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Systemic biochemical markers of joint metabolism and inflammation in relation to radiographic parameters and pain of the knee: data from CHECK, a cohort of early-osteoarthritis subjects

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SUMMARY

Objective: To investigate associations of biochemical markers of joint metabolism and inflammation with minimum joint space width (JSW) and osteophyte area (OP area) of knees showing no or doubtful radiographic osteoarthritis (OA) and to investigate whether these differed between painful and non-painful knees.
Design: Serum (s-) and urinary (u-) levels of the cartilage markers uCTX-II, sCOMP, sPIIANP, and sCS846, bone markers uCTX-I, uNTX-I, sPINP, and sOC, synovial markers sPIIINP and sHA, and inflammation markers hsCRP and erythrocyte sedimentation rate (ESR) were assessed in subjects from CHECK (Cohort Hip and Cohort Knee) demonstrating Kellgren and Lawrence grade $\leq$1 OA on knee radiographs. Minimum JSW and OP area of these knees were quantified in detail using Knee Images Digital Analysis (KIDA).
Results: uCTX-II levels showed negative associations with minimum JSW and positive associations with OP area. sCOMP and sHA levels showed positive associations with OP area, but not with minimum JSW. uCTX-I and uNTX-I levels showed negative associations with minimum JSW and OP area. Associations of biochemical marker levels with minimum JSW were similar between painful and non-painful knees, associations of uCTX-II, sCOMP, and sHA with OP area were only observed in painful knees.
Conclusions: In these subjects with no or doubtful radiographic knee OA, uCTX-II might not only reflect articular cartilage degradation but also endochondral ossification in osteophytes. Furthermore, sCOMP and sHA relate to osteophytes, maybe because synovitis drives osteophyte development. High bone turnover may aggravate articular cartilage loss. Metabolic activity in osteophytes and synovial tissue, but not in articular cartilage may be related to knee pain.

Introduction

Knee osteoarthritis (OA) is among the most disabling diseases in the developed world and its societal burden is only expected to increase further due to aging of the population, higher life expectancy, and the ongoing obesity epidemic[1]. Many approaches are followed to unravel the pathogenesis of OA and identify therapeutic targets. Biochemical markers have been proposed to be tools that could help along the challenging road to this point[2]. They can inform about the molecular events underlying the structural joint changes that characterize knee OA. Also, they can suggest what metabolic processes may be involved in the development of knee pain. In earlier studies, especially biochemical markers of cartilage degradation and synthesis, synovial activity (synovitis), and inflammation were associated with knee pain in OA[3–6]. Yet, how the presence of knee pain influenced associations of biochemical markers with individual radiographic OA features was not investigated.

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In the current study, systemic levels of multiple biochemical markers of joint metabolism and inflammation were assessed in subjects from CHECK (Cohort Hip and Cohort Knee), with painful and/or non-painful knees, with either no or doubtful radiographic OA (Kellgren and Lawrence, K&L, grade 0 or 1). Of these knees, minimum joint space width (JSW) and osteophyte area (OP area) were quantified in detail using semi-automated Knee Images Digital Analysis (KIDA). Then, associations of biochemical marker levels with minimum JSW and OP area were assessed to investigate what metabolic processes underlie these radiographic features. Finally, associations of biochemical marker levels and radiographic features were compared between painful and non-painful knees to determine whether this metabolic activity in articular cartilage and osteophytes might be relevant for having knee pain.

Method

Cohort characteristics

The current study was performed with baseline data from CHECK, a cohort of subjects age 45–65 years, with pain and/or stiffness of one or both knee(s) and/or hip(s), that had never or not longer than 6 months ago visited a general physician for these complaints for the first time[7]. Pain was classified as either present or absent according to the history of the patient that was obtained by an experienced rheumatologist. Subjects with any other pathological condition (e.g., other rheumatic disease, previous joint replacement) that could explain these symptoms were excluded.

Biochemical marker assessment

Biochemical marker levels were assessed in serum and second morning void urine samples, collected in a non-fasted state, between 8 and 12 AM. Marker levels were assessed by enzyme-linked immunosorbent assay or radioactive immunoassay, according to manufacturer instructions, as was described previously[8]. Intra-plate, inter-plate, and between-day coefficients of variation (standard deviation/mean*100%) 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%. Cartilage oligomeric matrix protein (COMP; AnaMar Med AB, Göteborg, Sweden): 5.0%, 4.0%, and 4.2%. N-terminal propeptide of procollagen type IIA (PIIANP; Millipore Corp, Billerica, MA, US): 15.8%, 7.0%, and 15.7%. Chondroitin sulfate 846 (CS846; IBEX, Montreal, Canada): 21.5%, 16.9%, and 15.3%. C-terminal telopeptide of collagen type I (CTX-I, Urine CrossLaps EIA, Immunodiagnostic systems Ltd., Boldon, UK): 9.7%, 6.1%, and 2.7%. N-terminal telopeptide of collagen type I (NTX-I, OSTEOMARK NTx Urine, Wampole laboratories, Princeton, US): 14.9%, 6.6%, and 10.7%. N-terminal propeptide of procollagen type I (PINP, UniQ, Orion Diagnostica, Espoo, Finland): 4.4%, 4.5%, and 6.2%. Osteocalcin (OC, N-MID Osteocalcin ELISA, Immunodiagnostic systems Ltd., Boldon, UK): 3.4%, 4.1%, and 4.3%. Hyaluronic acid (HA; Corgenix Inc, Westminster, CO, US): 15.1%, 13.0%, and 17.3%. N-terminal propeptide of procollagen type III (PIIINP; UniQ, Orion Diagnostica, Espoo, Finland): 5.4%, 3.2%, and 7.2%. Urinary biomarker levels were adjusted for urinary creatinine concentrations (automated kinetic assay, UniCel® DxC 800 Synchron® Clinical System, Beckman Coulter). Serum levels of high-sensitivity C-reactive protein (hsCRP) were assessed by an automated nephelometric assay (BN™ II analyzer, Siemens, routine clinical chemistry laboratory, University Medical Center Utrecht, Utrecht, The Netherlands). Erythrocyte sedimentation rate (ESR) was assessed according to clinical practice in each of the ten participating medical centers.

Acquisition of radiographic data

Radiographs of tibiofemoral knee joints were made in a posteroanterior view, weight-bearing, semiflexed (7–10°), according to Buckland–Wright[9]. Joint space narrowing (JSN) and osteophytosis were considered cardinal features of early-stage knee OA and were quantified on these radiographs using KIDA[10] (Fig. 1). Minimum JSW (in millimeters, mm) and OP area at the lateral and medial femur and tibia (in mm^2) were determined as was reported before[11]. Subchondral sclerosis was not selected as outcome of early-stage OA but was included as a potential confounder of associations of biochemical marker levels with minimum JSW and OP area. Subchondral bone density (subchondral BD), as a measure of sclerosis, was determined at the lateral and medial femur and tibia compartments (in mm aluminum equivalence, mmAl) by normalizing the grayscales of these compartments to grayscales of an aluminum step wedge that was included on all radiographs, as was explained before[12], and was averaged for all compartments. All measurements were performed by one observer, with intraclass correlation coefficients as determined by random reanalysis of 108 radiographs after a few months between 0.730 and 0.990.

Since other joints than the tibiofemoral knee joint would also contribute to systemic levels of biochemical markers and OA in this one joint may be a risk factor for OA in other joints, associations of systemic biochemical marker levels with radiographic parameters of the tibiofemoral knee joint needed to be tested (adjusted) for potential confounding by concurrent OA features in other joints. Radiographs were available for the patellofemoral knee joints and hip joints.

Radiographs of patellofemoral knee joints were made in a standing mediolateral and in a skyline (inferior superior) view,

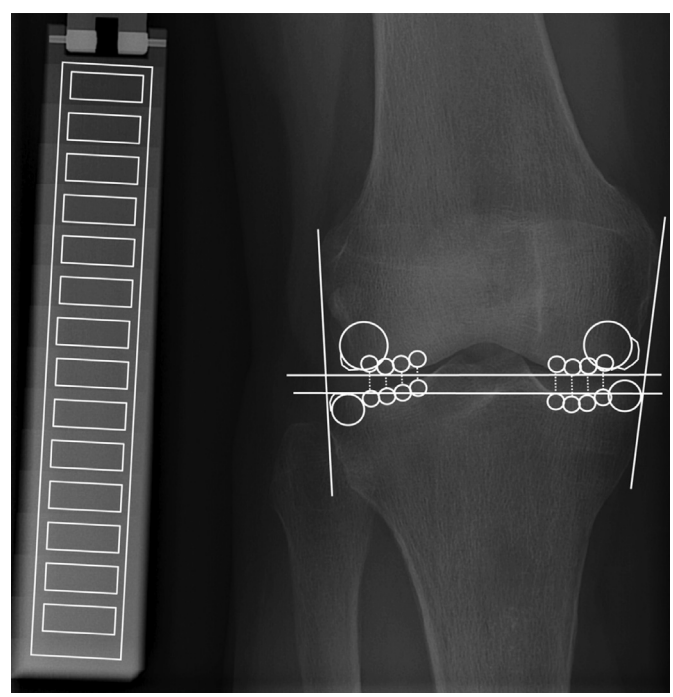
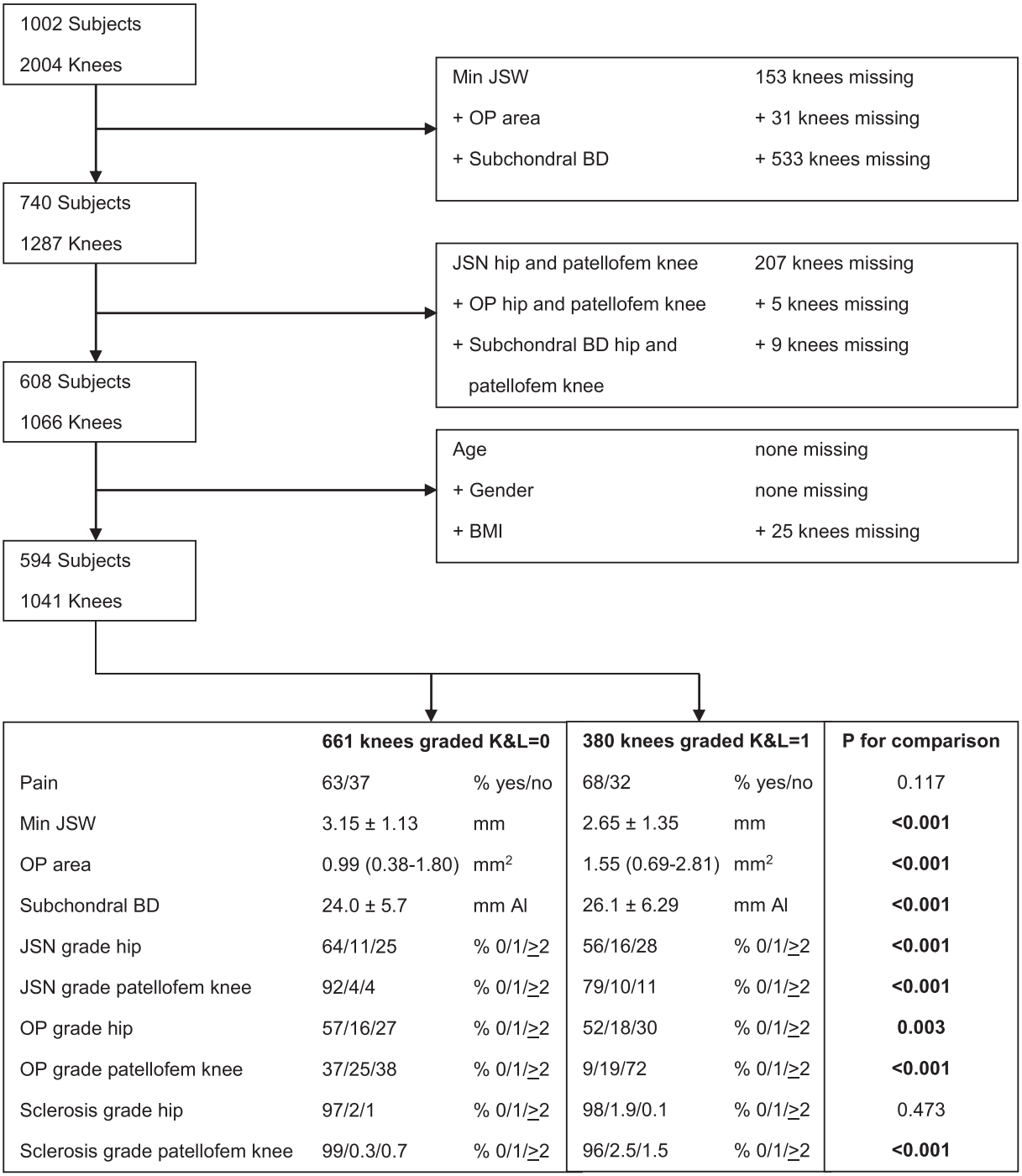


Fig. 1. Screenshot of KIDA. Small and larger circles are positioned in relation to a framework demarcating the knee joint. The framework and circles are positioned automatically, but their positions can be adjusted manually. JSW is assessed between the small circles aligning the bone-cartilage interface (dotted lines). Osteophyte area is determined outside the larger circles positioned at the corners of the lateral and medial femur and tibia. Bone density is assessed within the small circles, by normalization to an aluminum step wedge (rectangle) included on all radiographs. For details see references 10 and 12.

both in 30° flexion. JSN and osteophytes were scored on a 0–3 scale and sclerosis on a 0–1 scale, according to Burnett *et al.*[13]. When scores differed between mediolateral and the skyline views of the patellofemoral knee joint, the maximum score was used in our analyses.

Hip radiographs were made in an anteroposterior (AP) view of the pelvis, with hips in 15° internal rotation, and in a faux profil (FP) view of each hip, all weight-bearing. JSN and osteophytes were scored on a 0–3 scale and sclerosis on a 0–1 scale, according to Altman *et al.*[14]. JSN was scored at the superior and medial joint space on the AP radiographs and at the superior and posterior joint space on the FP radiographs. Scores were summed for each view. When these sum scores differed between views, the highest sum score was used in our analyses. Osteophytes were scored at the superior and inferior acetabulum and femur on the AP radiographs and scores were summed. Subchondral sclerosis was scored at the femoral compartment on the AP radiographs.



	661 knees graded K&L=0		380 knees graded K&L=1		P for comparison
Pain	63/37	% yes/no	68/32	% yes/no	0.117
Min JSW	3.15 ± 1.13	mm	2.65 ± 1.35	mm	**<0.001**
OP area	0.99 (0.38-1.80)	mm^2	1.55 (0.69-2.81)	mm^2	**<0.001**
Subchondral BD	24.0 ± 5.7	mm Al	26.1 ± 6.29	mm Al	**<0.001**
JSN grade hip	64/11/25	% 0/1/≥2	56/16/28	% 0/1/≥2	**<0.001**
JSN grade patellofem knee	92/4/4	% 0/1/≥2	79/10/11	% 0/1/≥2	**<0.001**
OP grade hip	57/16/27	% 0/1/≥2	52/18/30	% 0/1/≥2	**0.003**
OP grade patellofem knee	37/25/38	% 0/1/≥2	9/19/72	% 0/1/≥2	**<0.001**
Sclerosis grade hip	97/2/1	% 0/1/≥2	98/1.9/0.1	% 0/1/≥2	0.473
Sclerosis grade patellofem knee	99/0.3/0.7	% 0/1/≥2	96/2.5/1.5	% 0/1/≥2	**<0.001**

Fig. 2. Flow diagram of the inclusion procedure of 1002 CHECK subjects (2004 knees) for the current study. Data ranges are presented as mean ± standard deviation for normally distributed variables, as median (25–75% percentiles) for skewed variables, and as proportion (%) per category for discrete variables. BD, bone density; min JSW, minimum joint space width; JSN, joint space narrowing; K&L, Kellgren and Lawrence; OP, osteophyte/osteophytosis; patellofem, patellofemoral.

Statistical analysis

KIDA parameters of the both knees that are assessed within one subject cannot be considered independent[11]. Therefore, associations of biochemical marker levels with minimum JSW and OP area of the tibiofemoral knee joints in one subject were investigated by two-level linear mixed-effects models, the first level being the knees and the second level being the subjects, using a random intercept and fixed effects. In each of the models, either minimum JSW or OP area were used as outcome, while factors in each of the models were the biochemical marker of interest, radiographic parameters, demographic variables, and/or knee pain. Linear models assume that the relationship between the outcome and the fixed effects can be modeled through a linear function, that the variance is not a function of the mean, and that residuals of the model are normally distributed. Each of these assumptions was fulfilled in our linear mixed-effects models.

Associations of biochemical marker levels with minimum JSW and OP area were adjusted for potential confounding in three consecutive steps. In the first step, associations of biochemical marker levels with one of the KIDA parameters (e.g., minimum JSW) were adjusted for the other KIDA parameters of the tibiofemoral knee joint (e.g., OP area and subchondral BD). In the second step, associations between biochemical marker levels and KIDA parameters were adjusted for concurrent radiographic OA features of patellofemoral knee joints and hip joints. Finally, in the third step, associations were also adjusted for demographic variables (i.e., age, gender, and body mass index (BMI)). In these analyses, BMI and biochemical marker levels were logarithmically transformed and the square root of the OP area was used to obtain normally distributed residuals for the regression models. Confounding factors were selected in accordance with common use in biomarker literature and theoretical backgrounds.

Not all CHECK subjects were included based on knee pain and knee pain was not always bilateral, therefore not all knees were painful. To identify whether metabolic activity in articular cartilage and osteophytes was related to having knee pain, associations of biochemical marker levels with minimum JSW and OP area were evaluated for interaction with presence of knee pain. Statistically significant interaction indicates that the association of that marker with the outcome (i.e., minimum JSW or OP area) differs between painful and non-painful knees. Therefore, whenever interaction was statistically significant, the association of that marker with that KIDA parameter was determined for painful and non-painful knees separately.

To facilitate direct comparison between regression coefficients of variables and models, coefficients are expressed as standardized betas (stand betas). Standardized betas represent the number of standard deviations that the outcome will change as a result of one standard deviation change in the predictor. Therefore, they are independent of the units of measurement of the variables and can vary between −1 and 1.

All statistical analyses were performed using SAS, version 9.1. Statistical significance was defined as P values <0.05, except for interaction terms that were considered statistically significant at P values <0.100.

Results

Subject and knee characteristics

Fig. 2 shows for how many of the 1002 subjects (2004 knees) radiographic data were available for the tibiofemoral knee joint (KIDA) and for the patellofemoral knee joints and hip joints (Altman classification). Ultimately, for 594 subjects (1041 knees) all these radiographic data as well as demographic parameters were available. Of these knees, 661 knees were scored K&L grade 0 and 380 knees were scored K&L grade 1. K&L grade 1 knees expectedly showed a smaller minimum JSW, a larger OP area, and a higher subchondral BD. Interestingly, K&L grade 1 knees were also associated with more severe OA features of the patellofemoral knee joints and hip joints.

Table I demonstrates that subjects with and without complete radiographic data did not show clinically relevant, although sometimes statistically significant, differences for any of the demographics or biochemical markers.

For a maximum number of 12 samples per biochemical marker, marker levels were outside standard curves. Non-linear dilution effects hampered assessment of marker levels in diluted samples. To prevent bias from excluding these samples, marker levels in these samples were arbitrarily set at 80% of the minimum level or 120% of the maximum level that was assessed in samples inside standard curves[8]. Results did not differ essentially between analyses with and without these samples.

Minimum JSW

The putative cartilage degradation marker uCTX-II showed a negative association with minimum JSW (stand beta = −0.107, $P = 0.004$, adjusted for concurrent knee and hip OA features) that disappeared after adjustment for demographic variables, particularly BMI (data not shown). The bone degradation markers uCTX-I and uNTX-I showed negative associations with minimum JSW (stand beta = −0.128, $P = 0.001$, and stand beta = −0.130, $P < 0.001$, respectively, adjusted for concurrent knee and hip OA features) that remained for uCTX-I and disappeared for uNTX-I after adjustment for demographic variables (stand beta = −0.085, $P = 0.043$, stand beta = −0.076, $P = 0.064$, respectively), particularly gender (data not shown). The synovial markers sHA and sPIIINP showed positive associations with

Table I
Demographic parameters and biochemical marker levels for those subjects for whom radiographic data were and were not complete. To be included in the current study, radiographic OA features of the tibiofemoral and patellofemoral knee joints and hip joints were required. A flow diagram of the inclusion process is demonstrated in Fig. 2. Data ranges are presented as mean ± standard deviation for normally distributed variables, as median (25–75% percentiles) for skewed variables, and as proportion (%) per category for discrete variables. Comparison of variables between subjects with and without complete radiographic data was performed using independent-samples T test for continuous variables and Chi-square test for categorical variables. Statistically significant associations are in bold.

	608 subjects with one or two knees for which radiographic were complete	394 subjects with no knees for which radiographic data were complete		P for comparison
Age	55.9 ± 5.3	55.9 ± 5.1	years	0.933
BMI	25.2 (23.1–27.9)	25.9 (23.8–28.7)	kg/m^2	**0.007**
Gender	22/78	19/81	% male/female	0.296
uCTX-II	186 (129–282)	204 (138–285)	ng/mmol	**0.024**
sCOMP	8.5 (7.2–10.0)	8.3 (7.2–9.8)	U/l	0.405
sPIIANP	1380 (1072–1778)	1380 (1122–1799)	ng/ml	0.573
sCS846	67.6 (53.7–87.1)	72.4 (57.5–89.1)	ng/ml	0.431
uCTX-I	145 (98–219)	158 (105–240)	μg/mmol	0.192
uNTX-I	37.2 (26.9–50.1)	38.0 (28.8–51.3)	nM BCE/mmol	0.348
sPINP	41.7 (32.4–55.0)	42.7 (32.7–56.2)	ng/ml	0.315
sOC	12.9 (10.5–16.6)	13.2 (10.5–16.6)	ng/ml	0.848
sHA	25.7 (16.2–42.7)	28.8 (18.6–44.7)	ng/ml	**0.044**
sPIIINP	4.1 (3.5–4.9)	4.1 (3.5–4.9)	ng/ml	0.899
hsCRP	1.3 (0.6–3.0)	1.6 (0.8–3.5)	mg/l	**0.008**
ESR	7.9 (5.0–12.9)	7.3 (5.0–12.9)	mm/hr	0.442

minimum JSW, which became statistically significant for sHA only after adjustment for demographics (stand beta = 0.100, P = 0.022), particularly age (data not shown), and was strengthened for sPIIINP after adjustment for demographics (stand beta = 0.122, P = 0.002). The inflammation marker ESR showed a negative association with minimum JSW (stand beta = −0.091, P = 0.017, adjusted for concurrent knee and hip OA features) that disappeared after adjustment for demographic variables, gender in particular. The negative association of hsCRP with minimum JSW (stand beta = −0.074, P = 0.038, adjusted for OP area and subchondral BD) already disappeared after adjustment for concurrent patellofemoral and hip OA features.

None of the biochemical markers showed statistically significant interaction with presence of knee pain in their associations with minimum JSW (Table II, most right column).

OP area

First, all knees were analyzed, irrespective of presence of pain (Table III). uCTX-II and sCOMP, both putative cartilage degradation markers, showed positive associations with OP area (stand beta = 0.062, P = 0.044, and stand beta = 0.092, P = 0.005, respectively, adjusted for concurrent knee and hip OA features). The bone degradation markers uCTX-I and uNTX-I showed negative associations with OP area (stand beta = −0.072, P = 0.029, and stand beta = −0.077, P = 0.017, respectively, adjusted for concurrent knee and hip OA features). The synovial marker sHA showed a positive association with OP area (stand beta = 0.083, P = 0.011, adjusted for concurrent knee and hip OA features). All these associations disappeared after adjustment for demographic variables, mostly BMI (data not shown). In addition, the inflammation marker ESR showed a negative association with OP area only after adjustment for concurrent knee and hip OA features and demographic variables (stand beta = −0.076, P = 0.025).

Associations of OP area with uCTX-II, sCOMP, and sHA showed statistically significant interaction with the presence of pain ($P \leq 0.02$) and were therefore also analyzed in painful and non-painful knees separately. It appeared that the positive associations of uCTX-II, sCOMP, and sHA with OP area were only present in painful knees (stand beta = 0.100, P = 0.007, and stand beta = 0.140, P < 0.001, and stand beta = 0.116, P = 0.004, respectively, adjusted for concurrent knee and hip OA features), but not in non-painful knees (Table IV).

Table II
Associations of biochemical marker levels with minimum JSW of the knees as measured by KIDA and analyzed using mixed-effects models. From left to right, associations of biochemical marker levels with minimum JSW were consecutively adjusted for OP area and subchondral BD of the knees (both quantified using KIDA), for concurrent OA features of the patellofemoral knee joints and of the hip joints (scored according to Burnett and Altman, respectively), and for demographic variables (age, gender, and BMI). Standardized betas, 95% confidence intervals (95% CI), and P values are displayed. P values for the interaction of the biochemical markers with presence of knee pain in their association with minimum JSW are shown in the most right column. Statistically significant associations are in bold. Standardized betas represent the number of standard deviations that the outcome will change as a result of one standard deviation change in the predictor. Subch BD, subchondral bone density; min JSW, minimum joint space width; OP area, osteophyte area; rad OA, radiographic osteoarthritis

Independent	Dependent variable: Min JSW			
	Adjusted for			
	OP area, subch BD knee	+Rad OA features patellofemoral knee joint and hip	+Demographic variables	Interaction with presence of knee pain
uCTX-II	−0.115	−0.107	−0.046	
95% CI	−0.182 to −0.048	−0.180 to −0.035	−0.123 to 0.030	
P value	**<0.001**	**0.004**	0.238	0.255
sCOMP	0.045	0.042	0.043	
95% CI	−0.025 to 0.114	−0.035 to 0.119	−0.037 to 0.123	
P value	0.207	0.287	0.294	0.144
sPIIANP	−0.037	−0.029	0.009	
95% CI	−0.107 to 0.032	−0.106 to 0.048	−0.068 to 0.087	
P value	0.293	0.461	0.811	0.683
sCS846	−0.024	−0.036	−0.034	
95% CI	−0.093 to 0.0451	−0.114 to 0.042	−0.112 to 0.043	
P value	0.493	0.365	0.388	0.157
uCTX-I	−0.120	−0.128	−0.085	
95% CI	−0.190 to −0.051	−0.205 to −0.050	−0.167 to −0.003	
P value	**<0.001**	**0.001**	**0.043**	0.291
uNTX-I	−0.131	−0.130	−0.076	
95% CI	−0.199 to −0.062	−0.206 to −0.055	−0.157 to 0.004	
P value	**<0.001**	**<0.001**	0.064	0.921
sPINP	−0.012	−0.009	0.022	
95% CI	−0.081 to 0.057	−0.085 to 0.067	−0.055 to 0.098	
P value	0.724	0.821	0.576	0.948
sOC	0.014	0.018	0.059	
95% CI	−0.054 to 0.081	−0.058 to 0.094	−0.019 to 0.136	
P value	0.691	0.645	0.139	0.803
sHA	0.041	0.055	0.100	
95% CI	−0.028 to 0.110	−0.023 to 0.133	0.014 to 0.185	
P value	0.246	0.167	**0.022**	0.178
sPIIINP	0.076	0.102	0.122	
95% CI	0.007 to 0.146	0.027 to 0.177	0.045 to 0.198	
P value	**0.032**	**0.008**	**0.002**	0.830
ESR	−0.073	−0.091	−0.025	
95% CI	−0.142 to −0.005	−0.166 to −0.016	−0.107 to 0.056	
P value	**0.036**	**0.017**	0.539	0.374
hsCRP	−0.074	−0.057	−0.017	
95% CI	−0.144 to −0.004	−0.134 to 0.020	−0.101 to 0.066	
P value	**0.038**	0.145	0.681	0.466

Table III
Associations of biochemical marker levels with OP area of the knees as measured by KIDA and analyzed using mixed-effects models. From left to right, associations of biochemical marker levels with OP area were consecutively adjusted for minimum JSW and subchondral BD of the knees (both quantified using KIDA), for concurrent OA features of the patellofemoral knee joints and of the hip joints (scored according to Burnett and Altman, respectively), and for demographic variables (age, gender, and BMI). Standardized betas, 95% confidence intervals (95% CI), and *P* values are displayed. *P* values for the interaction of the biochemical markers with presence of knee pain in their association with OP area are shown in the most right column. Statistically significant associations are in bold. Standardized betas represent the number of standard deviations that the outcome will change as a result of one standard deviation change in the predictor. Subch BD, subchondral bone density; min JSW, minimum joint space width; OP area, osteophyte area; rad OA, radiographic osteoarthritis

Independent	Dependent variable: OP area			
	Adjusted for			
	Min JSW, subch BD knee	+Rad OA features patellofemoral knee joint and hip	+Demographic variables	Interaction with presence of knee pain
uCTX-II	0.086	0.062	0.052	
95% CI	0.024 to 0.148	0.002 to 0.123	−0.011 to 0.115	
P value	**0.007**	**0.044**	0.108	**0.015**
sCOMP	0.111	0.092	0.047	
95% CI	0.047 to 0.174	0.028 to 0.155	−0.019 to 0.114	
P value	**0.001**	**0.005**	0.164	**0.016**
sPIIANP	−0.033	−0.045	−0.057	
95% CI	−0.097 to 0.031	−0.109 to 0.019	−0.122 to 0.007	
P value	0.310	0.171	0.080	0.475
sCS846	0.042	0.029	0.012	
95% CI	−0.022 to 0.105	−0.035 to 0.094	−0.052 to 0.077	
P value	0.198	0.369	0.712	0.120
uCTX-I	−0.074	−0.072	−0.047	
95% CI	−0.138 to −0.009	−0.137 to −0.007	−0.115 to 0.022	
P value	**0.025**	**0.029**	0.181	0.254
uNTX-I	−0.071	−0.077	−0.063	
95% CI	−0.134 to −0.007	−0.140 to −0.014	−0.130 to 0.005	
P value	**0.030**	**0.017**	0.068	0.564
sPINP	−0.033	−0.029	−0.022	
95% CI	−0.096 to 0.0305	−0.093 to 0.034	−0.086 to 0.041	
P value	0.308	0.364	0.491	0.162
sOC	−0.024	−0.019	−0.004	
95% CI	−0.086 to 0.038	−0.082 to 0.045	−0.069 to 0.061	
P value	0.451	0.565	0.907	0.262
sHA	0.137	0.083	0.036	
95% CI	0.075 to 0.199	0.019 to 0.147	−0.035 to 0.107	
P value	**<0.001**	**0.011**	0.323	**0.020**
sPIIINP	0.054	0.036	−0.013	
95% CI	−0.011 to 0.118	−0.027 to 0.098	−0.078 to 0.052	
P value	0.101	0.261	0.695	0.175
ESR	−0.036	−0.057	−0.076	
95% CI	−0.099 to 0.027	−0.119 to 0.004	−0.142 to −0.010	
P value	0.267	0.069	**0.025**	0.474
hsCRP	0.024	−0.006	−0.065	
95% CI	−0.040 to 0.089	−0.070 to 0.058	−0.134 to 0.005	
P value	0.458	0.846	0.067	0.442

Discussion

The current study shows multiple associations of systemic biochemical marker levels with radiographic minimum JSW and OP area that were quantified in detail in knees with no or doubtful radiographic OA according to the K&L classification system. The putative cartilage degradation marker uCTX-II showed a negative association with minimum JSW and a positive association with OP area. The putative cartilage degradation marker sCOMP showed a positive association with OP area but no association with minimum JSW. The bone degradation markers uCTX-I and uNTX-I showed positive associations with minimum JSW and negative associations with OP area. The synovial markers sHA and sPIIINP showed positive associations with minimum JSW and the inflammation markers ESR and hsCRP showed negative associations with minimum JSW. Associations of biochemical marker levels with minimum JSW were independent of knee pain, whereas associations of uCTX-II, sCOMP, and sHA with OP area were only observed in painful knees. These data inform about possible metabolic processes underlying the radiographic features of OA and whether more active metabolism in articular cartilage and osteophytes may be related to knee pain.

CTX-II is proposed to be a marker of articular cartilage degradation. In our study, uCTX-II levels were indeed negatively associated with minimum JSW, which is in accordance with literature data[6,15–22]. uCTX-II levels were also positively associated with OP area, which again is in accordance with studies showing positive associations of uCTX-II levels with knee and/or hip osteophytes[18,20,23,24]. CTX-II release from osteophytes may originate from the osteoclastic resorption of calcified cartilage that is part of the endochondral ossification in osteophytes[25–28]. Associations of uCTX-II levels with minimum JSW did not differ between painful and non-painful knees, while associations of uCTX-II levels with OP area did. Apparently, CTX-II is released from articular cartilage irrespective of knee pain, but CTX-II is released from osteophytes in painful knees and not in non-painful knees (i.e., not to such an extent that this can be detected in urine and related to OP area). This indicates that metabolic activity in osteophytes may be involved in having knee pain, but metabolic activity in articular cartilage may not[29].

COMP is most frequently interpreted as a marker of cartilage degradation[30,31], but is present in other tissues as well[32–34]. In our study, both sCOMP and sHA did not show associations with minimum JSW, but showed positive associations with OP area. Both HA

Table IV
Associations of biochemical marker levels with OP area of the knees as measured by KIDA, analyzed in painful (65%) and non-painful (35%) knees separately. From left to right, associations of biochemical marker levels with OP area were consecutively adjusted for minimum JSW and subchondral BD of the knee (both quantified using KIDA), for concurrent OA features of the patellofemoral knee joints and of the hip joints (scored according to Burnett and Altman, respectively), and for demographic variables (age, gender, and BMI). Standardized betas, 95% confidence intervals (95% CI), and *P* values are displayed. Statistically significant associations are in bold. Standardized betas represent the number of standard deviations that the outcome will change as a result of one standard deviation change in the predictor. BD, bone density; min JSW, minimum joint space width; OA, osteoarthritis; OP, osteophyte area; rad OA, radiographic osteoarthritis

	Independent	Dependent variable: OP area		
		Adjusted for		
		Min JSW, subch BD knee	+Rad OA features patellofemoral knee joint and hip	+Demographic variables
Knee pain	uCTX-II	0.136	0.100	0.084
	95% CI	0.062 to 0.209	0.028 to 0.172	0.009 to 0.159
	P value	**<0.001**	**0.007**	**0.029**
No knee pain	uCTX-II	0.017	0.012	0.032
	95% CI	−0.088 to 0.122	−0.083 to 0.107	−0.070 to 0.136
	P value	0.744	0.801	0.537
Knee pain	sCOMP	0.141	0.140	0.108
	95% CI	0.062 to 0.220	0.061 to 0.219	0.024 to 0.192
	P value	**<0.001**	**<0.001**	**0.012**
No knee pain	sCOMP	0.071	0.038	−0.014
	95% CI	−0.025 to 0.168	−0.052 to 0.127	−0.104 to 0.076
	P value	0.145	0.404	0.758
Knee pain	sHA	0.170	0.116	0.069
	95% CI	0.094 to 0.245	0.039 to 0.193	−0.019 to 0.155
	P value	**<0.001**	**0.004**	0.122
No knee pain	sHA	0.110	0.040	0.005
	95% CI	0.014 to 0.209	−0.058 to 0.137	−0.100 to 0.111
	P value	**0.029**	0.424	0.918

and COMP are produced by synoviocytes[35,36] and are present in synovial tissue matrix[33,37]. Therefore, the positive associations of sHA and sCOMP levels with osteophytes may be considered in support of osteophyte development being driven by synovitis[26,38] as is syndesmophyte development in ankylosing spondylitis. Moreover, the positive associations of sHA and sCOMP with OP area were present in painful knees only, which suggests that metabolically active (inflamed?) synovial tissue, maybe (also) through osteophyte formation, is involved in having knee pain[39]. Alternatively, sCOMP may have originated from articular cartilage but its translocation from synovial fluid to blood may have increased in presence of synovitis[40].

ESR and hsCRP levels showed negative associations with minimum JSW, that only persisted for ESR after adjustment for concurrent patellofemoral and hip OA features. Assuming that ESR and hsCRP reflect synovitis in the current context, this confirms the notion that synovitis plays a role in cartilage damage[41]. This hypothesis contradicts the negative association of ESR with OP area, since also osteophyte growth may be driven by synovitis. This negative association appears counter-intuitive and may be a coincidental finding in multiple testing or be confounded by other, extra-articular, processes, (e.g., systemic inflammation unrelated to synovitis).

The positive associations of sHA and sPIIINP levels with minimum JSW were counter-intuitive as well, since it is unlikely that processes characterized by increased HA loss and/or production or by fibrosis[42–44] would protect against articular cartilage degradation. This unexpected finding may be attributable to confounding. First of all, both markers have limited tissue specificity[42–45]. Second, synovitis may drive articular cartilage degradation as well as increase clearance of biochemical markers from synovial fluid to blood. Finally, these associations may have been confounded by a positive association between JSW and total joint size.

In our study, high uCTX-I and uNTX-I levels were associated with more severe JSN (smaller minimum JSW) and less osteophytosis (smaller OP area). Assuming that elevated levels of these bone markers represent high rates of subchondral bone turnover, these findings would corroborate findings in animal models of early-stage OA. In these models, increased subchondral bone turnover preceded actual sclerosis and was related to a decrease in JSW[46]. Alternatively, biochemical markers released from subchondral bone turnover might be overwhelmed by biochemical markers released from systemic (skeletal) bone turnover. Assuming that these bone markers represent systemic bone metabolism, and high rates of bone turnover attenuate bone mineralization, this would mean that low systemic bone mineral density is detrimental for articular cartilage thickness but protects against osteophytosis. This would be in apparent accordance with studies showing that high systemic bone mineral density decreases the risk of progression of radiographic knee OA (mainly by reducing JSN)[47], but may be associated with an increased risk of incident radiographic knee OA (mainly by an increased risk of osteophyte development)[47–49].

All associations between tibiofemoral knee OA features and systemic biochemical marker levels were adjusted stepwise for potentially confounding factors (patellofemoral knee OA features, hip OA features, and demographics). Among the tested confounders, particularly patellofemoral OA features and demographics appeared to be associated with tibiofemoral OA features (data not shown). However, demographics showed stronger associations with systemic biochemical marker levels than patellofemoral OA features did. Accordingly, particularly (adjustment for) demographics appeared to affect associations between tibiofemoral KIDA parameters and systemic biochemical marker levels.

Obvious strengths of the current study are its large size, the large number and broad variety of biochemical markers that were simultaneously assessed, and the detailed quantification of radiographic knee parameters using KIDA. But, of course, this study has limitations also. First, serial biochemical marker data would have been preferable over the current baseline data but were not available. Second, not all KIDA data were available for all (knees of) CHECK subjects due to the high radiographic quality needed for quantitative evaluation of radiographs by KIDA. Since differences between subjects with and without complete data were minor, this will not have affected internal and external validity of the study. Third, in the current study K&L grade 0 and 1 knees were analyzed together, although these may in fact represent different entities[50]. However, sample size and effect sizes in this early-stage OA population were too small to allow separate analysis of K&L grade 0 and 1 knees. Fourth, MRI might have been preferable over the current X-ray imaging for assessing the early-stage OA parameters in our study population. Nevertheless, X-ray imaging is still the most widely used imaging modality in clinical practice and interpretation of MRI findings is not always straight forward either. Another limitation of our method for assessing JSW is that total joint size may have confounded JSW. Measures for total joint size were not available. Reassuring in this respect, however, is the positive association between our continuous measure of minimum JSW and the JSN grade according to the widely used categorical Altman classification system (data not shown). Finally, knee pain was classified as either present or absent according to the history of the patient. Use of a standardized instrument might have been better, although pain will always remain a subjective parameter and defining cut-offs for presence or absence of pain arbitrary. Moreover, currently

available standardized instruments are not developed for the very early-stage OA subjects that were part of the current study.

In conclusion, serum and urinary levels of markers of joint metabolism and inflammation showed multiple associations with minimum JSW and OP area when they were analyzed in detail in knees with no or doubtful OA according to the K&L classification system. In this context, urinary CTX-II levels may not only represent articular cartilage degradation, but also endochondral ossification in osteophytes. Similarly, COMP may originate from synovial tissue rather than from articular cartilage. Furthermore, high turnover of, either subchondral or systemic, bone may aggravate JSN. Finally, metabolic activity in synovial tissue and osteophytes but not articular cartilage were suggested to be involved in having knee pain. Synovitis may cause pain directly as well as indirectly through driving osteophyte development.

Author contributions

WvS, MK, and FL have all 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 and FL 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).

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Conflict of interest

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

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