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The ability of systemic biochemical markers to reflect presence, incidence, and progression of early-stage radiographic knee and hip osteoarthritis: data from CHECK

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SUMMARY

Objective: To relate systemic biochemical markers of joint metabolism to presence, incidence, and progression of early-stage radiographic knee and/or hip osteoarthritis (OA).
Method: The cartilage markers uCTX-II, sCOMP, sPIIANP, and sCS846, bone markers uCTX-I, uNTX-I, sPINP, and sOC, and synovial markers sHA and sPIIINP were assessed by enzyme-linked immunosorbent assay or radioactive immunoassay in baseline samples of CHECK (Cohort Hip and Cohort Knee), a cohort study of early-stage symptomatic knee and/or hip OA. Knee and hip radiographs were obtained at baseline and 5-year follow-up. Presence of OA at baseline was defined as Kellgren and Lawrence (K&L) = 1 (maximum observed). Incidence of OA was defined as K&L = 0 at baseline and K&L $\geq$ 1 at 5-year follow-up. Progression of OA was defined as K&L = 1 at baseline and K&L $\geq$ 2 at 5-year follow-up.
Results: Data were available for 801 subjects at baseline and for 723 subjects at both baseline and 5-year follow-up. Multiple cartilage and synovial markers showed positive associations with presence and progression of knee and hip OA and with incidence of hip OA, except for negative associations of uCTX-II and sCOMP with incidence of knee OA. uCTX-II and sCOMP showed multiple interactions with other biomarkers in their associations with knee and hip OA. Bone markers were positively associated with presence of radiographic knee OA, but negatively associated with progression of radiographic hip OA.
Conclusion: Especially metabolism in cartilage and synovial matrix appear to be of relevance in knee and hip OA. The role of bone metabolism appears to differ between knee and hip OA.
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Introduction

Biochemical markers (biomarkers) of joint metabolism have been proposed as tools that could help along the challenging road to efficacious diagnosis and treatment of OA[1]. Biomarkers are mostly tested in only small study populations and in isolation rather than in combination[2]. Moreover, many studies focus on subjects with advanced OA[2], while it are actually the early-stage disease subjects that would need to take advantage of any future biomarkers the most. They are the ones that are most likely to benefit from disease-modifying drugs and sensitive outcome measures for clinical trials in such early-stage subjects are eagerly awaited.

In the current study, ten systemic biomarkers of joint metabolism were simultaneously assessed in subjects with symptomatic knee and/or hip OA with no or minimum radiographic OA signs from CHECK (Cohort Hip and Cohort Knee). Earlier publications on these biomarkers in CHECK have demonstrated their associations

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with demographic variables[3,4], their mutual associations[3,5], and their associations with radiographic knee parameters and pain[6]. This time, we investigated to what extent these biomarkers reflected concurrent radiographic knee and hip OA and related to future incidence as well as progression of knee and hip OA during 5-year follow-up. With that, we tried to elucidate aspects of the pathogenesis of OA.

Method

Cohort characteristics

CHECK is a longitudinal cohort study of 1002 subjects, age 45–65 years at the time of inclusion, with pain and/or stiffness of one or both knee(s) and/or hip(s)[7]. They had never or not longer than 6 months ago consulted a physician for these symptoms for the first time. Subjects with any other pathological condition that could explain the symptoms (e.g., other rheumatic diseases, previous joint replacement) were excluded. Subjects needed to be sufficiently ambulatory to attend all follow-up visits.

At baseline, CHECK subjects (79.0% female) were age 56 ± 5 years (mean ± SD) and had a median (25–75% percentiles) BMI of 25.5 (23.3–28.4) kg/m^2. Median (25–75% percentiles) WOMAC scores, ranging between 0 and 100 and higher scores representing more complaints, were 25 (10–35) for pain, 38 (25–50) for stiffness, and 21 (10–35) for physical function.

Chronic liver disease was reported by three subjects (0.3%) and chronic renal disease by one subject (0.1%). Excluding these subjects did not change results essentially. Bisphosphonate use was not registered systematically. However, since osteoporosis was reported by only four subjects (0.04%), the use of these agents was presumably low.

Biochemical markers

Biomarker levels were assessed in baseline serum and second morning void urine samples from CHECK, collected once, in a non-fasted state, between 8 and 12 AM. Biomarker levels were assessed by enzyme-linked immunosorbent assay or radioactive immunoassay, as was described in more detail previously[3]. The large-scale assessment was performed over a number of days. Multiple quality controls were included and were unremarkable. Intra-plate, inter-plate, and between-day coefficients of variation (standard deviation/mean*100%) and median (interquartile range) biomarker levels were as follows: C-terminal telopeptide of collagen type II (CTX-II; Urine CartiLaps EIA, Immunodiagnostic systems Ltd., Boldon, UK): 10.0%, 9.3%, and 12.4%; 193 (132–281) ng/mmol. Cartilage oligomeric matrix protein (COMP; AnaMar Med AB, Göteborg, Sweden): 5.0%, 4.0%, and 4.2%; 8.5 (7.2–9.9) U/l. N-terminal propeptide of procollagen type IIA (PIIANP; Millipore Corp, Billerica, MA, US): 15.8%, 7.0%, and 15.7%; 1385 (1087–1771) ng/ml. Chondroitin sulphate 846 (CS846; IBEX, Montreal, Canada): 21.5%, 16.9%, and 15.3%; 70 (54–88) ng/ml. C-terminal telopeptide of collagen type I (CTX-I, Urine CrossLaps EIA, Immunodiagnostic systems Ltd., Boldon, UK): 9.7%, 6.1%, and 2.7%; 152 (100–225) μg/mmol. N-terminal telopeptide of collagen type I (NTX-I, OSTEOMARK NTx Urine, Wampole Laboratories, Princeton, US): 14.9%, 6.6%, and 10.7%; 37 (28–51) nM BCE/mmol. N-terminal propeptide of procollagen type I (PINP, UniQ, Orion Diagnostica, Espoo, Finland): 4.4%, 4.5%, and 6.2%; 42 (32–56) ng/ml. Osteocalcin (OC, N-MID Osteocalcin ELISA, Immunodiagnostic systems Ltd., Boldon, UK): 3.4%, 4.1%, and 4.3%; 13 (10–17) ng/ml. Hyaluronic acid (HA; Corgenix Inc, Westminster, CO, US): 15.1%, 13.0%, and 17.3%; 27 (17–43) ng/ml. N-terminal propeptide of procollagen type III (PIIINP; UniQ, Orion Diagnostica, Espoo, Finland): 5.4%, 3.2%, and 7.2%; 4.1 (3.5–4.9) ng/ml. Urinary biomarker levels were adjusted for urinary creatinine concentrations (automated kinetic assay, UniCel® DxC 800 Synchron® Clinical System, Beckman Coulter).

Radiographic data acquisition

Knee and hip radiography were performed at baseline and 5-year follow-up. Knee radiographs were made in a weight-bearing posteroanterior view, semiflexed. For the hip, weight-bearing anteroposterior radiographs of the pelvis were made with hips in 15° internal rotation. Radiographs were scored by five trained observers according to Kellgren & Lawrence (K&L)[8], in a paired fashion, with known sequence. A random subset of radiographs of 38 subjects was read by all observers, independently of each other, yielding moderate to substantial inter-observer agreement (Cohen's kappa = 0.60 for presence of K&L ≥ 2 in the knees at 5-year follow-up).

Definitions

Knee and/or hip pain were classified as either present or absent according to the history of the patient that was obtained by an experienced rheumatologist.

In each subject one index knee and one index hip were defined at baseline. When only one of both joints was painful that joint was considered the index joint. When both or none of the joints were painful the index joint was randomly selected from both.

Biomarkers were consecutively tested for associations with presence, incidence, and progression of radiographic OA of the index joint. Presence of radiographic OA of the index joint at baseline was defined as K&L = 1 (maximum observed, vs index joints that scored K&L = 0 at baseline). Incident radiographic OA of the index joint was defined as a K&L = 0 at baseline and K&L ≥ 1 at 5-year follow-up for the index joint (vs index joints scoring K&L = 0 at both baseline and 5-year follow-up). Progression of radiographic OA of the index joint was defined as K&L = 1 at baseline and a K&L grade ≥2 in the index joint at 5-year follow-up (vs index joints showing no increase at 5-year follow-up). These definitions were considered most appropriate in these early-stage OA subjects.

Statistical analysis

Cross-sectional associations between biomarkers (independent variables) and presence of radiographic OA in the index joint (dependent variable) were investigated by binary logistic regression, first adjusted for concurrent radiographic OA in the contralateral joint (either present or absent) and in hips or knees (either absent, unilateral, or bilateral) only, and in a next step also adjusted for age, gender, and BMI.

Associations of baseline biomarkers (independent variables) with incidence of radiographic OA or progression of radiographic OA in the index joint during follow-up (dependent variable) were first performed with adjustment for baseline radiographic OA in the index joint and its contralateral joint (present or absent) and in hips or knees (either absent, unilateral, or bilateral) as well as for radiographic OA changes during follow-up in the contralateral joint and hips or knees (occurrence of OA incidence or progression: yes or no). In a next step they were also adjusted for age, gender, and BMI.

To facilitate comparison between biomarkers and between analyses, biomarkers were logarithmically transformed and standardized as z-scores. Z-scores reflect how many standard deviations (SD) raw scores deviate from the population mean. As such, presented odds ratios (OR) indicate the change (ratio) of odds

per SD increase of the biomarker level. They are therefore independent of the units of measurement.

Note that not all index joints were painful, since CHECK subjects could have been included based on pain restricted to either knee(s) or hip(s). Therefore, interaction between biomarkers and presence of pain in the index joint could be investigated for all associations between biomarkers and radiographic OA. Statistically significant interaction between a biomarker and presence of pain indicates that the association of that biomarker with radiographic OA differs between painful and non-painful joints. Therefore, whenever interaction between a biomarker and presence of pain was statistically significant, the association of that biomarker with radiographic OA of the index joint was determined for painful and non-painful index joints separately.

All statistical analyses were performed using SPSS, version 15.0. Statistical significance was defined as P values <0.05, except for interaction that was considered statistically significant at P values <0.100.

Results

Available data

Of the 1002 CHECK subjects, biomarker data were available for 960 subjects. For those subjects, radiographic data were complete for 801 subjects at baseline and for 723 subjects at both baseline and 5-year follow-up, see Fig. 1. Subjects undergoing joint replacement surgery during follow-up were defined as missing cases at 5-year follow-up, since we were primarily interested in structural changes and the decision to undergo surgery is generally based on many factors that are not related to structural OA signs only[9]. At 5-year follow-up, 9 of the in total 1732 knees (0.5%) and 38 of the in total 1738 hips (2.2%) for which K&L grades were available at baseline had been replaced.

Cross-sectional associations between biomarkers and presence of OA

Presence of radiographic OA in the index knee appeared to be positively associated with markers of cartilage and synovial metabolism (uCTX-II OR/SD = 1.304, $P = 0.002$; sPIIANP OR/SD = 1.339, $P = 0.001$; sCS846 OR/SD = 1.253, $P = 0.007$; sHA OR/SD = 1.248, $P = 0.014$; OR/SD, odds ratio per standard deviation). Bone markers also showed statistically significant positive associations, mostly after adjustment for demographic variables and in painful knees only (uCTX-I OR/SD = 1.232, $P = 0.022$; sPINP OR/SD = 1.219, $P = 0.040$ for painful knees; sOC OR/SD = 1.281, $P = 0.016$ for painful knees) (Table I, top panel).

Presence of radiographic OA in the index hip was associated with markers of cartilage and synovial metabolism, especially with sHA (uCTX-II OR/SD = 1.262, $P = 0.021$; sCOMP OR/SD = 1.280, $P = 0.008$; sHA OR/SD = 1.437, $P < 0.001$) (Table II, top panel).

Associations of baseline biomarkers with incident OA

uCTX-II and sCOMP showed statistically significant, but unexpectedly negative associations with incidence of radiographic knee OA in subjects without radiographic OA of the index knee at

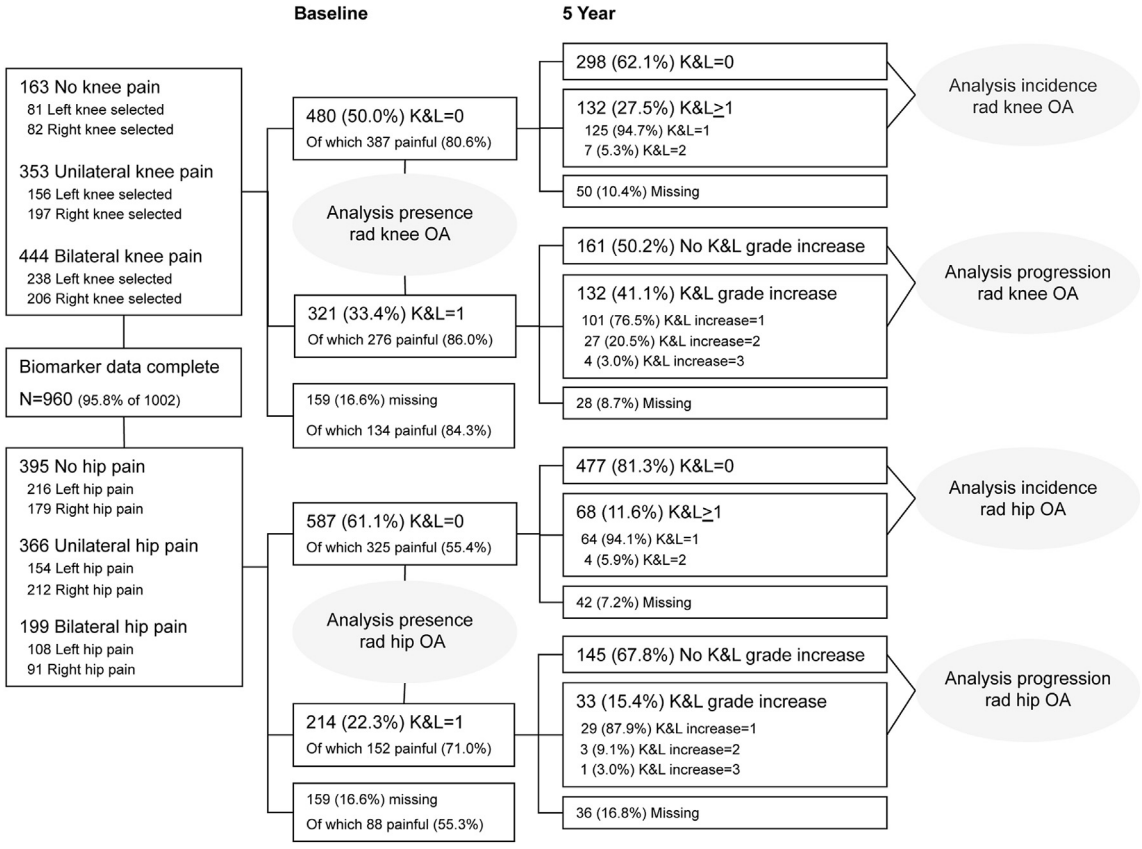

Fig. 1. Flow diagram of Kellgren and Lawrence grades of index knees and hips between baseline and 5-year follow-up. Only subjects with radiographic data available for the index joint and all three other joints at all relevant time points could be included. Subpopulations that were compared in each of the analyses are indicated. In CHECK as a whole, 41% of the subjects had knee pain only, 17% of the subjects had hip pain only, 42% of the subjects had pain of both knees and hips. K&L, Kellgren and Lawrence grade; rad, radiographic.

Table I
Associations between systemic biomarker levels and presence (top panel), incidence (middle panel), and progression (lower panel) of radiographic knee OA. Adjustment for covariates was performed stepwise. "Concurrent OA" includes OA in the contralateral joint (either present or absent) and in hips or knees (either absent, unilateral, or bilateral). "Demographics" includes age, gender, and BMI. Associations are displayed for painful and non-painful index knees separately when interaction between a biomarker and presence of pain was statistically significant. Statistically significant values are depicted in bold ($P < 0.05$, except $P < 0.100$ for interaction terms in the most right column). Numbers of subjects per (sub)group are shown in Fig. 1. OR/SD, odds ratio per standard deviation increase; 95% CI, 95% confidence interval; *P* significance

Knee OA presence	Adjusted for concurrent OA			Adjusted for demographics			Interaction with pain
	OR/SD	95% CI	*P*	OR/SD	95% CI	*P*	*P*
uCTX-II	1.304	1.105–1.538	**0.002**	1.264	1.058–1.508	**0.010**	0.765
sCOMP	1.108	0.940–1.305	0.220	0.997	0.838–1.187	0.973	**0.093**
- No pain	0.834	0.565–1.233	0.363	0.752	0.485–1.167	0.204	
- Pain	1.164	0.971–1.397	0.101	1.050	0.866–1.273	0.620	
sPIIANP	1.339	1.131–1.584	**0.001**	1.300	1.093–1.546	**0.003**	0.219
sCS846	1.253	1.065–1.475	**0.007**	1.273	1.076–1.506	**0.005**	0.819
uCTX-I	1.170	0.994–1.378	0.060	1.232	1.030–1.474	**0.022**	0.535
uNTX-I	1.063	0.904–1.249	0.462	1.080	0.904–1.289	0.395	0.720
sPINP	1.104	0.938–1.298	0.234	1.120	0.944–1.329	0.196	**0.024**
- No pain	0.713	0.468–1.087	0.116	0.676	0.429–1.063	0.090	
- Pain	1.192	0.997–1.425	0.054	1.219	1.009–1.473	**0.040**	
sOC	1.141	0.962–1.352	0.130	1.170	0.975–1.403	0.091	0.033
- No pain	0.733	0.467–1.149	0.176	0.705	0.429–1.157	0.166	
- Pain	1.239	1.026–1.498	**0.026**	1.281	1.046–1.568	**0.016**	
sHA	1.248	1.046–1.490	**0.014**	1.156	0.951–1.405	0.145	0.209
sPIIINP	1.093	0.924–1.293	0.300	1.009	0.847–1.203	0.916	0.263
Incidence							
uCTX-II	0.797	0.631–1.007	0.057	0.765	0.590–0.994	0.045	0.510
sCOMP	0.764	0.596–0.979	**0.033**	0.725	0.558–0.941	0.016	0.284
sPIIANP	1.004	0.787–1.282	0.972	0.996	0.777–1.276	0.972	0.964
sCS846	1.070	0.831–1.378	0.600	1.055	0.815–1.366	0.686	0.923
uCTX-I	1.150	0.904–1.464	0.255	1.248	0.956–1.629	0.103	0.859
uNTX-I	1.164	0.919–1.476	0.208	1.216	0.938–1.577	0.140	0.889
sPINP	1.136	0.905–1.426	0.272	1.166	0.920–1.478	0.204	0.869
sOC	1.253	0.996–1.576	0.054	1.273	1.000–1.620	0.050	0.533
sHA	1.267	0.971–1.654	0.081	1.309	0.971–1.764	0.077	0.296
sPIIINP	0.815	0.643–1.032	0.089	0.801	0.624–1.028	0.081	0.412
Progression							
uCTX-II	1.688	1.262–2.256	**<0.001**	1.653	1.222–2.235	**0.001**	0.805
sCOMP	1.481	1.137–1.928	**0.004**	1.496	1.123–1.992	**0.006**	0.628
sPIIANP	0.936	0.730–1.199	0.599	0.899	0.692–1.168	0.426	0.165
sCS846	0.938	0.729–1.207	0.618	0.909	0.699–1.181	0.474	**0.062**
- No pain	0.212	0.036–1.251	0.087	0.145	0.009–2.340	0.174	
- Pain	1.078	0.817–1.423	0.594	1.051	0.787–1.402	0.737	
uCTX-I	0.986	0.765–1.272	0.916	1.004	0.766–1.317	0.975	0.826
uNTX-I	1.159	0.898–1.496	0.258	1.204	0.911–1.591	0.191	0.441
sPINP	0.954	0.731–1.244	0.729	0.960	0.723–1.274	0.776	0.645
sOC	0.942	0.718–1.234	0.663	0.956	0.712–1.284	0.766	0.554
sHA	1.401	1.072–1.832	**0.014**	1.303	0.975–1.742	0.073	0.701
sPIIINP	1.248	0.956–1.631	0.104	1.191	0.897–1.581	0.226	0.101

baseline (uCTX-II OR/SD = 0.797, $P = 0.057$ before adjustment for demographics, OR/SD = 0.765, $P = 0.045$ after adjustment; sCOMP OR/SD = 0.764, $P = 0.033$). sOC, sHA, and sPIIINP showed associations with incident radiographic knee OA that approached statistical significance (sOC OR/SD = 1.253, $P = 0.054$; sHA OR/SD = 1.267, $P = 0.081$; sPIIINP OR/SD = 0.815, $P = 0.089$) (Table I, middle panel).

Both uCTX-II and sCOMP showed positive associations with incident radiographic OA in the index hip (uCTX-II OR/SD = 1.404, $P = 0.035$; sCOMP OR/SD = 1.396, $P = 0.020$) and for sPIIANP after adjustment for demographic variables only (OR/SD = 1.357, $P = 0.035$). For uCTX-II interaction with pain in the index hip was statistically significant ($P = 0.024$). When analysed separately, the association with incident radiographic OA appeared to hold true only in subjects with pain in that hip at baseline (OR/SD = 1.889, $P = 0.005$), but not in those without (OR/SD = 0.996, $P = 0.987$) (Table II, middle panel).

Associations of baseline biomarkers with OA progression

Progression of radiographic knee OA in subjects showing radiographic OA of the index knee at baseline was again associated with cartilage and synovial markers (uCTX-II OR/SD = 1.688, $P < 0.001$; sCOMP OR/SD = 1.481, $P = 0.004$; sHA OR/SD = 1.401, $P = 0.014$). sCS846 showed statistically significant interaction with presence of pain of the index knee at baseline. When analysed in subjects with and without pain separately, sCS846 approached a statistically significantly negative association with progression of radiographic knee OA in subjects without pain, but not in those with pain (OR/SD = 0.212, $P = 0.087$ in subjects without knee pain, OR/SD = 1.078, $P = 0.594$ in subjects with knee pain) (Table I, lower panel).

Progression of radiographic OA of the index hip appeared to be positively associated with sCS846 in subjects with pain of the index hip at baseline (OR/SD = 1.949, $P = 0.031$). Progression of hip OA was negatively associated with both uCTX-I and sOC (uCTX-I OR/SD = 0.572, $P = 0.014$; sOC OR/SD = 0.623, $P = 0.027$) and tended to be negatively associated with sPINP (OR/SD = 0.628, $P = 0.050$) (Table II, lower panel).

Interaction between biomarkers

Since uCTX-II and sCOMP showed the most consistent associations with radiographic knee and hip OA, they were tested for interaction with the other biomarkers in their associations with

Table II
Associations between systemic biomarker levels and presence (top panel), incidence (middle panel), and progression (lower panel) of radiographic hip OA. Adjustment for covariates was performed stepwise. "Concurrent OA" includes OA in the contralateral joint (either present or absent) and in hips or knees (either absent, unilateral, or bilateral). "Demographics" includes age, gender, and BMI. Associations are displayed for painful and non-painful index hips separately when interaction between a biomarker and presence of pain was statistically significant. Statistically significant values are depicted in bold ($P < 0.05$, except $P < 0.100$ for interaction terms in the most right column). Numbers of subjects per (sub)group are shown in Fig. 1. OR/SD, odds ratio per standard deviation increase; 95% CI, 95% confidence interval; *P* significance

Hip OA presence	Adjusted for concurrent OA			Adjusted for demographics			Interaction with pain
	OR/SD	95% CI	*P*	OR/SD	95% CI	*P*	*P*
uCTX-II	1.262	1.036–1.538	**0.021**	1.210	0.978–1.498	0.079	0.415
sCOMP	1.280	1.066–1.537	**0.008**	1.253	1.030–1.524	**0.024**	0.459
sPIIANP	0.956	0.799–1.144	0.626	0.953	0.793–1.147	0.613	0.998
sCS846	1.028	0.857–1.233	0.769	1.028	0.855–1.237	0.766	0.421
uCTX-I	1.002	0.838–1.198	0.985	0.986	0.809–1.201	0.886	0.780
uNTX-I	1.078	0.902–1.288	0.411	1.080	0.886–1.315	0.446	0.503
sPINP	0.922	0.770–1.105	0.379	0.899	0.742–1.089	0.277	0.290
sOC	0.859	0.711–1.036	0.112	0.802	0.653–0.984	**0.034**	0.874
sHA	1.437	1.172–1.760	**<0.001**	1.252	1.006–1.559	**0.044**	0.997
sPIIINP	1.036	0.860–1.247	0.711	1.030	0.845–1.255	0.770	0.120
Incidence							
uCTX-II	1.404	1.024–1.925	**0.035**	1.465	1.035–2.072	**0.031**	**0.024**
- No pain	0.996	0.612–1.620	0.987	1.077	0.624–1.857	0.790	
- Pain	1.889	1.213–2.941	**0.005**	1.872	1.156–3.032	**0.011**	
sCOMP	1.396	1.055–1.848	**0.020**	1.423	1.059–1.911	**0.019**	0.299
sPIIANP	1.280	0.968–1.692	0.083	1.357	1.022–1.804	**0.035**	0.865
sCS846	0.965	0.725–1.285	0.808	0.989	0.740–1.321	0.938	0.868
uCTX-I	1.108	0.855–1.437	0.437	1.083	0.819–1.432	0.578	0.451
uNTX-I	1.039	0.798–1.351	0.778	1.029	0.770–1.375	0.848	0.786
sPINP	1.133	0.865–1.483	0.364	1.064	0.802–1.410	0.667	0.556
sOC	0.976	0.748–1.272	0.856	0.859	0.643–1.148	0.305	0.303
sHA	0.943	0.728–1.220	0.654	0.839	0.628–1.122	0.237	0.940
sPIIINP	1.033	0.768–1.390	0.830	1.060	0.785–1.431	0.703	0.230
Progression							
uCTX-II	0.769	0.480–1.232	0.274	0.866	0.523–1.433	0.576	0.039
- No pain	0.467	0.161–1.353	0.161	0.508	0.158–1.627	0.254	
- Pain	0.858	0.488–1.512	0.597	0.917	0.510–1.651	0.773	
sCOMP	1.118	0.717–1.745	0.623	1.150	0.709–1.864	0.572	0.731
sPIIANP	0.851	0.557–1.300	0.455	0.777	0.491–1.230	0.281	0.179
sCS846	1.391	0.858–2.256	0.180	1.373	0.848–2.224	0.197	0.069
- No pain	0.679	0.266–1.736	0.419	0.620	0.221–1.746	0.366	
- Pain	1.949	1.064–3.571	0.031	1.977	1.068–3.661	0.030	
uCTX-I	0.572	0.366–0.894	0.014	0.537	0.326–0.884	0.015	0.225
uNTX-I	0.734	0.479–1.123	0.154	0.726	0.454–1.159	0.179	0.568
sPINP	0.628	0.394–0.999	0.050	0.627	0.382–1.030	0.065	0.150
sOC	0.623	0.409–0.948	0.027	0.558	0.350–0.888	0.014	0.360
sHA	0.922	0.622–1.368	0.687	0.925	0.586–1.462	0.739	0.398
sPIIINP	0.631	0.390–1.019	0.059	0.700	0.416–1.179	0.181	0.600

radiographic OA to see whether this could provide information on the pathogenetic mechanisms behind uCTX-II and sCOMP release. Statistically significant interaction between biomarkers indicates that the association of the one biomarker on the outcome is (partly) dependent on the other biomarker. Therefore, when interaction of uCTX-II or sCOMP with another biomarker was statistically significant ($P < 0.100$, data not shown) the association of uCTX-II or sCOMP with radiographic OA was tested at different levels of that other biomarker, as is shown in Fig. 2.

For the knee, especially at high sHA levels (tertiles) uCTX-II appeared to be positively associated with presence of radiographic OA. The negative association between uCTX-II and radiographic knee OA incidence that was observed in the population as a whole (OR/SD = 0.765, $P = 0.045$, Table I) generally appeared to hold true for lower levels of uCTX-I, uNTX-I, and sOC (Fig. 2(A), middle column) but not for higher levels. Finally, uCTX-II appeared to show positive associations with progression at lower sPIIANP levels, but not at higher sPIIANP levels. COMP did not show interaction with other biomarkers in its associations with radiographic knee OA (Fig. 2(A)).

For the hip, sCOMP showed statistically significant interaction with sCS846 in its association with presence of radiographic hip OA. It appeared that the positive association between sCOMP and presence of radiographic hip OA gradually increased with increasing sCS846 levels (Fig. 2(B), left column). uCTX-II appeared to be positively associated with radiographic OA incidence at low sOC levels, but not at higher sOC levels. Likewise, uCTX-II was positively associated with radiographic hip OA incidence at lower sHA and sPIIINP levels, but not at higher levels (Fig. 2(B), middle column).

Discussion

The current study illustrates how systemic biochemical marker levels relate to presence, incidence, and progression of early-stage radiographic knee and hip OA. Among the investigated markers, CTX-II and COMP appeared superior in their associations with radiographic knee and hip OA. Moreover, the observation that CTX-II was associated with progression of radiographic OA of the index knee at lower PIIANP levels only, confirmed earlier findings that dissociation between synthesis and degradation of collagen type II is relevant for radiographic knee OA progression[10–13]. We did, however, not find this for presence and incidence of radiographic knee OA and not at all for radiographic hip OA.

An unexpected finding on CTX-II and COMP, however, was that their levels were negatively associated with incidence of

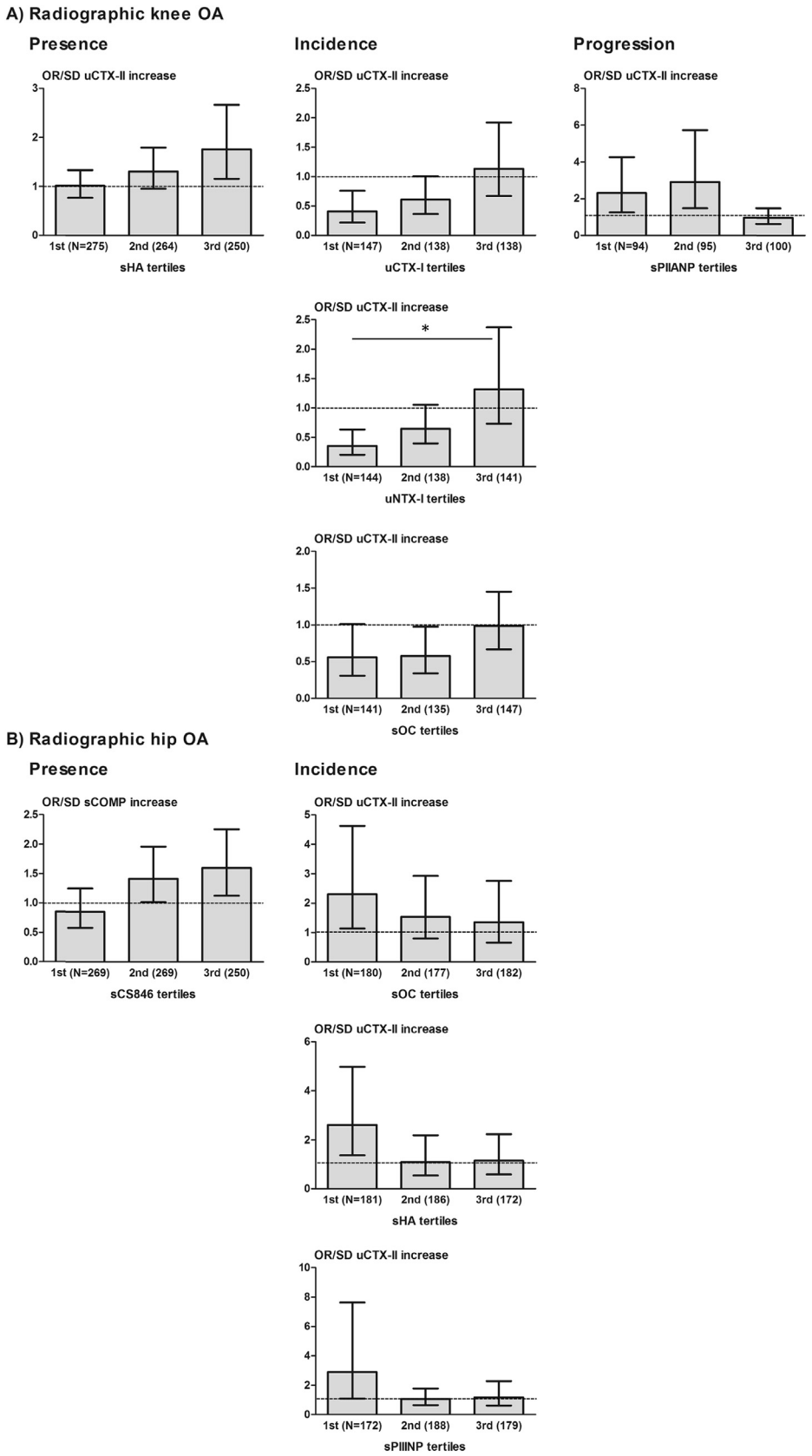

Fig. 2. Bar graphs showing associations of uCTX-II or sCOMP with presence, incidence, and progression of radiographic knee and hip OA at different levels of a second biomarker whenever justified based on statistically significant interaction. The number of subjects per tertile is indicated on the *x*-axis. Error bars represent 95% confidence intervals. Odds ratio = 1 is indicated by horizontal dotted lines in each of the graphs. Statistically significant differences between OR are indicated by an asterisk. OR/SD, odds ratio per standard deviation increase; P, significance.

radiographic knee OA. For CTX-II this negative association was especially found at lower levels of bone degradation and synthesis markers. This may indicate that a less dynamic (persistently low) cartilage and (subchondral) bone turnover (matrix turnover and with that adaptation and repair) are disadvantageous in the early phases of knee OA. However, the negative associations with incident knee OA were exactly opposite to the positive associations that were found in other analyses.

Previous data from CHECK suggest that CTX-II is intimately associated with bone metabolism, as opposed to other cartilage markers[3,4]. The current study again supports the hypothesis that CTX-II does not only relate to cartilage but also to bone metabolism, as CTX-II showed interaction with markers of both cartilage and bone metabolism. None of the other cartilage markers showed interaction with markers of bone metabolism.

The observation that serum PIIANP levels were increased in presence of radiographic knee OA and tended to be increased in subjects showing incident radiographic hip OA during follow-up adds to contradictory literature data showing both positive and negative associations between serum PIIANP levels and progression of radiographic knee OA[10,14]. This inconsistency between studies may result from the fact that the extent of collagen synthesis in osteoarthritic cartilage differs between histological-histochemical OA grades, first increasing between lower grades and then decreasing between higher grades[15]. The positive associations in our study may therefore be considered in line with the early-stage OA in this cohort, although cartilage damage has been shown to be already substantial in joints scoring K&L = 1[12].

We found serum CS846 levels to be positively associated with presence of radiographic knee OA, which would indicate that CS846 release is related to unbeneficial processes within the pathogenesis of OA. This was further substantiated by the observation that COMP levels were especially associated with presence of radiographic hip OA at higher CS846 levels. CS846 might represent the increased glycosaminoglycan turnover that eventually causes collagenolysis[16,17]. CS846 levels in synovial fluid were increased in various knee pathologies including primary OA[18] and experimental OA[19], were higher in late-stage as compared to early-stage OA, and were positively associated with COMP levels[18]. Most of the few human studies on CS846 levels in serum did not at all find associations with radiographic knee OA severity or progression[20–22], although one other study contradicted with ours in that mean CS846 levels in serial plasma samples appeared to be negatively associated with concurrent joint space narrowing of the knee[23].

The positive associations that were found between synovial markers and radiographic knee OA once more underscore the potential relevance of synovitis in early-stage knee OA[24–28]. Less is known about (markers of) synovitis in hip OA[2]. Serum HA and PIIINP levels were not associated with symptom severity, joint space width, and subchondral bone sclerosis of the hip in 376 hip OA patients of the ECHODIAH cohort when analysed cross-sectionally[29]. Serum HA levels were, however, shown to be associated with 3-year hip OA progression when defined as joint space narrowing $\leq$0.5 mm or requirement for total hip arthroplasty, but serum PIIINP levels were not[30]. In our study, serum HA levels were positively associated with presence of radiographic hip OA, but not with its incidence or progression. Neither did we find statistically significant associations between PIIINP levels and radiographic hip OA. However, PIIINP levels unexpectedly tended to be negatively associated with progression of radiographic hip OA and CTX-II levels were positively associated with radiographic hip OA incidence at lower PIIINP levels only. These findings were unexpected, since it is unlikely that any intra-articular process that is characterized by fibrosis would be associated with a favourable outcome in hip OA, and might have resulted from multiple testing or from its limited tissue specificity[31,32]. Likewise, the positive interaction between HA and CTX-II levels in the association with presence of radiographic knee OA seems more appropriate than the negative interaction between both in the association with radiographic hip OA incidence, also based on previous findings in other studies on OA[30,33] and RA[13,34].

Markers of bone metabolism showed apparently inconsistent associations with radiographic knee and hip OA. We did find positive associations of both bone degradation (CTX-I) and synthesis markers (PINP and OC) with presence of early-stage radiographic knee OA. This may indicate that a high bone turnover state is an aggravating factor in the early stages of radiographic knee OA. The bone markers may be related to the subchondral bone changes that happen in OA and, as such, our findings would be in line with the rationale for testing calcitonin[35], bisphosphonates[36], and risedronate[37] and strontium ranelate[38] in knee OA. Our findings are in accordance with the increased urinary CTX-I and NTX-I levels in progressive knee OA patients as compared to non-progressive knee OA patients and/or healthy controls[39] and the increased serum OC levels that were found in generalized OA patients[40]. However, they are in contradiction with other studies actually showing decreased bone marker levels in knee OA patients[33,41].

As opposed to our findings on knee OA, we found negative associations of bone marker levels with presence and progression of radiographic hip OA. Moreover, CTX-II appeared to be positively associated with radiographic hip OA incidence at lower OC levels only. These findings might indicate that high levels of bone turnover and decreased bone mass and/or mineral density would protect against hip OA and would argue for the hypothesis that osteoporosis and OA are inversely related[40]. Literature data on bone markers in hip OA are somewhat contradictory. Some studies did not find associations between systemic bone marker levels and concurrent or future hip OA[29,42], one found urinary CTX-I levels to be decreased in hip OA patients as compared to healthy controls but to be positively associated with radiographic progression and no association with hip OA progression for serum PINP levels[30], and one found increased serum ICTP (cross-linking C-terminal telopeptide of collagen type I) levels to be associated with rapidly destructive hip OA but none of the other bone markers[43,44].

The fact that associations with bone markers were different for knee and hip OA in our study can be due to pathogenetic differences between these joints[45]. It has been previously shown that the relationship between bone mineral density can differ between joint sites[5]. It can also be that our contradictory findings stem from the fact that K&L grades represent different OA stages between knee and hip[45,46] and that the influence of bone mineral density differs between OA stages[47]. Finally, it can be that the cause–effect relation is different between the associations that were found for both joints. Bone mineral density changes and according bone marker changes may have caused but may also have been the consequence of the OA process. Altered joint loading due to hip and/or knee OA symptoms has been shown to influence bone mineral density in the lower extremities[48,49].

In the current study on early-stage OA, radiographic OA needed to be defined as K&L = 1. However, joints that were scored K&L = 0 in these symptomatic subjects may have had structural OA changes not yet evident on radiographs[12] and K&L = 1 is considered only doubtful OA[45]. However, in the current study, presence of K&L = 1 changes in itself was statistically significantly associated with future K&L grade increase when all subjects were analysed together (OR = 1.884, 95% CI = 1.413–2.511, $P < 0.001$ for the knee; OR = 1.586, 95% CI = 1.037–2.427, $P = 0.033$ for the hip). Therefore, we think that these subcategories represented different populations in the current study and that separate analysis was justified. Notwithstanding, contrasts between subjects that were

defined as being with and without radiographic OA in the current study may not always have been maximum and differences of biomarker levels between them may be underestimated.

Strengths of the current study are its large size, its 5-year follow-up, and the large number of biomarkers that were simultaneously assessed. This study has limitations also. First, serial biochemical marker assessments would have been preferable over the current cross-sectional assessment. However, due to the large number of biomarkers this was infeasible at the moment, but may be reconsidered in the future. Second, coefficients of variation were >10% for some of the biomarker assessments, which may have caused that we missed some associations between biomarker levels and radiographic OA parameters. Third, radiographs at shorter intervals might have been informative, since OA progression may be phasic instead of linear[50]. The current design does not ascertain that subjects that showed OA progression at 5-year follow-up did actually have active disease at baseline. Fourth, comparison of biomarker levels between healthy and OA subjects would have been interesting, but healthy control subjects without any joint complaints were not included in the current study. Fifth, plain radiography has its limitations with regard to visualizing cartilage damage and does not visualize synovial tissue changes. Moreover, confounding by concurrent metabolism in other joints than knee and hip cannot be ruled out. Finally, using the K&L classification system did not allow investigation of associations between biomarkers and specific radiographic OA features. Fourth, direct measures of bone density (e.g., dual-energy X-ray absorptiometry, DEXA) would have supported our tentative conclusions on the associations between bone metabolism and OA. Such data were not available however.

In conclusion, especially biomarkers of cartilage and synovial metabolism were found to be associated with radiographic knee and/or hip OA in this cohort of symptomatic subjects with only early-stage radiographic OA. As such, higher levels of cartilage and synovial matrix turnover apparently are important characteristics of knee and hip OA in their early stages already. This does not exclude involvement of subchondral bone turnover in OA, since markers of bone metabolism may reflect systemic bone metabolism as a whole rather than subchondral bone metabolism only. Bone markers showed associations that differed between knee and hip, which might indicate a different role of bone metabolism between knee and hip OA.

Author contributions

WvS, FL, JB have made substantial contributions to the conception and design of the study, obtaining of funding, and acquisition of data. WvS, FL, and PW were primarily involved in the analysis and interpretation of data. WvS wrote article drafts that were critically revised for important intellectual content by all authors. All authors gave their approval of the final version to be submitted. WvS, FL, and SBZ (radiographic data) take responsibility for the integrity of the work as a whole, from inception to finished article (w.e.vanspil@umcutrecht.nl; f.lafeber@umcutrecht.nl; s.bierma-zeinstra@erasmusmc.nl).

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Competing interests

There are no competing interests to be declared by any of the authors.

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