

RESEARCH ARTICLE

Serum cartilage oligomeric matrix protein and development of radiographic and painful knee osteoarthritis. A community-based cohort of middle-aged women

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Abstract

Context and objective: We evaluated the predictive value of serum cartilage oligomeric matrix protein (sCOMP) levels over 20 years on the development of radiographic (RKO) and painful knee osteoarthritis (KOA) in a longitudinal cohort of middle-aged women.

Materials and methods: Five hundred and ninety-three women with no baseline KOA underwent 5-year knee radiographs over 20-years and were asked about knee pain a month before each assessment. A repeated measures logistic regression model was used where the outcomes were recorded at 5, 10, 15 and 20-years follow-up.

Results: The highest quartile of sCOMP was associated with increased risk of RKO with overall OR of 1.97 (95% CI: 1.33–2.91) over 20 years when compared with the lowest sCOMP quartile. The association with painful KOA was similar and also independent, but only when the fourth and third sCOMP quartiles were compared.

Discussion and conclusion: This study demonstrates that sCOMP levels are predictive of subsequent structural changes and incidence of painful KOA, independently of age and BMI.

Keywords

Cohort, COMP, epidemiology, knee, osteoarthritis

History

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Introduction

Knee osteoarthritis (KOA) is a highly prevalent disease of the largest synovial joint affecting quality of life, mobility and it is linked with an excess mortality (Cooper & Arden, 2011; Kluzek et al., 2015; Liu et al., 2015; Murphy et al., 2008; Nüesch et al., 2011; Semanik et al., 2015; Szoek et al., 2006). The lifetime risk of diagnosed symptomatic KOA is around 45%, occurring relatively early, with a median age of 55 years at diagnosis (Losina et al., 2013; Murphy et al., 2008). It is estimated that ~10% of the UK population over 55 years has disabling symptoms due to KOA (Peat, 2001). The lifetime risk of receiving a total knee replacement is also substantial, with values of 8.1% for men and 10.8% for

women, respectively (Culliford et al., 2012). KOA has many known risk factors, including age, female gender, obesity, history of knee injury and occupation. There is a need to find reliable markers that identify individuals with increased risk of developing KOA.

The cartilage oligomeric matrix protein (COMP) is a pentameric glycoprotein that was first identified in hyaline cartilage and fibrocartilage by Fife and colleagues (Fife, 1988; Fife et al., 1991). COMP is derived from several tissue types, including cartilage, synovium and menisci, and composed of five equal subunits, each 110 kDa (Müller et al., 1998). It is synthesised by chondrocytes and synovial cells activated by proinflammatory cytokines (Zivanović et al., 2011). The role of COMP is not fully understood. The presence of a pentameric COMP complex increases the collagen fibril formation while the presence of the monomeric COMP decreases it (Halász et al., 2007). Mutations of the COMP gene are linked with multiple epiphyseal dysplasia, a disorder of cartilage and bone development (Kim et al., 2011). Structure and biochemical properties of COMP suggest it might be involved in tissue storing of vitamin D (Guo et al., 1998; Ozbek et al., 2002). Synovial COMP concentrations have been correlated with serum COMP (sCOMP) levels (Addison et al., 2009). The precise factors

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associated with variation of serum COMP levels remain incompletely understood. The results from The Johnston County Osteoarthritis Project demonstrate that ethnicity, sex and radiographic hip or knee changes are associated with variation of sCOMP levels (Jordan et al., 2003). Age, weight, gender and mechanical loading affect the level of sCOMP in healthy individuals (Bartels et al., 2014; Niehoff et al., 2010; Verma & Dalal, 2013). Serum COMP levels increase significantly from 1 h after rising from bed and are affected by exercise (Kong et al., 2006; Neidhart et al., 2000).

Elevated sCOMP levels have been reported in patients with chronic inflammatory arthritis compared to healthy children (Saxne & Heinegård, 1992). High levels of sCOMP have been observed after a knee injury, with active synovitis and in knee osteoarthritis (Dahlberg et al., 1994; Lohmander et al., 1994; Vilím et al., 2001). Several studies showed a cross-sectional association of sCOMP with radiographic KOA (RKO) (Fernandes et al., 2007; Kong et al., 2006).

Associations with painful KOA are more complicated. Higher serum concentrations are linked to self-reported pain (Kluzek et al., 2014). Feldman and colleagues reported significantly higher levels in a population with painful RKO than in age and sex-matched healthy controls (Fernandes et al., 2007). However, other studies have shown that sCOMP levels do not correlate with severity of KOA symptoms (Lai et al., 2012; Verma & Dalal, 2013) and in a population with knee pain, no association was found between sCOMP levels and structural radiographic or MRI changes (Cibere et al., 2009). In a meta-analysis of cohort and case-controlled studies, Hoch et al. showed that sCOMP levels are significantly higher in patients with knee osteoarthritic symptoms (Hoch et al., 2011).

In longitudinal studies, individuals with low sCOMP levels and KOA were found to have a significantly lower rate of medial cartilage volume loss during the follow-up (Berry et al., 2010) and those with higher sCOMP levels had a significantly increased risk of subsequent cartilage loss (Hunter et al., 2007). In women with RKO, upward trajectories of longitudinally acquired sCOMP levels were associated with higher knee pain and stiffness scores and increased RKO severity (Sowers et al., 2009). Individuals with clinical OA and high sCOMP levels have been found to be at risk of rapidly progressing debilitating joint disease in a longitudinal study (Verma & Dalal, 2013).

Blumenfeld and colleagues showed that high baseline sCOMP levels predict the development of RKO over 10 years in middle-aged women (Blumenfeld et al., 2013). Longitudinal associations of baseline COMP levels with incidence of RKO in more than 10 years and incidence of painful KOA are unknown.

The aim of this study is to look at the predictive value of sCOMP levels over 20 years on the development of RKO and the painful KOA in a longitudinal cohort of middle-aged women.

Methods

Design

Prospective, population-based cohort study of middle-aged women.

Study population

The Chingford cohort study has been shown to be representative of middle-aged women in England and their characteristics have been described in detail previously (Hart & Spector, 1993a,b). All participants aged 45–64 years were registered in the same large general practice. Among 1353 women contacted and asked to participate in 1988–1989, 1003 (78% response rate) attended the baseline visit and have since been examined annually.

Inclusion and exclusion criteria

Only subjects with K/L scores of 0 at baseline in both knees were included in the analysis. We also excluded women with any of the following exclusion criteria at baseline or at any point during follow-up: rheumatoid arthritis, psoriatic arthritis, gout, Paget's disease, polio, cerebral palsy and chronic inflammatory demyelinating neuropathy. Women also had to have pain and radiographic knee data at baseline and during at least one 5-year interval over a 20-year follow-up. Five hundred and ninety-three women were included in the analysis with available biomarkers. The local ethics committee approved the study protocol and all participants signed an informed consent form.

Outcomes

The outcomes of interest were the incidence of subject level: (a) radiographic KOA (RKO); (b) painful radiographic KOA and (c) symptomatic KOA during the 20-year follow-up. RKO assessed from weight bearing, extended knee X-rays were obtained every 5 years (years 5, 10, 15 or 20). *Incident RKO* was determined using a K/L score of 2 or more at any point during follow-up with radiographic data. Women were asked if they had felt any knee pain in the last month and the number of painful days of knee pain they had experienced in the last month. Site-specific knee pain was classified as positive if they answered "yes" and more than 0 d were reported. *Painful RKO* was classified as positive if they had both any knee pain and K/L scores of 2 or more, affecting the same knee joint during the same follow-up visit. Subjects were classified as having *symptomatic RKO* if they had both knee pain for 15 d or more in the preceding month and K/L scores of 2 or more, affecting the same knee joint during the same follow-up time. Those who underwent total knee replacement during the 20-year follow-up were classified as having incident RKO and painful RKO.

Radiographic KOA assessment

Weight-bearing anteroposterior (AP) plain film extended knee radiographs were obtained at baseline, year 5, year 10 and year 15. Digital X-rays were taken in year 20 using protocols as similar as possible to previous visits (Leyland et al., 2012). Radiographs were scored individually by a single trained reader who was blinded to all clinical information except for study visit and study number. The presence of RKO was determined using the Kellgren/Lawrence (K/L) score (Kellgren & Lawrence, 1957; Kellgren et al., 1963). The baseline and year 5 radiographs were read by the same two

observers (DH and TS) and a single reader (DH) graded the year 10 and year 15 radiographs. As previously reported, reproducibility of the grading system was high (Spector et al., 1993). Year 20 radiographs were read by an additional observer who trained using comparable methods under the guidance of a previous observer (DH).

Serum COMP levels

Sera were obtained from venous fasting blood during the second- and third-year follow-ups and stored at -80°C . This study used an enzyme-linked immunosorbent assay (ELISA) with an ELISA kit manufactured by AnaMar Medical (Uppsala, Sweden). The intra- and inter-assay variations were below 4% and 8%, respectively. The samples were measured in 2009, when the format was a serum standard. The assay was validated before testing the samples, a process that included the sample dilution, determination of measurement range and day-to-day variation. The quantification range was determined internally by the lab as 2 (lower limit of quantification) to 35 (upper limit of quantification) U/L. All samples were measured in the duplicates and within the measuring range when diluted 4–10 times. Samples were re-run if intra assay coefficients of variability (CVs) were above 15% or if their values were outside the quantification range. Each plate included two kit controls, as well as three internally identified quality control samples. From these, the inter-assay CVs were monitored and controlled for being $<10\%$. No measurements were removed by the lab. Distribution of the values in the cohort without baseline RKO is presented in the Appendix.

Statistical analysis

Five hundred and ninety-three patients had no KOA (K/L grade 0 in either knee at baseline) and available sCOMP data. A repeated measures logistic regression model was used where the outcomes were recorded at 5, 10, 15 and 20-years follow-up. The primary exposure of interest in this model was sCOMP levels (categorised into quartiles due to the non-linear effect of sCOMP on outcomes). This model estimates the impact of predictors on the average outcome over the 4 follow-up time points. An interaction of terms was fitted between sCOMP and follow-up time to assess whether the association of sCOMP on outcome changed between 5 and 20-years follow-up. This technique allowed us to include participants meeting baseline inclusion criteria with complete radiographic and pain follow-up data set available ($n=328$), along with those who had data available during any 5-year interval follow-up over 20 years. This method allowed us to utilise the longitudinal data with interval-censored data when precise time to RKO was not available. The models were adjusted for age and BMI. We reported risks using odds ratios (OR) and adjusted predictions for each sCOMP quartile during 5-year intervals. This was performed using STATA/IC package version 12.1 (StataCorp., College Station, TX).

Potential confounders

We assessed information on potential confounders at Y1. Covariates, including age and BMI, were incorporated into

the analysis. Health-related behaviours that have been shown to modify incidence of RKO or levels of sCOMP were assessed: physical activity at baseline and smoking status.

Physical activity was assessed via the following question: “How many times per week are you engaged in activity that makes you sweat?” The participant was classified as active if she reported more than an hour of strenuous activity each week. Smoking habits were divided into the categories of non-smoker or currently smoking. Non-steroidal anti-inflammatory drug (NSAIDs) use was coded as a dichotomous variable.

Results

Of the original 1003 women who were seen at baseline, 970 underwent radiography at baseline and 750 had a baseline K/L score of 0 (Figure 1). Of these, 646 had at least 1 follow-up X-ray and 377 had X-rays at 20 years. Five hundred and ninety-three women had a baseline and follow-up radiographs and serum COMP measured at years 2 or 3. Comparison of women's characteristics who have not been included into analyses (due to lack of biomarker data or loss to follow-up) with those who have had been is summarised in Table 1 (Appendix).

Distribution of potential confounders of the study sample ($n=593$) according to quartiles of the sCOMP levels is summarised in Table 2. Subjects with the 1st quartile of COMP were younger (mean age 52 versus 55) and slimmer (mean BMI 23 versus 26) when compared to the fourth. There were no significant differences in terms of physical activity levels, NSAID use, smoking, previous knee injury history or type of work undertaken.

Radiographic OA

The cumulative incidence of RKO was 8% by year 5, 24% by year 10, 36% by year 15 and 81% by year 20. A significant independent association was found between higher sCOMP quartile incidence of RKO (OR = 1.97, 95% confidence interval (CI) = 1.33–2.91) when compared with the lowest sCOMP quartile (Figure 2).

Painful radiographic OA

A significant independent association was found between the highest quartile compared to the lowest quartile of sCOMP quartile (OR 1.8 with 95% CI: 1.18–2.75) (Figure 3). After adjusting for age and BMI, women with the highest baseline quartile of sCOMP levels had a 48% increase in the risk of developing painful RKO, compared to women with the lowest sCOMP level, which has become borderline significant (OR = 1.48 with 95% CI 0.95–2.3).

Interestingly, a significant independent association was found between the higher sCOMP quartile and risk of developing painful RKO (OR = 1.95, 95% CI = 1.25–3.05) when compared with the third sCOMP quartile.

We found a similar association when we used 15 or more days of pain to classify symptomatic RKO. The association between the baseline fourth quartile and risk of developing symptomatic RKO was non-significant (OR = 1.27 with 95% CI = 0.74–2.2) when compared with the first quartile of sCOMP. However, the association between the highest

Figure 1. The Venn diagrams illustrate the relationship between the radiographic, painful and symptomatic KOA, during each follow-up visit of the cohort with no RKOA at baseline ($n = 593$). RKOA, radiographic knee osteoarthritis; painful RKOA, any knee pain reported in the last month and K/L score ≥ 2 on the same site; symptomatic RKOA, knee pain for 15 d or more in the last month and the K/L score ≥ 2 .

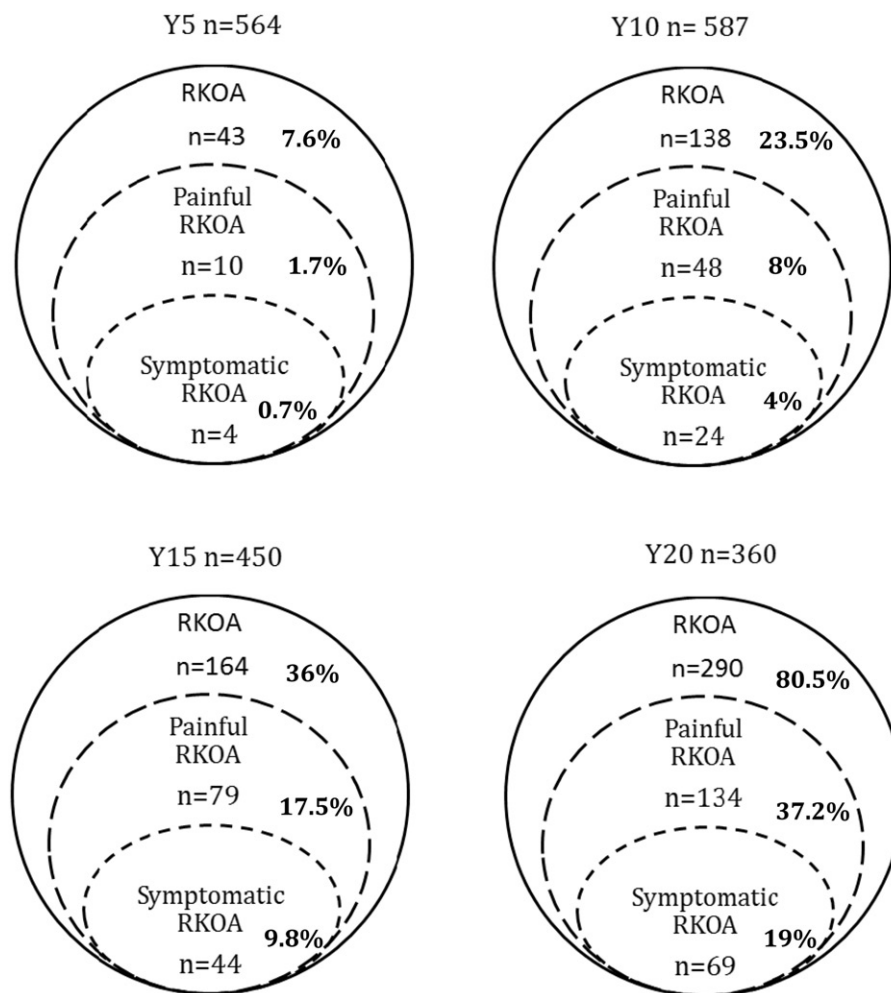


Table 1. Comparison of women's characteristics who have not been included into analyses (due to lack of biomarker data or loss to follow-up) with those who have had been.

Characteristic	Included into analysis ($n = 593$)	Not included into analysis ($n = 157$)	p value
sCOMP levels (U/L)	2.7–43.3	13.9	N/A
Age, years, mean (SD)	53.2 (5.7)	54.7 (6.5)	0.005
Body mass index, mean (SD)	24.9 (3.7)	25.9 (4.42)	0.146
Smoking (%)			0.300
Non-smoker	55%	53%	
Ex-smoker	23.9%	21%	
Current smoker	21%	26%	
Alcohol units per week (%)			0.945
0	29%	35%	
1–14	68%	58%	
>14	2.8%	7%	
Manual/non-manual work (%)			0.668
Manual	17.3%	16%	
Non-manual	82.6%	84%	
Previous knee injury n (%)	57 (10.7)	19 (14)	0.3468
Physical activity, (>1 h of strenuous physical activity a week) n (%)	105 (18)	29 (19)	0.846
Non-ASA NSAIDs medication n (%)			0.571
No	563 (96)	108 (95)	
Yes	24 (4)	6 (5)	
Creatinine mg/L, mean(SD)	830 (433)	659 (116)	

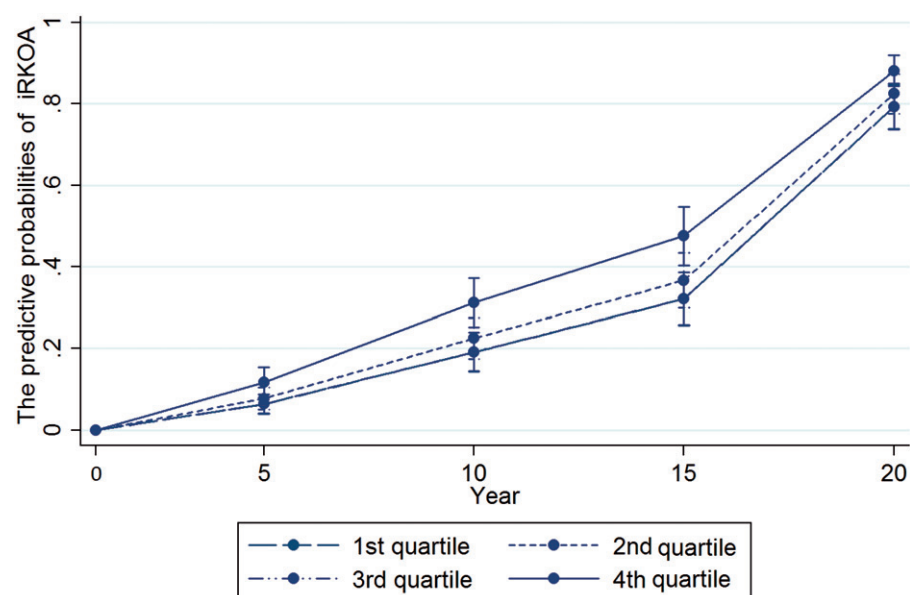
SD, standard deviation; NSAIDs, non-steroidal anti-inflammatory drugs.

Table 2. Women's characteristics across sCOMP quartiles.

Characteristics	First COMP quartile (n = 149)	Second COMP quartile (n = 148)	Third COMP quartile (n = 149)	Fourth COMP quartile (n = 147)	p value
sCOMP levels (U/L)	2.7–7.6	7.7–9.4	9.5–11.8	11.9–42	
Age, years, mean (SD)	51.9 (5.6)	52.9 (6.0)	53.0 (5.5)	55.2 (5.3)	0.000
Body mass index, mean (SD)	23.9 (3.1)	25.2 (3.6)	24.7 (3.7)	25.9 (4.2)	0.004
Smoking (%)					0.743
Non-smoker	60%	54%	52%	55%	
Ex-smoker	21%	24%	26%	25%	
Current smoker	19%	22%	22%	20%	
Alcohol units per week, (%)					0.259
0	24%	23%	34%	36%	
1–14	73%	75%	62.5%	61%	
>14	2%	2%	3.5%	3%	
Manual/non-manual work (%)					0.679
Manual	15%	15%	25.5%	17.5%	
Non-manual	85%	85%	74.5%	82.5%	
Previous knee injury (n)	14	11	14	18	0.490
Physical activity, (>1 h of strenuous physical activity a week) n (%)	28 (19)	17 (12)	32 (21.4)	28 (19)	0.125
Non-ASA NSAIDs medication n (%)					0.792
No	143 (96)	140 (95)	140 (94)	140 (95)	
Yes	4 (3)	6 (4)	7 (5)	7 (5)	
Creatinine mg/L, mean(SD)	844 (468)	867 (442)	824 (391)	786 (429)	0.467

SD, standard deviation; NSAIDs, non-steroidal anti-inflammatory drugs.

Figure 2. Serum COMP quartile sand the risk of developing RKOA. The results are adjusted for baseline age and BMI. The bottom line consists of the overlapping lines for the first and second sCOMP quartiles.



quartile and the risk remained statistically significant in multivariate analysis when compared with the third quartile (OR = 2.05 with 95% CI = 1.15–3.67) (Figure 4).

Discussion

This study demonstrated that the highest sCOMP quartile predicts the incidence of RKOA over 20 years in the cohort of middle-aged women with no baseline KOA. The association between the highest quartile of sCOMP with the incidence of painful and symptomatic KOA was significant in multivariate analyses only when it was compared to the third quartile.

Our study supports the previously reported significant association between baseline sCOMP levels and the risk

of developing RKOA in the cohorts with K/L <2 at baseline and follow-up to 10 years (Blumenfeld et al., 2013; Valdes et al., 2014) and additionally shows that this relationship is significant over a period of 20 years of follow-up, independent of baseline age and BMI in a cohort with no baseline knee OA (K/L 0). This is an important difference from previous studies, as K/L 1 has been shown to be one of the strongest predictors of future RKOA as defined by K/L >2 (Kerkhof et al., 2014).

Despite the significant variability, self-reported knee pain in KOA has been associated with structural radiographic changes (Soni et al., 2012). Interestingly, despite significantly increased the incidence of RKOA over time in our cohort, we found that the majority of the patients with RKOA reported

Figure 3. Serum COMP quartiles and the risk of developing painful RKOA. The results are adjusted for baseline age and BMI.

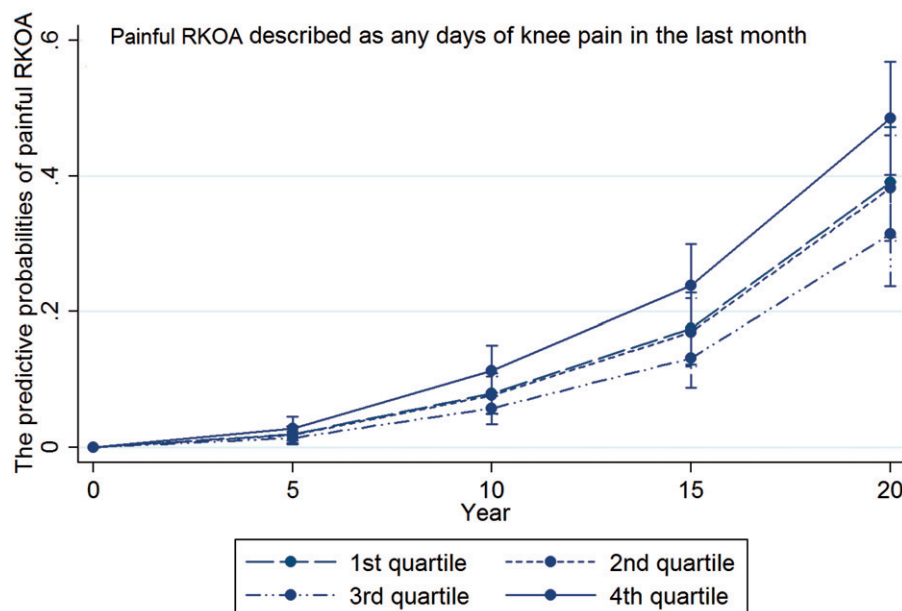
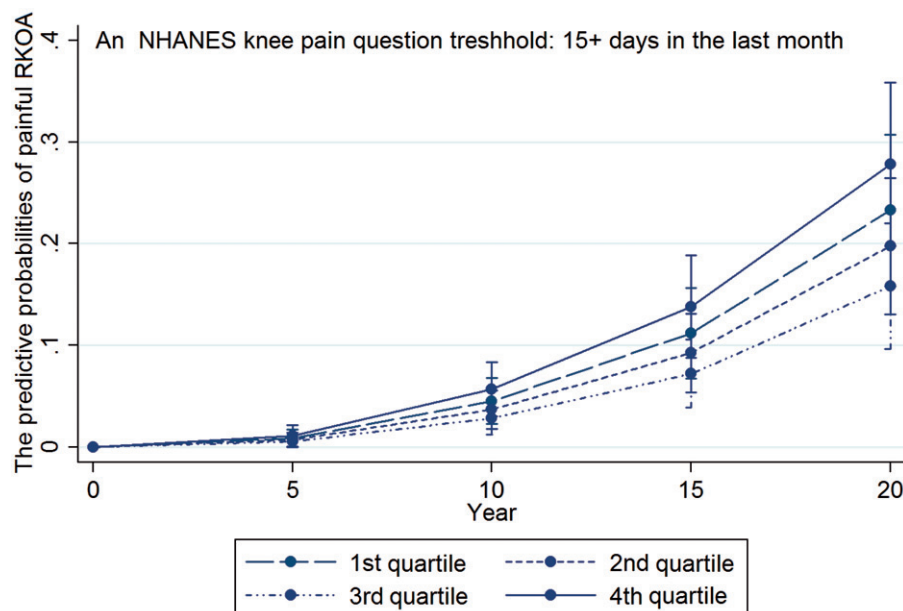


Figure 4. Serum COMP quartiles and the risk of developing symptomatic RKOA. The results are adjusted for baseline age and BMI.



no pain in the affected knee and only a small minority report a significant knee pain (15d or more), during each 5-year follow-ups over 20-years.

We also demonstrated that high sCOMP levels are a predictor of subsequent structural knee changes, but that individuals with middle range levels of sCOMP (third quartile) are less likely to develop painful KOA.

Unlike other forms of arthritis linked with inflammation, knee OA is characterised by new bone formation (Suri & Walsh, 2012). Knee pain linked with osteophytes is likely representing unsuccessful remodelling process probably mediated by inflammatory responses (Daghestani et al., 2015; Ene et al., 2015; Stoppiello et al., 2014). Radiographic changes without any significant symptoms might be also associated with high makers of tissue turnover (Karsdal et al., 2014). Differences between the probability of

developing painful or symptomatic RKOA in fourth and third quartile of sCOMP might reflect those differences between a successful and failed remodelling/repair process in KOA.

Strengths and potential limitations

This cohort study is representative for the UK population of middle-aged women. With 5-year interval follow-up knee radiographs obtained over 20 years, and self-reported knee pain data collected contemporaneously, this is by far the longest observational study data looking at the predictive value of sCOMP for the development of KOA. A discrete-time survival analysis model used in this study allowed us to maximise the utility of follow-up data available.

The research has some limitations, including losses to follow-up and results being limited to women only. The serial

knee radiographs were not obtained in the semiflexed AP view, as they were not available 25 years ago when the study started and for consistency the same technique was used during follow-up. The levels of sCOMP can reflect morphological and pathological changes in any cartilaginous joint. In this study, we did not assess osteoarthritis affecting other joints.

Loss of follow-up represents a major limitation in all long-term cohort studies. There is potential for bias due to subjects' mortality, or withdrawal, and the general selection of a healthier cohort to attend the follow-up visits. We minimised this healthier selection bias by using an analysis model that allowed us to include results from all individuals who met baseline inclusion criteria and attended any 5-year interval follow-up over 20 years.

Conclusion

In a population of middle-aged women without baseline RKO, the highest sCOMP levels were linked with an increased risk of developing structural changes consistent with RKO and painful KO over 20 years. This study highlights the fact that further research is needed to understand differences in markers of tissue turnover to differentiate populations at risk of developing radiographic and painful RKO.

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Declaration of interest

ACBJ and MK are full-time employees and shareholders of Nordic Bioscience, a privately owned biotech company involved in biomarker development and validation. However, they have no commercial interest in the specific biomarker described in this paper sCOMP.

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Appendix

Serum Cartilage Oligomeric Matrix Protein Distribution

Serum cartilage oligomeric matrix protein quartile values in a population of middle-aged women without KOA.

Quartile	Minimum	Maximum
1	2.7	7.6
2	7.7	9.4
3	9.5	11.8
4	11.9	43