C level (Fig. 3B). This association was strongest for the TF joint. Uc2c level was not associated with differences in the JSN status within the same Oph grade (Fig. 3C).

The results of linear regression analysis were similar to those of unadjusted comparisons of distinct KOA features (Table 3). Pronounced sex-related differences were observed. The ability of the Oph and JSN status to predict uC2C level was much lower in males than in females (adjusted $R^2 = 0.09–0.27$ vs. $0.35–0.40$). The best uC2C prediction was achieved with the combined use of TF Ophs and JSN in both joint compartments among females (adjusted $R^2 = 0.43$).

Linear regression also revealed that the SFA score predicted uC2C level well (adjusted $R^2 = 0.59$; Table 4). The addition of the Oph status to the SFA score significantly improved the model of uC2C prediction.

The best predictive models for uC2C level (adjusted $R^2 = 0.75$, Table 4) included combinations of OA features (e.g., presence of a macroscopic cartilage lesion and Ophs in the TF joint with JSN in the PF joint). Both the presence of Ophs and cartilage damage were associated statistically significantly and independently with uC2C level.

4. Discussion

The C2C assay that detects intrachain epitopes in 45 residues of the Col2 neoepeitope on the carboxy-terminal 3/4 fragment (IB–C2C-HUSA) has been used in a small number of studies [6-8]. Previously we have shown that increased output of uC2C correlates with knee pain, decline of the functional abilities of lower limb, and with the results of lower limb performance tests [8]. Other researchers [6] have observed that uC2C levels were increased in subjects with KL grade 2, which is definitive for the radiographic diagnosis of OA [15]. Hence, uC2C has the potential to be both an indicator of pathogenic processes and a clinical endpoint.

To our knowledge, this study is the first to demonstrate the increased output of uC2C in subjects with minimal radiographic findings of OA (gOA grade 1 vs. 0). KL grade 1 is generally considered to indicate that the presence of Ophs is “doubtful,” and its significance has been underestimated in many publications and in clinical practice [16]. Recently, researchers specified that magnetic resonance imaging (MRI)-detected Ophs (but not those detected by standard radiographic evaluation) were associated with OA changes over time [17,18]. Despite MRI advances in recent decades, radiography has remained the primary imaging modality for the definition of inclusion and exclusion criteria for OA-related clinical trials [19]. Our results confirm that uC2C concentration reflects the early molecular disturbance of Col2 in joint tissues. The JSN and the Oph status are often merged into a single OA severity scale. Our data demonstrate that the use of the Nottingham scoring system [12] helps to overcome the limitations of the KL system.

Like the current study, previous publications [20,21] have shown that Oph formation is an important feature of early OA stages. Moreover, we found that uC2C level was associated significantly not only with the JSN, but also with the Oph status. Histomorphologically, Ophs appear as fibrocartilage with an admixture of cartilaginous and fibrous matrix components, such as Col2 and aggrecan on the one hand and type I collagen (Col1) on the other hand [22]. At the same time, osteophytic chondrocytes demonstrate increased expression of tissue-remodeling enzymes (MMP-9, MMP-13, and hyaluronan synthase 1) [23], possibly...
Table 3
Ln-models predicting the increase of uC2C by radiographic features and their combinations.

| Parameters | Whole group | Males (n = 119) | Females (n = 183) |
|------------|-------------|-----------------|-------------------|
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.21 (0.15-0.28)*** | 0.12 (0.02-0.22)* | 0.28 (0.2-0.37)*** |
| BMI        | -0.1 (-0.16-0.03)*** | 0.05 (-0.04-0.15) | 0.07 (-0.02-0.16) |
| gOA        | 0.27 (0.2-0.34)*** | 0.28 (0.18-0.38)*** | 0.25 (0.16-0.34)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.22 (0.15-0.28)*** | 0.14 (0.03-0.24)** | 0.28 (0.19-0.36)** |
| Gender     | -0.11 (-0.17-0.04)*** | 0.07 (-0.04-0.17) | 0.07 (-0.02-0.15) |
| BMI        | 0.08 (0.01-0.14)* | 0.23 (0.12-0.33)*** | 0.26 (0.17-0.35)*** |
| FFOA       | 0.25 (0.19-0.32)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.24 (0.16-0.3)*** | 0.15 (0.05-0.25)*** | 0.3 (0.21-0.39)*** |
| Gender     | -0.08 (-0.14-0.01)* | 0.04 (-0.06-0.14) | 0.1 (0.01-0.19)* |
| BMI        | 0.08 (0.02-0.15)* | 0.28 (0.17-0.37)*** | 0.2 (0.11-0.29)*** |
| FFOA       | 0.24 (0.17-0.3)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.73-8.95) | 8.7 (8.61-8.78) |
| Age        | 0.25 (0.18-0.32)*** | 0.17 (0.06-0.28)** | 0.32 (0.23-0.41)** |
| BMI        | 0.11 (0.04-0.18)** | 0.1 (-0.01-0.21) | 0.11 (0.03-0.2)** |
| TFJSN      | 0.15 (0.08-0.23)*** | 0.00 (-0.08-0.14) | 0.18 (0.09-0.27)** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.22 (0.15-0.28)*** | 0.15 (0.05-0.25)** | 0.28 (0.19-0.36)** |
| Gender     | -0.10 (-0.19-0.05)*** | 0.11 (-0.01-0.21) | 0.11 (0.03-0.2)** |
| BMI        | 0.11 (0.05-0.18)** | 0.03 (-0.08-0.14) | 0.18 (0.09-0.27)** |
| TFJSN      | 0.25 (0.19-0.32)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.69-8.82) | 8.7 (8.62-8.78) |
| Age        | 0.23 (0.16-0.3)*** | 0.15 (0.16-0.29)** | 0.3 (0.21-0.39)** |
| Gender     | -0.08 (-0.14-0.01)* | 0.04 (-0.02-0.15) | 0.11 (0.02-0.19)* |
| BMI        | 0.09 (0.02-0.16)** | 0.27 (0.17-0.3)** | 0.19 (0.1-0.28)** |
| FFOph      | 0.23 (0.16-0.3)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.21 (0.14-0.28)*** | 0.15 (0.05-0.25)** | 0.26 (0.17-0.35)** |
| BMI        | 0.07 (0.01-0.14)* | 0.07 (-0.03-0.17) | 0.05 (-0.03-0.14) |
| TFJSN      | 0.23 (0.16-0.3)*** | 0.26 (0.14-0.37)*** | 0.22 (0.13-0.31)** |
| TFJSN      | 0.07 (-0.14) | -0.03 (-0.15-0.08) | 0.12 (0.03-0.21)** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.22 (0.15-0.29)*** | 0.15 (0.05-0.25)** | 0.28 (0.19-0.37)** |
| Gender     | -0.09 (-0.16-0.03)*** | 0.04 (-0.06-0.14) | 0.1 (0.01-0.19)* |
| BMI        | 0.08 (0.02-0.15)* | 0.28 (0.17-0.38)*** | 0.13 (0.02-0.23)* |
| FFOph      | 0.2 (0.13-0.27)*** | -0.02 (-0.13-0.08) | 0.12 (0.02-0.22)* |
| FFOA       | 0.2 (0.16-0.3)*** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.73-8.95) | 8.7 (8.62-8.78) |
| Age        | 0.23 (0.16-0.3)*** | 0.17 (0.06-0.28)** | 0.28 (0.19-0.37)** |
| BMI        | 0.1 (0.03-0.16)** | 0.1 (-0.01-0.21) | 0.08 (0.07-0.17) |
| TFJSN      | 0.11 (0.04-0.18)** | 0.09 (-0.03-0.2) | 0.12 (0.03-0.21)* |
| FFOph      | 0.13 (0.06-0.2)*** | 0.01 (-0.11-0.12) | 0.15 (0.06-0.24)** |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.78) |
| Age        | 0.21 (0.15-0.28)*** | 0.14 (0.04-0.24)** | 0.27 (0.18-0.36)*** |
| Gender     | -0.09 (-0.15-0.02)*** | 0.04 (-0.06-0.14) | 0.08 (-0.01-0.17) |
| BMI        | 0.076 (0.01-0.14)* | 0.1 (-0.05-0.24) | 0.21 (0.09-0.33)** |
| TFJSN      | 0.18 (0.09-0.27)*** | 0.2 (0.05-0.35)** | 0.06 (-0.06-0.17) |
| FFOph      | 0.11 (0.02-0.2)* |
| Intercept  | 8.76 (8.69-8.82) | 8.84 (8.74-8.94) | 8.7 (8.62-8.79) |
| Age        | 0.2 (0.13-0.27)*** | 0.15 (0.05-0.25)** | 0.25 (0.16-0.33)*** |

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causing generation of C2C during enchondral ossification in Oph.

As expected, we demonstrated that uC2C excretion is associated substantially with the presence of knee cartilage lesions, as determined directly by macroscopic examination. This finding is consistent with immunohistochemical findings indicating that areas of cartilage damage have significantly increased levels of C2C [21]. The low values of medial SFA scores were characteristic of the subjects with mainly early stages of kOA in our study.

Radiographically assessed JSN is known to be insensitive in assessment of early-stage OA [3,24,25]. In addition to cartilage thickness, the position and degeneration of the meniscus account for substantial proportions of the explained variance in JSN [26]. Convincing evidence indicates that degenerative meniscal lesion often suggests only early-stage kOA [27]. The menisci are structurally analogous to the surfaces of articular cartilage [28], and are composed of Col1 as well as Col2 [29]. Thus, we propose that meniscal degeneration is one of the sources of uC2C during OA development.

Two decades ago, it was recognized that knee OA is characterized by several simultaneous radiological changes [30]. In early kOA, the radiographic presence of marginal Ophs has high sensitivity, specificity, and positive predictive value for presence of MRI-detected cartilage defects in the TF joint and meniscal abnormalities, irrespective of the presence of JSN [30]. We demonstrated in our similarly aged cohort that the presence of cartilage lesions, indicated by the SFA score, might precede radiographic JSN. uC2C prediction was the best with the combined use of different kOA features, mainly the SFA score in combination with TF Ophs and PF JSN.

To our knowledge, this study is the first to show that uC2C production increases continuously with kOA structural changes. We found that uC2C level could be used to discriminate subjects with early and advanced gOA. In normal cartilage, matrix turnover is regulated strictly, with a delicate balance maintained between synthesis and degradation. In OA, this balance is disturbed, usually with enhanced Col2 degradation and synthesis [31]. OA is characterized by excessive damage of the collagen fibrillar network, which appears to be mediated primarily by collagenases, mainly MMP-1 and MMP-13 [32]. Thus, uC2C level reflects severity of OA changes and can indirectly reflect MMP-13 activity [33]. For this reason, it is a marker of the disease burden and we believe that it could be useful for the monitoring of disease activity and treatment effects.

OA has been proposed to be a systemic disease [34], and its burden can thus be thought of as severity in terms of the number of joints involved [35]. Unexpectedly, we found that uC2C level did not differ between unilateral and bilateral OA cases. However, we do not claim that uC2C level is not significantly influenced by the number of involved knee joints. Considering that our unilateral OA cases had only grade 1 severity, a portion of the uC2C present in these cases must have come from another source.

kOA has been conceptualized as a multicompartmental disease [36]. Although PFOA may occur in the absence of TFOA [37], few studies have focused on the PF compartment alone. In this study, OA occurrence in an isolated compartment (TF or PF) was characteristic of the early stages of the disease. We demonstrated a significant association between uC2C level and TFOA, but found only a tendency toward association with PFOA, probably due to the small number of isolated PFOA cases in our sample. Nevertheless, the inclusion of the statuses of both compartments in the models increased the power to predict uC2C level. Thus, in interpretation of uC2C results, especially in early-stage kOA when isolated occurrence is more common, the influence of the processes occurring in both compartments (as sources of uC2C) cannot be ignored.

Sex-based differences in the incidence and course of kOA have been reported previously [38,39]. Although we found no difference in uC2C excretion between the sexes, we noted several features in females exclusively. First, cases with Ophs and JSN were more common in females. Second, older age and higher BMI were associated more frequently with bilateral kOA in females than in males. Moreover, uC2C prediction by Ophs and JSN, and especially by TFOA, was better in individuals with higher BMI.

Table 3

| Parameters | Whole group n = 302 | Males n = 119 | Females n = 183 |
|------------|---------------------|---------------|-----------------|
|            | R²                  | R²            | R²              |
| Intercept  | 9.22 (8.97–9.47)    | 0.38          | 0.43            |
| Age        | 0.13 (0.43–0.16)    | 0.03 (0.37–0.31) | 0.01 (0.01–0.15) |
| Gender     | 0.05 (0.01–0.05)    | 0.00 (0.01–0.05) | 0.05 (0.01–0.05) |
| BMI        | 0.07 (0.06–0.08)    | 0.06 (0.05–0.07) | 0.07 (0.06–0.07) |
| SFA        | 0.01 (0.01–0.02)    | 0.01 (0.01–0.02) | 0.01 (0.01–0.02) |
| TF Oph     | 0.21 (0.18–0.23)    | 0.21 (0.18–0.23) | 0.21 (0.18–0.23) |
| TFOA       | 0.25 (0.22–0.26)    | 0.25 (0.22–0.26) | 0.25 (0.22–0.26) |
| TFJSN      | 0.26 (0.24–0.28)    | 0.26 (0.24–0.28) | 0.26 (0.24–0.28) |
| PF Oph     | 0.07 (0.05–0.09)    | 0.07 (0.05–0.09) | 0.07 (0.05–0.09) |
| PFJSN      | 0.08 (0.06–0.10)    | 0.08 (0.06–0.10) | 0.08 (0.06–0.10) |
| Gender     | 0.01 (0.01–0.02)    | 0.01 (0.01–0.02) | 0.01 (0.01–0.02) |
| BMI        | 0.02 (0.01–0.03)    | 0.02 (0.01–0.03) | 0.02 (0.01–0.03) |
| SFA        | 0.01 (0.01–0.02)    | 0.01 (0.01–0.02) | 0.01 (0.01–0.02) |
| TF Oph     | 0.07 (0.05–0.09)    | 0.07 (0.05–0.09) | 0.07 (0.05–0.09) |
| TFOA       | 0.07 (0.05–0.09)    | 0.07 (0.05–0.09) | 0.07 (0.05–0.09) |
| TFJSN      | 0.08 (0.06–0.10)    | 0.08 (0.06–0.10) | 0.08 (0.06–0.10) |
| PF Oph     | 0.09 (0.07–0.11)    | 0.09 (0.07–0.11) | 0.09 (0.07–0.11) |
| PFJSN      | 0.09 (0.07–0.11)    | 0.09 (0.07–0.11) | 0.09 (0.07–0.11) |

gOA – global osteoarthritis score; TFOA, PFOA – osteoarthritis score in TF or PF joint; TFJSN, PFJSN – joint-space narrowing score in TF or PF joint; TFOph, PFOph – osteophytes score in TF or PF joint; *P < 0.05; **P < 0.01; ***P < 0.001. In bold: R² of the best models.

Table 4

| Parameters | Estimates (CI 95%) n = 14 | R² |
|------------|---------------------------|----|
| Intercept  | 9.22 (8.97–9.47)          | 0.59 |
| Age        | 0.38 (0.35–0.41)          | 0.75 |
| Gender     | 0.15 (0.13–0.17)          | 0.82 |
| BMI        | 0.12 (0.09–0.15)          | 0.89 |
| SFA        | 0.08 (0.06–0.10)          | 0.92 |
| TF Oph     | 0.21 (0.18–0.24)          | 0.97 |
| TFOA       | 0.25 (0.22–0.26)          | 1.01 |
| TFJSN      | 0.26 (0.24–0.28)          | 1.03 |
| PF Oph     | 0.07 (0.05–0.09)          | 1.05 |
| PFJSN      | 0.08 (0.06–0.10)          | 1.07 |

SFA – medial SFA score; TFOph – osteophytes score in TF joint; PFJSN – joint space narrowing score in PF joint; n – number of cases; *P < 0.05.
females than in males. The best model with radiographic features predicted uC2C level almost twice as well in females as in males. Recently, we demonstrated the existence of significant sex-dependent differences in cytokine production, with predomination of angiogenesis upregulation in females with grade 1 kOA, whereas activation of tissue remodeling was predominated in males [40], suggesting the presence of sex-related differences in the pathways of kOA pathogenesis. Recognition of the sex-based differences related to uC2C revealed in the present study may aid the assessment of the efficacy of novel therapeutic agents that directly suppress MMP gene expression [41] or angiogenesis [42].

The modeling performed in this study demonstrated clearly that the mechanisms underlying uC2C excretion are complex and are associated with the Oph and the JSN status in both knee joint compartments. Our findings indicate that TF and PF Oph formation, minor cartilage damage, PF JSN, and meniscal lesion development may occur in early kOA. These features, except for meniscal lesion development, which we did not include in our analysis (although over a half of the patients underwent arthroscopic surgery due to degenerative meniscal lesions), formed the basis of our best model for uC2C prediction. Thus, uC2C could serve as a parameter that integrates several important features of early kOA and may reflect pathogenic processes in any knee joint structure in which Col2 is dismantled. As direct examination of cartilage is not suitable for early diagnostic assessment, and as the performance of multiple radiological examinations of the knee joint is time-consuming, we propose the use of uC2C as an early dynamic marker for the evaluation of cartilage status and associated processes in the context of kOA.

This study has several limitations. First, both knee compartments were involved in a large number of study subjects, even those with early-stage kOA. This distribution complicated the study of isolated changes, especially isolated JSN. Second, because SFA scores and uC2C concentrations were determined simultaneously in a small number of subjects, the statistical power of the models including SFA determination was low, which could have reduced the chance of detecting a true effect. Finally, we adjusted for age, sex, and BMI as generally accepted OA risk factors, but not for menopausal status, in the main analyses. As C2C was also observed in deep calcified cartilage [21], it may, like markers of bone turnover, be influenced by menopause.

Despite these limitations, our study had several strengths. First, the sample was population-based, and thus included sufficient numbers of subjects with early-stage kOA and asymptomatic controls for the drawing of statistically based conclusions about the early stages of kOA. Second, our study group was well characterized by detailed radiographic evaluation. The radiographic evaluation of both the TF and PF compartments using a detailed and standardized scoring system is essential for OA research. Finally, the direct examination of the cartilage status in subjects who underwent arthroscopic surgery eliminated the difficulty of detecting early cartilage damage by X-ray. Although the study findings suggest the possible role of uC2C as a diagnostic marker, further validation and qualification are needed. In future studies, sex-specific differences in the pathogenesis of kOA should also be addressed.

In conclusion, the results of this study confirm that uC2C is an integrative and potential diagnostic marker for kOA. It can be used as a sensitive marker for the burden of this disease. Especially, we want to emphasize its value for the assessment of early-stage kOA when radiographic changes are still difficult to detect. As an integrative marker, uC2C is derived simultaneously from the main OA processes of the main knee compartments: cartilage degradation and Oph formation in the PF and TF joints, probably involving also the meniscus. In our opinion, uC2C has the potential to be a marker for disease-modifying treatment evaluation in the future.

Author contributions

All co-authors made a substantial contribution to the conception and design of the study, acquisition, analysis and interpretation of the data. All authors involved in the drafting and the revising of the manuscript, have read and approved its final version.

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Declaration of competing interest

None of the authors has any conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocarto.2020.100096.

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