Research Article

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Biomarkers in Medicine

Biochemical, clinical, demographic and imaging biomarkers for disease progression in knee osteoarthritis

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Aim: To identify prognostic biomarker(s) for knee osteoarthritis (OA) in the Osteoarthritis Initiative (OAI) cohort. **Methods:** Multilevel regression was used to determine the association between baseline biomarkers and change in biomarkers from baseline to 24 months with clinical and radiographic OA progression over 48 months of follow-up. **Results:** Higher values of baseline urinary CTXII were consistently associated with an increased risk of OA disease progression outcomes: Kellgren & Lawrence grade (odds ratio [OR]: 1.15, 95% CI: 1.03–1.28); medial joint space narrowing (OR: 1.06, 95% CI: 1.02–1.10); lateral osteophytes (OR: 1.05, 95% CI: 1.01–1.10); joint space width (regression coefficient: -0.005, 95% CI: -0.008–0.001); and Western Ontario and McMaster Universities Arthritis Index pain scores (OR: 1.02, 95% CI: 1.01–1.04). Changes in serum PIIANP and serum COMP over 24 months were associated with clinical disease progression. **Conclusion:** Urinary CTXII showed stronger associations with radiographic OA and appears to be a reliable prognostic marker, while changes in other biomarkers were found in early symptomatic OA, supporting the phasic nature of OA.

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Keywords: biomarkers • knee • osteoarthritis • progression • regression

Background

Osteoarthritis (OA) is the most common chronic degenerative disease affecting mainly older people. OA affects more than 500 million people worldwide, and the incidence of OA is increasing [1]. About 9 million people suffer from OA in the UK [2], costing the economy approximately £3.2 billion every year in lost working days [3]. OA is a multifactorial disease of unknown etiology and is an increasing burden on the UK National Health Service and providers of healthcare in other countries [4]. The disease develops slowly over the years and is characterized by progressive loss of articular cartilage and bone remodeling leading to the loss of joint function. The disease mechanism involves a combination of cellular and biochemical changes in the joint tissues that causes structural changes over the years [5].

Currently, the diagnosis of OA is based on symptoms and is usually confirmed with an x-ray. However, radiography is insensitive for early diagnosis and monitoring disease progression [6,7]; by the time OA is diagnosed, it has progressed to late-stage disease and caused irreparable damage to the joints. Other imaging methods, such as MRI and dual-energy x-ray absorptiometry (DXA), may be useful for early diagnosis and monitoring of OA [8]. However, availability and costs limit their application for routine clinical use. Therefore, research has focused on the development of disease-specific biomarkers [9]. Unfortunately, the evolution of biomarkers in OA has met many challenges. OA is a heterogeneous disease and the exact cause of OA is unknown. The heterogeneity in onset, lack of consistency for a surrogate measure, efficacy of intervention and definition of clinical outcomes are other issues that have hampered the development of an OA-specific biomarker [10]. In addition, some molecules, such as cartilage oligomeric matrix protein (COMP), a potential marker for assessing cartilage pathway, may also be found

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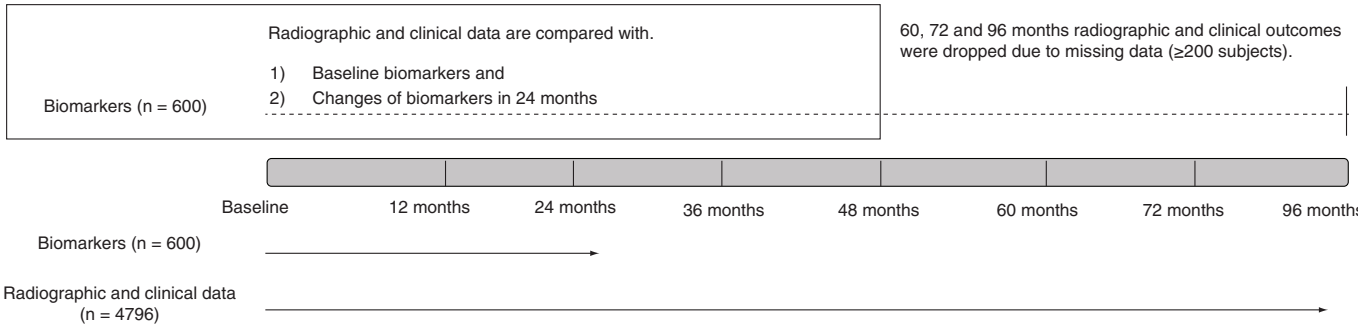

Figure 1. Study design. Radiographic and clinical data were followed up for 96 months while biomarkers data were available for up to 24 months.

in other arthritic diseases such as rheumatoid arthritis (RA) [11,12], which is a chronic autoimmune disease that attacks the joints.

The National Institutes of Health Osteoarthritis Initiative (OAI) cohort was created to address many of the challenges just discussed and to provide the scientific community with longitudinal biomarker, imaging and clinical data to identify biomarkers that may be useful for diagnosis and prediction of disease progression as well as monitoring of the efficacy of new treatments [13]. The authors recently used the data from the OAI cohort and identified biomarkers (urinary CTXII, serum COMP, serum CS846 and serum Coll2-1 NO2) that can be considered clinically useful [14]. Earlier, Kraus *et al.* used the database to identify urinary CTXII as a promising biomarker for monitoring disease progression in OA [15]. Several other studies have also sought to find biomarkers that would be useful for monitoring OA progression in smaller independent cohorts. For example, fibulin-3 fragments were elevated and associated with joint stability in obese patients with OA, suggesting that it can be considered a prognostic marker in the OA subpopulation [16]. Similarly, elevated levels of high sensitivity C-reactive protein (hsCRP) are associated with cartilage loss related to OA [17]. This study aims to use the OAI database to carry out a comprehensive analysis of both the short- and long-term prognostic value of baseline and change in biochemical markers and clinical, demographic and radiographic markers in OA.

Methods

Study design

The OAI is a longitudinal multicenter observational study designed to study and understand how to prevent the development of knee OA [13]. Four clinical sites in the USA were set up to recruit adults with or at risk for knee OA. It is one of the largest and most comprehensive cohorts to date, consisting of 4796 participants aged 40–79. The OAI study was approved by the institutional review board at each clinical site, the NIH, OAI investigators and private funding partners. All participants gave consent for the original observational study so no additional/further approval was needed. All participants were followed up annually for up to 10 years, and demographic, clinical, radiographic, blood and urine data were collected yearly. The clinical, radiographic, demographic and biomarkers data are publicly available [13]. The current study used a subset of 600 patients who were Kellgren & Lawrence (K & L) grade 1 or more and had complete data for biochemical markers (biomarkers), and clinical, demographic and imaging data at baseline and 24-month follow-up. This study was designed to predict biomarkers of knee OA progression over 48 months of follow-up (Figure 1). The clinical and radiographical features of OA [14] used in this study were Knee Injury and Osteoarthritis Outcome Score (KOOS) pain/symptoms score [18], Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain/stiffness score [19], K & L grades [20], joint space width (JSW), joint space narrowing (JSN) [21] in medial and lateral sites (JSN medial/JSN lateral) and osteophytes in medial and lateral sites. A patient level-based analysis using the maximum K & L grade for the worst knee for each patient was completed.

Biochemical markers

All available biomarker data at baseline, 12 and 24 months for the subsets of 600 patients (Figure 1) were extracted from the OAI dataset. Data for 19 biomarkers were available. Of these biomarkers, some were removed (sNTXI, uC2C, uCTXIα, uCTXIβ) due to collinearity or many missing values (uColl2-1 NO2), leaving 13

Table 1. The association between changes in biomarkers and radiographic and clinical outcomes.

Parameter	Inclusion criteria	Exclusion criteria
Do K & L grades <3 affect the measurement of biomarkers and predict the likelihood of OA worsening over 4 years of follow-up?		
Population	Adults with K & L grade 0, 1, 2, 3	Adults with K & L grade 4, TKR
Intervention (exposure)	Changes of biomarkers over time (baseline – 24 months)	12 months biomarker data
Comparison/outcome	OA progression of K & L grade 0, 1, 2, 3 over 4 years of follow-up.	
Do JSN OARSI scores <2 affect the measurement of biomarkers and predict the likelihood of OA worsening over 4 years of follow-up?		
Population	Adults with JSN in medial/lateral site OARSI score 0, 1, 2	Adults with JSN 3, TKR
Intervention (exposure)	Changes of biomarkers over time (baseline – 24 months)	12 months biomarker data
Comparison/outcome	OA progression of JSN medial/lateral OARSI score 0, 1, 2 over 4 years of follow-up	
Do OARSI grade >2 osteophytes affect the measurement of biomarkers and predict the likelihood of OA worsening over 4 years of follow-up?		
Population	Adults with osteophytes medial side OARSI score 0, 1, 2	Adults with osteophytes medial side OARSI 3, TKR
Intervention (exposure)	Changes of biomarkers over time (baseline – 24 months)	12 months biomarker data
Comparison/outcome	OA progression of in osteophytes OARSI score 0, 1 over 4 years of follow-up	
Do mild, moderate, severe WOMAC pain scores affect the measurement of biomarkers and predict the likelihood of OA worsening over 4/6 years of follow-up?		
Population	Adults with mild, moderate and severe pain	TKR
Intervention (exposure)	Changes in biomarkers over time (baseline – 24 months)	12 months biomarker data
Comparison	Adults with no pain <3	
Outcome	OA progression of WOMAC pain score ≥3 over 4 years of follow-up	
Do mild, moderate, severe WOMAC stiffness scores affect the measurement of biomarkers and predict the likelihood of OA worsening over 4/6 years of follow-up?		
Population	Adults with mild, moderate and severe stiffness (score 1–8)	TKR
Intervention (exposure)	Changes of biomarkers over time (baseline – 24 months)	12 months biomarker data
Comparison	Adults with no stiffness <3	
Outcome	OA progression of WOMAC pain score ≥3 over 4 years of follow-up	

JSN: Joint space narrowing; K & L: Kellgren & Lawrence; OA: Osteoarthritis; OARSI: Osteoarthritis Research Society International; TKR: Total knee replacement; WOMAC: Western Ontario and McMaster Universities Arthritis Index.

biomarkers, including urinary creatinine, for analysis. Some biomarkers were measured in serum and others in urine (Supplementary Table 3). All urine biomarkers are adjusted for creatinine levels. The protocol for collecting and processing the blood samples and selecting and quantifying the biomarkers has been described elsewhere [12]. The biomarker data are collected at the person level. The biomarkers included for analysis were types I and II collagen markers (C2C, C1,2C), C-propeptide of type II collagen (CPII), COMP, N-propeptide of collagen IIA (PIIANP), chondroitin sulfate 846 (CS846), C-terminal cross-linked telopeptide of type I collagen (CTXI), hyaluronic acid (HA), MMP-3, the nitrated form of the α-helical region of type II collagen (Coll2-1 NO2), cross-linked N-telopeptide of type I collagen (NTXI) and C-terminal cross-linked telopeptide of type II collagen (CTXII). The predictors used in the study were the baseline biomarkers and the changes in biomarkers over 24 months.

Definition of outcome variables

The definitions of outcome variables for OA disease progression are described in Supplementary Tables 1 & 2, where the relationships between baseline biomarkers and the progression outcomes are described. Measures of OA progression outcomes included: K & L grade, JSN medial, JSN lateral, osteophytes medial, osteophytes lateral, WOMAC pain score, WOMAC stiffness score, KOOS pain score and KOOS symptoms scores. Outcome variables are measured at yearly intervals for up to 48 months of follow-up. The relationship between the changes in biomarkers in 24 months and disease progression was also examined. Disease progression was defined as an increase of K & L grade/JSN medial and lateral Osteoarthritis Research Society International (OARSI) grade/osteophytes medial and lateral OARSI grade within the follow-up years. Clinical outcomes (WOMAC and KOOS scores) were categorized as mild, moderate and severe. Clinical progression outcomes were considered when participants had progressed into the next category within the follow-up years. Participants that had K & L grade 4, JSN medial and lateral OARSI grade 3, osteophytes medial and lateral OARSI grade 4, and total knee replacements were excluded. Details on the inclusions and exclusion criteria for each outcome measure are described in Table 1.

Table 2. Univariate model. The association of baseline biomarkers and Kellgren & Lawrence grade.

	K & L grade outcome		
	Univariate		
Parameter	OR (95% CI)	p-value	Likelihood-ratio test
Follow-up			
12 months	**3.508585 (1.440229,8.547364)**	**0.0060**	
24 months	**4.450909 (1.787663,11.08184)**	**0.0010**	
36 months	**10.06467 (3.726081,27.18607)**	**0.0000**	
48 months	**11.08278 (4.086839,30.05453)**	**0.0000**	
Biomarkers			
sC1,2C	0.9893783 (0.9543644,1.025677)	0.5610	0.0029
sC2C	1.009436 (0.997998,1.021005)	0.1060	0.2924
sCPII	1.000008 (0.9999899,1.000027)	0.3720	0.9748
sPIIANP	1.0005 (0.9997764,1.001224)	0.1760	0.0019
sColl2-1 NO2	**1.186603 (1.018414,1.382569)**	**0.0280**	1.0000
sCS846	0.9951469 (0.9855555,1.004832)	0.3250	0.5582
sMMP3	0.9967338 (0.9552624,1.040006)	0.8800	1.0000
sCTXI	0.998655 (0.9959298,1.001388)	0.3340	1.0000
sCOMP	0.9859741 (0.9671159,1.0052)	0.1520	0.2786
sHA	1.011633 (0.9916988,1.031967)	0.2550	0.8211
uCTXII	**1.038892 (1.001685,1.077481)**	**0.0400**	0.0068
uC1,2C	1.131627 (0.6895662,1.857079)	0.6250	0.0012
uNTXI	1.003756 (0.9710075,1.03761)	0.8250	1.0000

Likelihood ratio test is to test for the evidence of time interaction with the biomarkers and <0.05 is considered as significant. Statistically significant values are shown in bold.
K & L: Kellgren & Lawrence; OR: Odds ratio.

Statistical analysis

Data cleaning was performed as described in a recent study [14]. The observational data have a clustered structure, with each individual participant having repeated measures of outcomes at 12 monthly intervals over a 48-month total follow-up. To account for outcome measures nested within subjects, generalized estimating equations (GEE) with population average-effects models were used. Logistic GEE regression models are used for binary outcomes and linear GEE regression models for continuous outcomes. First, a simple univariate regression model was used to test the association of a single biomarker with the binary outcome (Table 2). To assess for evidence of interaction between biomarkers and follow-up time on OA progression outcomes, random-effects regression models were fitted, using likelihood-ratio tests for evidence of interactions with time. Where there was no evidence of interaction, a single overall effect size is reported, as the association is consistent over the 48-month follow-up period. Where there is a significant interaction with time, individual effect sizes are reported for each follow-up time point. Multivariable regression models were then fitted, including all 13 biomarkers and confounding variables of age, BMI, gender and race. Receiver operator characteristic (ROC) curves were used to evaluate the baseline biomarkers' predictive ability to predict OA progression in 48 months.

Results

Subject characteristics

A total of 600 subjects with biomarker measurements were identified from the OAI cohort. Fifty-nine percent were women, and more than 55% of the cohort were above 60 years of age. The cohort seemed to lean toward the obesity range, where three-quarters of the participants being overweight. Additionally, more than half of the population had been identified as having K & L grade ≥2, joint space narrowing, osteophytes and OA symptoms at baseline (Table 3). In other words, the cohort selection was subjective and more than half of the population already had established OA.

Association between baseline biomarkers and osteoarthritis progression over 4 years of follow-up

The results of the univariate analysis showed higher values of sColl2-1 NO2 (OR: 1.1866, 95% CI: 1.0184–1.3825) and uCTXII (OR: 1.0389, 95% CI: 1.0016–1.0774) were associated with K & L grade progression (Table 2) by

Table 3. Demographic data for the Osteoarthritis Initiative cohort.

Cohort demographics (n = 600)			
Variables	**Frequency**	**Percentage**	**Cumulative**
Age (years)			
40–50	53	8.83	8.83
50–60	212	35.33	44.17
60–70	198	33	77.17
70–80	137	22.83	100
Gender			
Male	247	41.17	41.17
Female	353	58.83	100
Race			
Other nonwhite	11	1.83	1.83
White or Caucasian	475	79.17	81
Black or African–American	109	18.17	99.17
Asian	5	0.83	100
BMI at baseline			
Normal	60	10	10
Overweight	219	36.5	46.5
Class I obese	212	35.33	81.83
Class II obese	83	13.83	95.67
Class III obese	25	4.17	99.83
Missing	1	0.17	100
K & L grade at baseline			
<2	50	8.33	8.33
>2	550	91.67	100
JSN present at baseline			
No	117	19.5	19.5
Yes	483	80.5	100
Osteophytes present at baseline			
No	39	6.5	6.5
Yes	561	93.5	100
Symptoms presence			
No	294	49	49
Yes	306	51	100

JSN: Joint space narrowing; K & L: Kellgren & Lawrence.

18% and 4%, respectively, per unit increase in biomarker value. There was a significant effect of follow-up time for the whole cohort. The odds of progression to OA were 11-times higher at a 48-month follow-up when compared with baseline (Table 2).

Multivariate analysis was used to assess the relationship between all biomarkers (predictors) and outcomes (OA) adjusted for age, BMI, gender and race. The multivariate analysis showed that BMI (whether obese or not) was associated with a tenfold increased risk of OA when followed up for over 48 months (Table 4). Age was found to be associated with most of the radiographic and clinical variables (JSN medial, osteophytes medial, JSW, WOMAC stiffness, KOOS pain and KOOS symptoms), while race had a negative association with three out of the four clinical variables (Table 4). Nine out of the 13 biomarkers were associated with either one of the radiographic or clinical outcomes. Higher ORs were observed in the multivariate analysis of K & L grade and were found to be significant for sCOMP (OR: 0.9493, 95% CI: 0.9116–0.9886), sColl2-1 NO2 (OR: 1.7217, 95% CI: 1.2254–2.4191) and uCTXII (OR: 1.1497, 95% CI: 1.0296–1.2839). Only sColl2-1 NO2 and uCTXII were associated with a higher OR and increased risk of OA in both univariate and multivariate K & L grade models (Tables 2 & 4).

There was evidence of an interaction between sCOMP and time of K & L grade progression (Supplementary Table 4), and there was a negative association at 12 months (OR: 0.9878, 95% CI: 0.9760–0.9998, p = 0.045),

Table 4. Multivariate analysis of the association between baseline biomarkers and outcomes.

Parameter	K & L grade	JSN medial	JSN lateral	Osteophytes medial	Osteophytes lateral	Joint space width	WOMAC pain	WOMAC stiffness	KOOS pain	KOOS symptoms
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	Coefficient (95% CI)	OR (95% CI)	OR (95% CI)	Coefficient (95% CI)	Coefficient (95% CI)
12 months	5.2455 (1.82829,15.04974)‡	ns	ns	3.85002 (1.49896,9.88860)‡	8.22742 (2.8368,23.86161)†	0 (0,0)†	ns	0.72944 (0.53407,0.99627)‡	ns	ns
24 months	7.0396 (2.38145,20.80916)†	9.52426 (3.12442,29.03304)†	ns	4.37077 (1.68420,11.34285)‡	15.03208 (5.10534,44.26025)†	-0.09711 (-0.15111,-0.04311)†	ns	ns	ns	ns
36 months	15.97259 (4.92149,51.83871)†	17.88754 (5.68319,56.30007)†	ns	12.48064 (4.15219,37.51427)†	12.95126 (4.39155,38.19499)	-0.30303 (-0.35659,-0.24948)†	ns	ns	-1.51319 (-2.49335,-0.53303)‡	ns
48 months	21.78328 (6.47551,73.27791)†	34.03892 (10.61453,109.1568)†	3.50027 (1.12585,10.88234)‡	20.08868 (5.95863,67.72617)†	27.11711 (9.10339,80.7763)†	-0.49011 (-0.54452,-0.4357)†	1.44765 (1.05401,1.9883)‡	ns	-1.78566 (-2.76686,-0.80446)†	ns
Predictors										
sC2C	ns	ns	ns	ns	ns	0.00253 (-0.00588, 0.01095)‡	ns	ns	ns	ns
sCPII	ns	ns	ns	ns	ns	-0.00326 (-0.00576,-0.00075)‡	ns	ns	ns	ns
sPIIANP	1.00198 (1.00046,1.00349)‡	1.00114 (1.00028,1.00201)‡	ns	ns	ns	ns	ns	ns	ns	ns
sColl2-1 NO2	1.72176 (1.22544,2.4191)‡	ns	1.18254 (1.01679,1.37531)‡	ns	ns	ns	ns	ns	ns	ns
sCS846	ns	ns	ns	ns	ns	ns	ns	1.00386 (1.00022,1.00752)‡	ns	ns
sCOMP	0.94934 (0.91164,0.98861)‡	ns	1.00294 (1.00044,1.00545)‡	ns	ns	ns	ns	ns	ns	ns
sHA	ns	ns	ns	ns	ns	0.00345 (-0.00021,0.00711)‡	ns	ns	ns	ns
uCTXII	1.14977 (1.02962,1.28396)‡	1.06082 (1.02255,1.10052)‡	ns	ns	1.05277 (1.01036,1.09696)‡	-0.00462 (-0.00774,-0.00149)†	1.02167 (1.00705,1.03649)‡	ns	-0.0824 (-0.1392,-0.02559)‡	-0.07352 (-0.11974,-0.02729)‡
uC1,2C	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.79708 (0.00973,1.58442)‡
Age	ns	4.13329 (1.85874,9.19124)†	ns	3.06849 (1.40759,6.68919)‡	ns	0.00602 (-0.01899,0.03103)‡	ns	0.75732 (0.58896,0.97382)‡	1.25867 (0.11136,2.40598)‡	1.66678 (0.73323,2.60033)†
Race	ns	ns	ns	ns	ns	ns	1.99612 (1.14707,3.47364)‡	ns	-4.93249 (-7.14995,-2.71503)†	-3.30593 (-5.11022,-1.50164)†
Gender	ns	ns	ns	ns	ns	ns	ns	ns	ns	-1.96853 (-3.84144,-0.09562)‡
BMI	10.60795 (2.77042,40.61787)†	ns	2.96077 (1.3128,6.67743)‡	ns	3.83827 (1.59937,9.21135)‡	ns	1.50664 (1.16799,1.94349)‡	1.62717 (1.29969,2.03717)†	-1.72893 (-2.74115,-0.71672)†	-1.49849 (-2.32212,-0.67485)†

Radiographic outcomes: JSN medial, JSN lateral, osteophytes medial, osteophytes lateral, K & L grade, JSW. Clinical outcomes: WOMAC pain, WOMAC stiffness, KOOS pain, KOOS symptoms.

†‡p-value < 0.001; p-value < 0.05.

JSW: Joint space width; K & L: K & L: Kellgren & Lawrence; KOOS: Knee Injury and Osteoarthritis Outcome Score; ns: Nonsignificant; OR: Odds ratio; sC2C: Serum type 1 and 2 collagen markers; sCPII: Serum C-propeptide of type II collagen; sColl2-1 NO2: Serum nitrated form of α-helical region of type II collagen; sCS846: Serum chondroitin sulfate 846; sCOMP: Serum cartilage oligomeric matrix protein; sPIIANP: Serum N-propeptide of collagen IIA; uCTXII: Urinary C-terminal crosslinked telopeptide of type II collagen (CTXII); WOMAC: Western Ontario and McMaster Universities Arthritis Index.

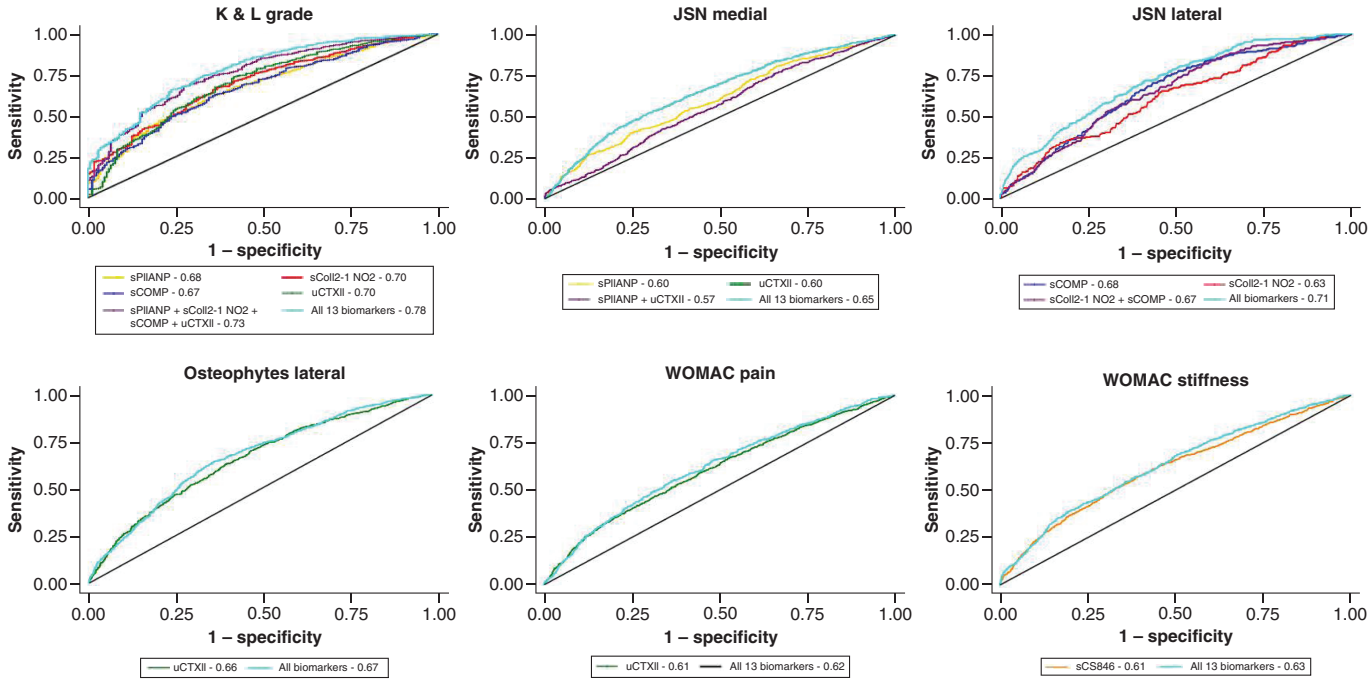

Figure 2. Receiver operating characteristic curves evaluating the predictive ability of baseline biomarkers to predict osteoarthritis at 48 months.
JSN: Joint space narrowing; K & L: Kellgren & Lawrence; WOMAC: Western Ontario and McMaster Universities Arthritis Index.

24 months (OR: 0.9841, 95% CI: 0.9720–0.9964, p = 0.011), 36 months (OR: 0.9839, 95% CI: 0.9711–0.9968, p = 0.015) and 48 months (OR: 0.9828, 95% CI: 0.9707–0.9950, p = 0.006). The radiographic and clinical outcomes showed a very similar pattern of association where uCTXII had the highest ORs and showed significant results for the radiographic and clinical outcome variables (JSN, JSW, osteophytes, WOMAC pain/stiffness score and KOOS pain/symptoms score; Table 4). Serum Coll2-1 NO2 was shown to increase, at least 72% and 18%, the risk of developing OA for K & L grade and JSN on the lateral side, respectively.

ROC curves were used to assess the discriminatory ability of the biomarkers to explain variations in OA progression outcomes. The results showed that sCOMP, sPIIANP, sColl2-1 NO2 and uCTXII had the highest areas under the curve of 0.67, 0.68, 0.70 and 0.70, respectively, in association with K & L grades (Figure 2). When all three biomarkers were combined, the area under the curve increased to 0.73. Furthermore, when all the biomarkers with demographic predictors were included, the area under the curve was 0.78. The trend for other radiographic and clinical outcomes was similar (Figure 2).

Do changes in biomarker values between baseline & 24 months predict osteoarthritis disease progression over 48 months?

A change in sCS846 (OR: 1.0256, 95% CI: 1.0093–1.0423) was significantly associated with JSN medial progression while uNTXI (OR: 0.9310, 95% CI: 0.8837–0.9809) was associated with osteophyte lateral progression (Table 5). A higher OR was observed for sColl2-1 NO2 (OR: 1.1830, 95% CI: 1.0797–1.2961), whereas sHA (OR: 0.98942, 95% CI: 0.98154–0.99738) had a negative association with KOOS symptoms. Serum C2C (OR: 1.00584, 95% CI: 1.00056–1.01117) and PIIANP (0.99966 95% CI: 0.99941–0.99992) were also found to have significant associations with WOMAC stiffness (Table 5).

Discussion

Biomarker studies have been in the limelight for detecting the presence and monitoring the progression of OA for several decades. Many studies, including the authors', have provided evidence that demographic risks [22], radiographic features [23] and symptoms [24] of OA are associated with molecular biomarkers and predict disease progression. A few studies have shown that clinical features of OA, such as pain scores [25] and joint effusion [26], are also predictive of OA progression. However, these studies have used different criteria to recruit subjects and widely

Table 5. Changes in biomarkers and radiographic and clinical outcomes.

Parameter	K & L grade	JSN medial	JSN lateral	Osteophytes medial	Osteophytes lateral	WOMAC pain	WOMAC stiffness	KOOS pain	KOOS symptoms
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
24 months	5.52318 (2.56911,11.87396)†	15.30975 (7.16084,32.73196)†	ns	3.90177 (1.9533,7.7939)†	2.6743 (1.32129,5.41281)‡	2.97424 (1.8052,4.90037)†	1.59461 (1.1543,2.20289)‡	1.9929 (1.31447,3.02149)†	2.22645 (1.31783,3.76156)‡
36 months	21.73236 (9.64758,48.95479)†	46.07624 (20.59993,103.0596)†	18.56927 (1.2614,273.3619)‡	11.54083 (5.53386,24.06831)†	4.6685 (2.28792,9.52607)†	4.7317 (2.68092,8.35122)†	2.66702 (1.86828,3.80723)†	2.8001 (1.78797,4.38519)†	4.00639 (2.16681,7.40774)†
48 months	59.4839 (25.06182,141.1842)†	98.09148 (42.12909,228.3918)†	161.5617 (10.08864,2587.284)†	35.45106 (15.75094,79.79067)†	7.80987 (3.7731,16.16547)†	4.36242 (2.49886,7.61575)†	3.52883 (2.4138,5.15894)†	3.05633 (1.93046,4.83882)†	4.24972 (2.26951,7.95773)†
Predictors									
sC2C	ns	ns	ns	ns	ns	ns	1.00584 (1.00054,1.01117)‡	ns	ns
sPIIANP	ns	ns	ns	ns	ns	ns	0.99966 (0.99941,0.99992)‡	ns	ns
sColl2-1 NO2	ns	ns	ns	ns	ns	ns	ns	ns	1.18301 (1.07977,1.29614)†
sCS846	ns	1.02568 (1.0093,1.04232)‡	ns	ns	ns	ns	ns	ns	ns
sHA	ns	ns	ns	ns	ns	ns	ns	ns	0.98942 (0.98154,0.99738)‡
uNTXI	ns	ns	ns	ns	0.93108 (0.88376,0.98094)‡	ns	ns	ns	ns
Age	ns	1.97216 (1.20357,3.23155)‡	ns	ns	0.52498 (0.29784,0.92534)‡	ns	ns	ns	1.36418 (1.01627,1.83119)‡
Race	ns	ns	ns	ns	5.84592 (1.92711,17.73372)‡	ns	ns	ns	ns
Gender	ns	0.37107 (0.14782,0.93148)‡	ns	ns	3.40809 (1.17214,9.90927)‡	ns	ns	ns	ns
BMI	ns	ns	ns	ns	ns	ns	ns	ns	ns

Radiographic outcomes: JSN medial, JSN lateral, osteophytes medial, osteophytes lateral, K & L grade, JSW. Clinical outcomes: WOMAC pain, WOMAC stiffness, KOOS pain, KOOS symptoms.
†‡p-value <0.001; p-value <0.05.
JSN: Joint space narrowing; JSW: Joint space width; K & L: K & L: Kellgren & Lawrence; KOOS: Knee Injury and Osteoarthritis Outcome Score; ns: Nonsignificant; OR: Odds ratio; sC2C: Serum type 1 and 2 collagen markers; sPIIANP: Serum N-propeptide of collagen IIA; sColl2-1 NO2: Serum nitrated form of α-helical region of type II collagen; sCS846: Serum chondroitin sulfate 846; sHA: Serum hyaluronic acid; uNTXI: Urinary NTXI: cross-linked N-telopeptide of type I collagen; WOMAC: Western Ontario and McMaster Universities Arthritis Index.

differing definitions of OA progression, resulting in conflicting or inconclusive data. In the current study, data from one of the largest and most highly characterized OA cohorts were used to investigate the prognostic values of biomarkers at baseline and change in biomarkers over 24 months, on a wide range of clinical and radiographic measures of OA disease progression. This study is a comprehensive investigation of both the short- and long-term prognostic value of baseline and change in biochemical markers and clinical, demographic and radiographic variables in OA. The unique study design and the use of complex statistical methodology have led to a number of new and important observations.

The results showed that the risk of OA development increases every year, and demographic factors (age and BMI) play an important role in OA progression. In our previous cross-sectional study, uCTXII was identified as a clinically useful biomarker for OA [14]. In this study of OA disease progression, the data showed that baseline uCTXII was associated with disease progression for all the clinical (KOOS and WOMAC) and several radiographic outcomes (K & L grades, JSW, JSN medial and osteophytes lateral) during the 48 months of follow-up, which reflect cartilage degradation in OA progression, and is a potential clinically relevant prognostic marker. Furthermore, other baseline biomarkers in this study (sColl2-1 NO2, sPIIANP and sCOMP) were also associated with at least one of the radiographic outcomes, which suggests that the OA progression could be phasic and may not be reflected by a single biomarker. The changes in biomarkers over 24 months were also investigated in association with radiographic or clinical outcomes. Changes in several biomarkers (sC2C, sPIIANP, sColl2-1 NO2 and sHA) predicted OA disease progression and were elevated early in the disease course, which also supports the contention that OA is cyclic and phasic [27].

Urinary CTXII had been studied extensively in both humans and animals [27–30], and reports indicate that CTXII is associated with radiographic severity of OA [30,31]. Therefore, the current findings are consistent with other studies and provide new evidence that CTXII level reflects articular cartilage and calcified cartilage turnover. Urinary CTXII data had significant associations with most of the outcome measures in this study and, overall, the data suggest that uCTXII is a predictor of structural damage, worsening radiographic changes and clinical symptoms, pain and stiffness. Thus, to date, urinary CTXII appears to be the most effective cartilage degradation marker that fits into the Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic (BIPED) criteria [32]. These observations are in general agreement with several recent studies that used data from the OAI cohort to investigate uCTXII as a surrogate marker of OA [15]. However, there are some fundamental differences in the current study design and approach to data analysis. A wide range of individual radiographic and clinical OA disease progression outcomes were investigated. The analyses explored both baseline biomarkers and changes in biomarker values as predictors of disease progression. The methods of statistical analysis, using GEE to account for repeated measures of outcome over time, ensure appropriate accounting for the clustered nature of the data in estimating standard errors and effects sizes, but also minimize missing data in outcomes, by including patients with at least one follow-up measure. For example, in the study by Kraus *et al.*, disease progression was defined as radiographic and pain increase, radiographic but no pain and pain but no radiographic progression [15,33]. Instead, the current study investigated at a wide range of individual measures of OA disease progression, rather than a composite measure of outcome combining clinical and radiographic data, as described in Table 2 & Supplementary Table 1. Several other studies using the OAI cohort demonstrated that structural damage leads to a gradual onset of accelerated knee OA. In the study by Driban *et al.*, OA patients who were more than 65 years of age, had a BMI of >35 and had a K & L grade ≥ 2 were reported to develop accelerated knee OA over 4 years [34]. This is consistent with the current data, in that BMI and aging play a significant role in OA progression.

In this study, uCTXII was associated with the radiographic outcomes K & L grade (OR: 1.15), JSN medial (OR: 1.06) and osteophytes lateral (OR: 1.05). The presence of osteophytes and JSN is generally accepted as an indication of the severity of radiographic OA. In another study using the OAI cohort, Wang *et al.* demonstrated that age, BMI, K & L grade and JSN are significant contributors to OA severity [35]. This study was focused on the evaluation of risk factors associated with the disease progression. Another study looking at disease progression over 48 months in the same cohort reported that age, glucose concentrations, BMI and static alignment are risk factors for developing accelerated OA [36]. Finally, an MRI study by Dam *et al.* showed that elevated baseline CTXII predicted cartilage loss over 21 months and suggested that CTXII may be used to identify patients at high risk of progression. However, in the study, the association of elevated baseline uCTXII with radiographic progression (K & L or JSN) was not statistically significant [30]. The current data show baseline uCTXII had a significant association with K & L grade, JSN in the medial site, osteophyte lateral, JSW and KOOS and WOMAC scores. The main

reason for the discrepancy in the results may be a short follow-up in the Dam study compared with the current work.

The biomarker changes (sC2C, sPIIANP, sColl2-1 NO2, sHA, uNTXI) over 4 years were only associated with either one clinical or one radiographic outcome. A change in uCTXII over 4 years was not associated with radiographic or clinical outcomes. This is a surprising finding since many studies, including our own, have consistently demonstrated links between this biomarker at baseline and disease progression according to both radiographic and clinical outcomes. A recent study also showed that elevated uCTXII predicted a higher risk of total joint replacement [37]. There may be several possible explanations for this result. First, the nature of disease progression in OA is such that the disease usually develops slowly over many years and symptoms appear in later years. Second, 50% of the OAI cohort used in this study had a K & L grade of >2 and had osteophytes. Therefore, the cohort is not the best cohort to demonstrate whether a change in biomarkers early in the disease course predicts progression. In K & L grade ≥2 patients, the cartilage may have already been damaged, and the measurement of the biomarker may remain the same or slightly increase each year. These findings supported our previous study showing that the levels of sPIIANP and uCTXII are higher in knee OA progression [27]. Serum PIIANP increased rapidly while uCTXII remained high and stable during a 5-year follow-up, suggesting that type II collagen degradation is active throughout disease progression [27]. The present results also showed that both sPIIANP and uCTXII (regardless of baseline and changes in biomarkers) are associated with symptomatic and radiographic progression, indicating that there is an imbalance in tissue homeostasis and these biomarkers remain active in OA progression. This explains the unique pathophysiological mechanism in OA development and suggests that OA may have a phasic disease activity. The data also suggested the potential to identify patients from early progressors and monitor OA using uCTXII, sColl2-1 NO2 and sPIIANP. Previous studies indicate that patients with knee OA are characterized by an uncoupling of type II collagen synthesis and degradation, which can be detected by assays for type II collagen-derived biomarkers such as sPIIANP and uCTXII [38]. The present data suggest that type II collagen-derived cartilage degradation markers may not predict short-term progression, possibly because the metabolism of cartilage is very slow [39]. Cartilage degradations start with collagenases and aggrecanases, leading to collagen type II fragments being released into serum or urine. The pathway occurs slowly, and the metabolites released into the bloodstream or urine can potentially be used to detect asymptomatic OA. Still, for this to happen, a more sophisticated monitoring system and outcome measures are needed.

Limitations of the study

First, the OAI cohort used in this study is heavily selected such that almost 50% of the subjects had established OA, 47% were over 60 years old and 75% were overweight. These are important risk factors for OA, therefore, this is a high-risk cohort for OA progression by study design. Consequently, selection bias and effects on the generalizability of the findings cannot be ruled out. Second, some of the biomarkers were excluded due to collinearity (e.g., sNTXI, uC2C, uCTXIα, uCTXIβ and uColl2-1 NO2) to account for missing data in the analysis, which may lead to bias and loss of precision. However, the results clearly showed that most of the type II collagen cleavage markers were associated with radiographic damage and clinical symptoms reflecting the clinical presentation in OA patients and, therefore, these biomarkers will be useful in the investigation of the disease pathophysiology. In this study, the biomarkers do not distinguish between right or left knees, and the worst affected knee was chosen to analyze the relationship between biomarkers and the progression outcomes, therefore, we were unable to account for other joint sites. The results should therefore be interpreted with caution, and further research in other joint sites is needed to support the findings of this study. However, most OA studies are focused on the knees, which are commonly affected by the disease. Lastly, the OAI dataset had a large number of missing data beyond year 5 of follow-up. Therefore, we could not assess the relationship between biomarkers and disease progression for a longer period. OA is degenerative and considered part of the aging process, so, it requires a longer period of observation to understand its pathophysiology and disease progression. It is also worth noting that JSN as an outcome measure may not be best, as it often requires longer follow-up times before it can be detected.

Conclusion

In line with previous studies, age and BMI were found to play an essential role in OA progression in the current study. Seven out of 13 biomarkers (sC2C, sCPII, sPIIANP, sColl2-1 NO2, sCOMP, sHA and uCTXII) at baseline were associated with radiographic progression while three biomarkers (sCS846, uCTXII and uC1,2C) were able to predict symptomatic OA. Four out of 13 biomarkers (changes in biomarkers over 24 months) appear to be

linked to the early course of OA and of these four biomarkers, sPIIANP and sColl2-1 NO2 were found to be statistically significant and correlated with symptoms, but not with structural damage (radiographic feature). These observations support the phasic nature of OA. Finally, uCTXII appears to be a predictor of structural damage as well as clinical symptoms and, therefore, may be the best current biomarker for the investigation of OA.

Summary points

- Baseline prognostic markers are informative for identifying patients at risk of osteoarthritis (OA) progression.
- A higher odds ratio for urinary CTXII is associated with an increased risk of disease progression.
- Urinary CTXII is a clinically useful prognostic marker.
- OA may be a phasic disease as changes in some biomarkers (serum PIIANP and serum Coll2-1 NO2) were found in early symptomatic OA while others (urinary CTXII) were more strongly associated with radiographic OA.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/bmm-2021-0579

Author contributions

The study design was prepared by M Sharif and A Judge. The main manuscript text was written by M Sharif, Y Liem, Y Li and A Judge. The data was prepared by Y Liem and Y Li. Y Liem analyzed the data and prepared all tables and figures. All authors read and approved the final version of the manuscript.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The OAI study was approved by the institutional review board at each clinical site, the NIH, OAI investigators and private funding partners. All participants gave consent for the observational study.

Data sharing statement

All analyzed data generated for this study are included in this article.

References

Papers of special note have been highlighted as: •• of considerable interest

1. Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. *Lancet* 396(10264), 1711–1712 (2020).
2. National Health Service Overview Arthritis. www.nhs.uk/conditions/arthritis/
3. Chen A, Gupte C, Akhtar K, Smith P, Cobb J. The global economic cost of osteoarthritis: how the UK compares. *Arthritis* 2012, 698709 (2012).
4. Hallo De Wolf A, Toebes B. Assessing private sector involvement in health care and universal health coverage in light of the right to health. *Health Hum. Rights* 18(2), 79–92 (2016).

5. Xia B, Di C, Zhang J, Hu S, Jin H, Tong P. Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif. Tissue Int.* 95(6), 495–505 (2014).
6. Attur M, Krasnokutsky-Samuels S, Samuels J, Abramson SB. Prognostic biomarkers in osteoarthritis. *Curr. Opin. Rheumatol.* 25(1), 136–144 (2013).
7. Braun HJ, Gold GE. Diagnosis of osteoarthritis: imaging. *Bone* 51(2), 278–288 (2012).
8. Betancourt M, Linden J, Rivadeneira F *et al.* Dual energy x-ray absorptiometry analysis contributes to the prediction of hip osteoarthritis progression. *Arthritis Res. Ther.* 11(6), R162 (2009).
9. Henrotin Y. Osteoarthritis year 2011 in review: biochemical markers of osteoarthritis: an overview of research and initiatives. *Osteoarthritis Cartilage* 20(3), 215–217 (2012).
10. Hunter DJ, Nevitt M, Losina E, Kraus V. Biomarkers for osteoarthritis: current position and steps towards further validation. *Best Pract. Res. Clin. Rheumatol.* 28(1), 61–71 (2014).

•• **A comprehensive review discussing the challenges in osteoarthritis biomarkers research and the need to investigate biomarkers for validation and qualifying for osteoarthritis clinical trials.**

11. Skoumal M, Haberhauer G, Feyertag J, Kittl EM, Bauer K, Dunky A. Serum levels of cartilage oligomeric matrix protein (COMP): a rapid decrease in patients with active rheumatoid arthritis undergoing intravenous steroid treatment. *Rheumatol. Int.* 26(11), 1001–1004 (2006).
12. Wisłowska M, Jabłońska B. Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis and knee osteoarthritis. *Clin. Rheumatol.* 24(3), 278–284 (2005).
13. The Osteoarthritis Initiative. https://nda.nih.gov/oai/
14. Liem Y, Judge A, Kirwan J, Ourradi K, Li Y, Sharif M. Multivariable logistic and linear regression models for identification of clinically useful biomarkers for osteoarthritis. *Sci. Rep.* 10(1), 11328 (2020).

•• **Identified four biomarkers that may have diagnostic value.**

15. Kraus VB, Collins JE, Hargrove D *et al.* Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers Consortium. *Ann. Rheum. Dis.* 76(1), 186–195 (2017).

•• **Demonstrates that a panel of biomarkers are promising candidates for clinical trial usage and for predicting disease outcomes.**

16. Runhaar J, Sanchez C, Taralla S, Henrotin Y, Bierma-Zeinstra SM. Fibulin-3 fragments are prognostic biomarkers of osteoarthritis incidence in overweight and obese women. *Osteoarthritis Cartilage* 24(4), 672–678 (2016).
17. Smith JW, Martins TB, Gopez E, Johnson T, Hill HR, Rosenberg TD. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther. Adv. Musculoskelet. Dis.* 4(5), 315–325 (2012).
18. Roos EM, Lohmander LS. *Health Qual. Life Outcomes* 1(1), 64 (2003).
19. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J. Rheumatol.* 15(12), 1833–1840 (1988).
20. Kohn MD, Sassoon AA, Fernando ND. Classifications in brief: Kellgren-Lawrence classification of osteoarthritis. *Clin. Orthop. Relat. Res.* 474(8), 1886–1893 (2016).
21. Dam EB, Byrjalsen I, Karsdal MA, Qvist P, Christiansen C. Increased urinary excretion of C-telopeptides of type II collagen (CTX-II) predicts cartilage loss over 21 months by MRI. *Osteoarthritis Cartilage* 17(3), 384–389 (2009).
22. Mazzuca SA, Brandt KD, Katz BP, Ding Y, Lane KA, Buckwalter KA. Risk factors for early radiographic changes of tibiofemoral osteoarthritis. *Ann. Rheumatic Dis.* 66(3), 394–399 (2007).
23. Golightly YM, Marshall SW, Kraus VB *et al.* Biomarkers of incident radiographic knee osteoarthritis: do they vary by chronic knee symptoms? *Arthritis Rheumatism* 63(8), 2276–2283 (2011).
24. Haraden CA, Huebner JL, Hsueh M-F, Li Y-J, Kraus VB. Synovial fluid biomarkers associated with osteoarthritis severity reflect macrophage and neutrophil related inflammation. *Arthritis Res. Ther.* 21(1), 146 (2019).
25. Sofat N, Ejindu V, Heron C *et al.* Biomarkers in painful symptomatic knee OA demonstrate that MRI assessed joint damage and Type II collagen degradation products are linked to disease progression. *Front. Neurosci.* 13, 1016 (2019).
26. Wang Y, Teichtahl AJ, Pelletier J-P *et al.* Knee effusion volume assessed by magnetic resonance imaging and progression of knee osteoarthritis: data from the Osteoarthritis Initiative. *Rheumatology* 58(2), 246–253 (2019).
27. Sharif M, Kirwan J, Charni N, Sandell LJ, Whittles C, Garnero P. A 5-yr longitudinal study of type IIA collagen synthesis and total type II collagen degradation in patients with knee osteoarthritis–association with disease progression. *Rheumatology* 46(6), 938–943 (2007).

•• **Demonstrate that sPIIANP and uCTXII predict disease outcome over 5 years but act differently in different stages of osteoarthritis.**

28. Arunrukthavon P, Heebthamai D, Benchasiriluck P, Chaluay S, Chotanaphuti T, Khuangsirikul S. Can urinary CTX-II be a biomarker for knee osteoarthritis? *Arthroplasty* 2(1), 6 (2020).

29. Chmielewski TL, Trumble TN, Joseph AM *et al.* Urinary CTX-II concentrations are elevated and associated with knee pain and function in subjects with ACL reconstruction. *Osteoarthritis Cartilage* 20(11), 1294–1301 (2012).
30. Dam EB, Byrjalsen I, Karsdal MA, Qvist P, Christiansen C. Increased urinary excretion of C-telopeptides of type II collagen (CTX-II) predicts cartilage loss over 21 months by MRI. *Osteoarthritis Cartilage* 17(3), 384–389 (2009).
31. Sowers MF, Karvonen-Gutierrez CA, Yosef M *et al.* Longitudinal changes of serum COMP and urinary CTX-II predict x-ray defined knee osteoarthritis severity and stiffness in women. *Osteoarthritis Cartilage* 17(12), 1609–1614 (2009).
32. Lotz M, Martel-Pelletier J, Christiansen C *et al.* Republished: value of biomarkers in osteoarthritis: current status and perspectives. *Postgrad. Med. J.* 90(1061), 171–178 (2014).
33. Hunter DJ, Deveza LA, Collins JE *et al.* Multivariable modeling of biomarker data from the phase 1 Foundation for the NIH Osteoarthritis Biomarkers Consortium. *Arthritis Care Res.* doi:10.1002/acr.24557 (2021) (Epub ahead of print).
34. Driban JB, Eaton CB, Lo GH *et al.* Overweight older adults, particularly after an injury, are at high risk for accelerated knee osteoarthritis: data from the Osteoarthritis Initiative. *Clin. Rheumatol.* 35(4), 1071–1076 (2016).
35. Wang Y, You L, Chyr J *et al.* Causal discovery in radiographic markers of knee osteoarthritis and prediction for knee osteoarthritis severity with attention – long short-term memory. *Front. Public Health* 8, 604654 (2020).
36. Driban JB, Mcalindon TE, Amin M *et al.* Risk factors can classify individuals who develop accelerated knee osteoarthritis: data from the osteoarthritis initiative. *J. Orthopaedic Res.* doi:10.1002/jor.23675 (2017) (Epub ahead of print).
37. Bihlet AR, Bjerre-Bastos JJ, Andersen JR, Byrjalsen I, Karsdal MA, Bay-Jensen AC. Clinical and biochemical factors associated with risk of total joint replacement and radiographic progression in osteoarthritis: data from two phase III clinical trials. *Semin. Arthritis Rheum.* 50(6), 1374–1381 (2020).
38. Garnero P, Ayral X, Rousseau J-C *et al.* Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. *Arthritis Rheumatism* 46(10), 2613–2624 (2002).
39. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. *Sports Health* 1(6), 461–468 (2009).