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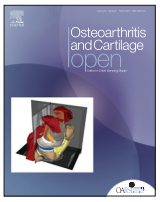

Pathological tissue formation and degradation biomarkers correlate with patient reported pain outcomes: an explorative study

Anne C. Bay-Jensen [a,*], Mukundan Attur [b], Jonathan Samuels [b], Christian S. Thudium [a], Steven B. Abramson [b], Morten A. Karsdal [a]

[a] Nordic Bioscience, Biomarkers & Research A/S, Herlev, Denmark
[b] Division of Rheumatology, Department of Medicine, NYU Grossman School of Medicine, NYU Langone Health, New York, NY-10003, USA

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ABSTRACT

Background: The lack of disease modifying drugs in Osteoarthritis (OA) may be attributed to the difficulty in robust response based on patient-reported outcomes (PROs) linked to drug mechanism of action. Joint tissue turnover biomarkers are associated with disease progression. A subset of patients has elevated serum levels of CRP metabolite (CRPM). This explorative study investigates the associations between PROs and joint tissue turnover markers in patients with high or low CRPM.

Methods: Serum of 146 knee OA patients of the New York Inflammation cohort and 21 healthy donors were assessed for biomarkers of collagen degradation (C1M, C2M, C3M, C4M), formation (PRO-C1, PRO-C2, PRO-C3, PRO-C4), and CRPM. Mean (SD) age was 62.5 (10.1); BMI, 26.6 (3.6); 62% women; and, 67.6% had symptomatic OA. WOMAC pain, stiffness, function, and total were recorded at baseline and at two-year follow-up. Associations were adjusted for race, sex, age, BMI, and NSAID.

Results: There was no difference in markers between donors and patients. C2M correlated with the WOMAC scores in all CRPM groups. Significant correlations were observed between PROs and PRO-C4, C1M, and C3M in the $CRPM_{high}$ group. The best predictive models for improvement were found for function and total with AUCs of 0.74 ($p < 0.01$) and 0.78 ($p < 0.01$). The best predictive models for worsening were found for function and total with AUCs of 0.84 ($p < 0.01$) and 0.80 ($p < 0.05$).

Conclusion: We hypothesize that collagen markers are prognostic tools for segregating patient populations in clinical trials.

1. Introduction

Osteoarthritis (OA) is a chronic disease characterized by pain and disability. Currently, there are no approved treatments that modify the structure of OA. One of the reasons for this is the challenge in obtaining reliable patient-reported outcomes (PROs) that can demonstrate the effectiveness of a drug. Two factors contribute to this challenge: 1) Placebo responses often overshadow the actual drug effect [1]; and 2) Patients with OA often experience long periods of stability without significant changes [2]. To address this, there is a need for biomarkers that can identify patients who are more likely to have active progressive disease during drug trials, allowing for a more targeted selection of patients who will respond to treatment.

Tissue regeneration is an ongoing process in the body, including within the joints. In many cases, this process occurs silently without any symptoms [3,4]. However, in chronic diseases like OA, tissue remodeling can lead to the loss or increase of tissue, altering its composition and resulting in organ dysfunction. These changes can manifest as prolonged symptomatic tissue remodeling [5,6].

Collagens play a vital role in the structure of tissues, with several types being abundant in the body [7]. Type I collagen is the most prevalent protein in the body, and it is found in various organs, including the joints [6]. Consequently, collagens in various organs are constantly being broken down and synthesized in response to strain and stress on the body. The four most abundant collagens of the joint are Type I (mainly bone), Type II (cartilage), Type III, and Type IV (synovium). They are all maintained and

Abbreviations: AUC, area under the curve; ECM, extracellular matrix; OA, osteoarthritis; MMP, matrix metalloproteinase; PRO, patients reported outcomes; TLR, toll-like receptor.

* Corresponding author. Nordic Bioscience, Herlev Hovedgade 207., DK-2730, Herlev, Denmark.
E-mail address: acbj@nordicbio.com (A.C. Bay-Jensen).

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remodeled in both the healthy and the OA joint. In OA, Type I collagen turnover becomes elevated, leading to subchondral thickening, while Type II collagen is gradually lost [8]. Type III and Type IV collagen expressions increase as the synovium thickens and the vascular network expands [9,10]. Consequently, it is interesting to quantify collagen epitopes related to either the formation or degradation of tissues [11]. Collagens are ideally suited for this purpose, as they contain pro-peptides that are released during the formation of the collagens, and consequently may be a surrogate for tissue formation, while degradation fragments of the helical chain can be used as degradation biomarkers [12]. This approach has been extensively used in the osteoporosis field [13], in which all efficacious treatments affect the tissue balance [14]. However, it has also been used in the OA field; an example is the FNIH/OAI biomarkers analysis, in which formation and degradation as well as bone formation and bone resorption were associated with structural and symptomatic progression [15]. The first biomarker data from the APPROACH study, which includes synovial markers, showed that tissue turnover markers were able to identify tissue endotypes, which were differentially associated with pain and structural outcomes [16].

CRPM, a degradation fragment of CRP (C-reactive protein) [17], has recently shown promise in assessing structural damage and disease activity in OA [18,19]. Unlike CRP, which is an acute-phase reactant produced in the liver, CRPM is derived from CRP but is processed in the tissues by matrix metalloproteinases (MMPs), resulting in the formation of CRPM. Thus, CRPM can be considered a marker of tissue inflammation. While CRP levels can vary significantly due to acute reactions, the chronic tissue inflammation reflected by CRPM is more stable [18].

In the present explorative and hypothesis-generating study, we test the association between Types I, II, III, and IV collagen formation, degradation biomarkers, and PROs.

2. Patients and methods

2.1. The OA patient population

A total of 146 knee OA patients from the New York Inflammation cohort [20] were included in this post hoc biomarker study according to the American college of rheumatology (ACR) criteria: 62% were women, the mean (standard deviation, SD) age was 62.5 (10.1), BMI was 26.6 (3.6), 32% were NSAID users, and 67.6% had symptomatic disease. WOMAC pain, stiffness, function, and total scores were recorded in 141 patients at baseline (BL), and 134 had scores recorded at the two-year follow-up (FU). The data are summarized in Table 1.

Symptomatic improvement and worsening were defined as a 20-mm decrease or a 10-mm increase in the individual WOMAC scores (on a 100-mm scale), respectively, from BL to FU. A 20-mm decrease corresponds to a 20% improvement in pain, which we consider a statistical and clinically relevant change that would support a real improvement for a chronic progressive disease such as OA. A 10-mm increase in the individual WOMAC, corresponding to a worsening, was considered clinical relevant for assessing progression in the disease. Our cutoffs are exploratory and inspired by Erdogan et al. and Angst et al. discussing minimal clinically significant differences and the smallest detectable difference [21–24].

2.2. Collagen expression in human cartilage and synovial samples

The use of all surgically discarded human cartilage and synovial tissue was approved by the New York University Institutional Review Board. The knee OA patients were free of nonsteroidal anti-inflammatory and steroid drugs for at least two weeks before surgery. Tissues were obtained from OA patients with advanced end-stage OA at the time of knee joint replacement surgery (age 40–80 years). Control, nonarthritic knee tissues were obtained from autopsy patients within 24 h (NDRI, Philadelphia, PA, USA) and were within the same age range (40–80 years) as the OA specimens. Total RNA was extracted from frozen cartilage, as were synovial biopsies from seven healthy donors and seven OA patients [25]. Briefly, cartilage tissues were milled to powder using Freezer Miller (SPEX, Metuchen, NJ, USA). RNA was extracted in TRI Reagent (MRC Labs, Cincinnati, OH, USA). Total RNA from the synovium was extracted as reported previously [26]. Total RNA was precipitated with equal volumes of isopropanol and purified using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA, USA). All procedures were carried out according to the manufacturer's directions. Complete details of cartilage RNA extraction and pooling of RNA for microarray analysis can be accessed on the NCBI site (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169077 and GSE206848). The fragmented cDNA (target) was hybridized against human U133A arrays, as suggested by the manufacturer (Affymetrix), and expression was normalized as described previously [27]. The cartilage and synovium microarray data can be accessed at the GEO repository (GSE169077 and GSE206848). Please also refer to Attur et al. for further description of the samples and methodology [25].

Table 1
Patient demographics and characteristics.

Characteristics	All OA patients	controls	Comparison between OA patient and controls
N (n, with full dataset)	146	21	
Females, n (%)	90 (62.0)	9 (42.9)	0.102[a]
Mean age (SD), years	62.5 (10.1)	56.2 (8.8)	0.008[b]
BMI (kg/m2) (mean, SD)	26.6 (3.6)	26.7 (4.1)	0.929[b]
Race, n (African, Asian, European, other)	32/11/102/1	7/3/11/0	0.090[a]
NSAID use (n, %)	32 (22.1)	-	
Contra-lateral RKOA (n, %)	61 (59.8)	-	-
Signal knee clinical assessments (mean, SD)			
WOMAC total (0-300)	111.7 (66.8)	-	
WOMAC pain (0-100)	35.4 (22.9)	-	
WOMAC function (0-100)	32.4 (23,3)	-	
WOMAC stiffness (0-100)	40.8 (25.7)	-	
VAS knee pain (0-100)	41.7 (28.3)	-	
KL grade (0-4)			
0	14	15	<0.0001
1	22	6	
2	20	0	
3	47	0	
4	8	0	
NA	36		
Serum Biomarkers (estimated marginal means, SE)[c]			
S-C1M	3.95 (0.04)	3.95 (0.10)	0.984
S-C2M	-1.14 (0.03)	-1.07 (0.08)	0.403
S-C3M	2.93 (0.03)	2.86 (0.07)	0.417
S-C4M	3.64 (0.05)	3.72 (0.13)	0.585
S-PRO-C1	4.87 (0.04)	4.74 (0.10)	0.234
P-PRO-C2	0.52 (0.05)	0.69 (0.12)	0.224
S-PRO-C3	2.55 (0.03)	2.65 (0.09)	0.301
S-PRO-C4	5.43 (0.03)	5.31 (0.08)	0.137

[a] Chi-squared test (α=0.05).
[b] Mann-Whitney test.
[c] ANCOVA using LN transformed data and adjusting for age, gender, race and BMI, Bonferroni corrected.

2.3. Biochemical markers

Collagen markers measured Type I, II, III, and IV collagen formation and degradation, in serum samples at BL and in 21 healthy donor samples

from the same institute. All markers have been described previously: PRO-C1 [28], PRO-C2 [28], PRO-C3 [29], PRO-C4 [30], C1M [31], C2M [32], C3M [33], and C4M [34]. All markers were measured in manual competitive immune assays. Samples were measured in duplicate with an intra-assay variation of <15%. Samples with a variation >15% were rerun. Three control samples were added to all plates to monitor the inter-assay variation. A variation of 15% was allowed. All plates met the target. All assays were technically validated according to EMA guidelines for analytical method validation [35].

2.4. Statistics

All analyses were considered post hoc and exploratory. No missing data were imputed; no data were excluded from the dataset. Given the explorative nature of the analyses we did not corrected for multiple testing. Significance levels are given as follows: #$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$. Demographic and clinical data are shown as mean and SD. Comparison between healthy controls on the demographics was done by a chi-square test or the Mann-Whitney test ($\alpha = 0.05$). The biomarkers were not normally distributed and, therefore, ln-transformed. Comparing ln-transformed biomarkers between patients and controls was done by ANCOVA, adjusting for age, gender, race, and BMI. Differential expression was investigated by the mean of each transcript, assessing the transcription of the particular collagen chain. A two-tailed *t*-test ($\alpha = 0.05$), not assuming equal variance, was done to assess differential expression between OA and non-OA samples. Verification of CRPM cutoff was done by receiver-operator curve (ROC) analysis, setting a precision range of 20% of the previously identified cutoff for a low and high level of the marker: 7.2–10.8 ng/mL [18]. Multivariate linear regression was conducted to test the correlation between ln-transformed biomarker data and WOMAC scores, adjusting for age, gender, race, BMI, and NSAID use. Backward logistic regression was used to find predictors for the outcomes, and the AUCs with 95% confidence intervals were used to assess the model's power to predict the outcome.

3. Results

3.1. Cohort description

The percentage of females and the mean age were lower and the race distribution differed in the control group (Table 1). Mean BMI was no different between the two groups. NSAID use and PROs were not recorded for the control group. Six of the 21 controls had a Kellgreen-Lawrance grade of 1. None of the measured biomarkers were different between the OA and healthy control groups (Table 1). Twenty-two percent of the OA patients were on NSAIDs at BL, and 60% had contralateral knee OA.

The collagen degradation and formation serum markers correlated to other serum markers associated with inflammation and tissue remodeling (see Supplementary Table S1).

3.2. Collagen expression in OA tissues

Multiple probes represent some of the collagen genes in the Affymetrix U133A array, and the values are averaged and presented in Table 2. We first looked for the expression of collagens in non-OA and end-stage OA at cartilage and synovium tissue levels. The following collagen chains were significantly and differentially expressed in OA cartilage relative to non-OA cartilage: Type Iα1 and Type Iα2, Type IIα1, Type IIIα1, and Type IVα1 and Type Ivα4 (Table 2). In contrast, in the synovium, Type Iα2, Type IIα1, Type IVα3, and Type IVα6 were significantly expressed in OA relative to non-OA synovium (Table 2). Type IVα6 in the synovium was the only collagen chain that was less expressed in OA tissue compared to non-OA. Supplementary Tables S2 and S3 provide a list of the transcripts assessed.

3.3. Verifying cut-off for high and low CRPM

Previous studies have shown, High CRPM (>9 ng/ml) is associated with radiographic OA progression due to an active inflammatory profile, contrasting with OA patients with low CRPM [18]. By ROC analysis, OA vs. healthy controls, we verified this cut-off at 8.72 using Youden index associated criterion value (Fig. 1), which is within 20% precision range of the previously identified cut-off of 9 ng/mL. The specificity and sensitivity mean [ranges] at this cut-off were 81.0 [58.1–94.6] and 43.7 [35.4–52.2]. A clinical descriptive table can be found in the supplementary section (Table S4).

3.4. Correlation between PROs and collagen formation and degradation markers

We correlated tissue biomarkers with WOMAC scores and divided patients into two groups based on a CRPM threshold (9 ng/mL) to explore

Table 2
Fold mRNA expression of collagens from OA cartilage (n = 6) and synovium (n = 7) compared with non-OA tissues (5 cartilage and 7 synovial biopsies). The *P*-values are not corrected for multiplicity.

Gene		Non-OA		OA			
Cartilage	No. of transcripts	Average	SD	Average	SD	Fold Change	T-test
COL1A1	5	2614	2367	9239	9421	3.53	0.0021
COL1A2	2	5842	4564	27,264	10,293	4.67	0.00005
COL2A1	2	18,796	8951	40,893	9887	2.18	0.00006
COL3A1	3	10,522	9493	26,734	8608	2.54	0.013
COL4A1	2	1233	342	1488	297	1.21	ns
COL4A2	2	791	304	1107	509	1.40	ns
COL4A3	7	384	124	389	130	1.01	ns
COL4A4	1	340	16	395	34	1.16	0.018
COL4A5	1	395	101	452	90	1.15	ns
COL4A6	5	721	246	763	282	1.06	Ns
Synovium							
COL1A1	4	1086	1630	1698	2512	1.56	ns
COL1A2	2	6386	3408	7216	2883	1.13	0.028
COL2A1	2	6.5	3.3	9.7	9.2	1.50	0.000031
COL3A1	3	7588	3408	8935	1846	1.18	ns
COL4A1	2	907	575	1210	698	1.33	ns
COL4A2	2	907	757	1351	976	1.49	ns
COL4A3	7	13.8	11.8	31.8	98.5	2.30	0.0063
COL4A4	1	9.3	4.5	23.1	21.9	2.49	ns
COL4A5	1	21.1	14.0	24.3	20.0	1.15	ns
COL4A6	3	12.5	6.2	9.9	6.0	0.79	0.040

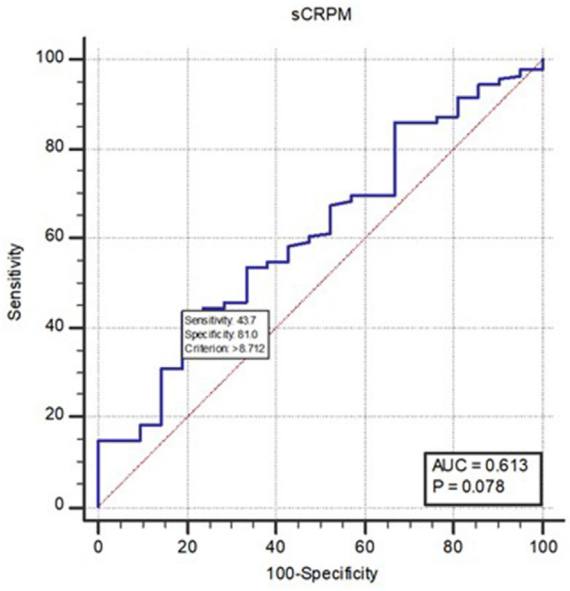

Fig. 1. The area under the curve (AUC) for OA vs. healthy controls.

the association between CRPM, inflammation, and symptom severity [18].

The Type II collagen degradation marker C2M was negatively correlated with WOMAC pain, function, and stiffness before segregation into high and low CRPM. In contrast, C3M was correlated with WOMAC stiffness and C4M with WOMAC pain (Table 3).

The pattern of correlations was markedly different in the two subgroups, as observed by the color coding in Table 3. The Type II collagen formation marker PRO-C2 was negatively correlated with WOMAC stiffness in the high CRPM group ($\beta_{PRO\text{-}C2} = -2.5$, $p < 0.05$), but no correlation was found in the low CRPM group. The Type IV collagen turnover marker PRO-C4 was negatively correlated with WOMAC pain in the low CRPM group ($\beta_{PRO\text{-}C4} = -16.3$, $p < 0.05$), whereas it was positive correlated with WOMAC pain ($\beta_{PRO\text{-}C4} = 40.4$, $p < 0.01$), stiffness ($\beta_{PRO\text{-}C4} = 37.5$, $p < 0.05$), and total ($\beta_{PRO\text{-}C4} = 115.5$, $p < 0.05$) in the high CRPM group (Table 3). The Type I collagen degradation marker C1M was positively correlated with all WOMAC scores ($\beta_{C1M} > 18$, $p < 0.05$) in the high CRPM, but no correlations were found in the low group. C2M was negatively correlated with all WOMAC scores ($\beta_{C2M} < -19$, $p < 0.01$) in the high group but also with WOMAC pain, function, and total in the low CRPM group ($\beta_{C2M} < -16$, $p < 0.05$) and in the high CRPM group. The Type III collagen degradation marker C3M was negatively correlated with WOMAC function ($\beta_{C3M} = -33.9$, $p < 0.05$) while being positively correlated with WOMAC pain ($\beta_{C3M} = 24.1$, $p < 0.05$) and function in the low CRPM group ($\beta_{C3M} = 27.0$, $p < 0.01$) (Table 3).

We also tested whether a combination of the biomarkers and demographics could predict the presence of symptomatic knee OA. In the ALL population, the AUC was 0.69 [0.61–0.77] ($p = 0.0042$), where the predictors were PRO-C3, C2M and NSAID-use. No predictor was found in the low CRPM group, while the combination of C1M and C3M was predictive with an AUC of 0.75 [0.61–0.86] ($p = 0.0048$) (data not shown).

3.5. Collagen formation and degradation as predictors of symptomatic improvement

Next, we investigated whether any markers were predictors of symptomatic improvement (Table 4). Based on results in Table 3, $CRPM_{low/high}$ was a predictor in the logistic regression analyses. C2M and BMI were independent predictors of a 20-mm decrease in WOMAC pain with an AUC of 0.68 ($p < 0.01$), and C2M alone was a predictor of WOMAC stiffness regression (AUC = 0.64, $p < 0.01$). The combination of C1M, C2M, C3M, and $CRPM_{low/high}$ was predictive of function improvement (AUC = 0.74, $p < 0.01$), while the combination of PRO-C2, C1M, C2M, NSAID use, and age was predictive of a 60-mm improvement in the total score (AUC = 0.78, $p < 0.01$).

Patients with marked symptomatic disease are statistically more likely to regress than those with mild symptomatic disease. Thus, we also tested the ability of the markers to predict the level in patients with marked symptomatic disease (Table 4, last column). The rate of patients experiencing improvement in the subpopulation ranged between 33% and 43%, in contrast to the population ranging from 16% to 26%. Together with C4M, BMI and age were predictive of pain improvement (AUC = 0.67, $p < 0.05$). C2M was still the only predictor of stiffness improvement. However, the predictor was no longer significant. C1M,

Table 3
Multiple variate regression. Regression coefficient β for the correlation between PROs and ln transformed collagen biomarker adjusted for age, gender, race, BMI, contra-lateral knee OA, and NSAID use. Significance levels are given as follows: #p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. Red and blue indicate positive and negative correlations, respectively. The *P*-values are not corrected for multiplicity.

	ALL (n=141)				Low CRPM (n=82)				High CRPM (n=59)			
	WOMAC				WOMAC				WOMAC			
	Pain	Function	Stiffness	Total	Pain	Function	Stiffness	Total	Pain	Function	Stiffness	Total
PRO-C1	4.4	2.6	-0.8	4.1	7.3	8.7	9.6	-22.5	2.1	-4.6	1.3	-5.0
	-	-	-	-	-	-	-	#	-	-	-	-
PRO-C2	0.7	-2.1	1.3	2.5	-2.4	-3.0	-0.3	-1.3	-5.1	-11.2	-22.5	-43.4
	-	-	-	-	-	-	-	-	-	-	*	-
PRO-C3	2.7	3.9	-3.3	-0.4	-4.4	4.4	1.1	1.0	-9.4	-19.3	-20.0	-48.7
	-	-	-	-	-	-	-	-	-	-	-	-
PRO-C4	-0.9	-0.1	0.2	4.0	-16.3	-6.9	-5.1	-25.2	40.4	23.6	37.5	115.5
	-	-	-	-	*	-	-	-	**	#	*	**
C1M	2.8	7.2	-0.1	11.2	1.8	14.4	6.2	22.1	19.0	18.9	22.1	65.2
	-	-	-	-	-	-	-	-	*	*	*	*
C2M	-25.8	-20.9	-25.7	-73.1	-19.8	-16.4	-12.2	-50.0	-34.4	-19.7	-35.4	-96.0
	****	***	***	****	*	*	#	*	***	**	****	****
C3M	5.5	-10.1	-21.2	-26.0	24.1	27.0	7.9	58.3	-17.4	-33.9	-24.5	-71.6
	-	-	**	-	*	**	-	#	-	*	#	#
C4M	-5.5	-1.1	-1.0	-5.2	-13.5	-9.2	-2.7	-27.5	2.5	13.2	14.9	33.1
	**	-	-	-	#	-	-	-	-	-	-	-

Table 4
Prediction of symptomatic improvement OA. Multiple variate logistic regression, including LN-transformed biomarkers (PRO-C1, PRO-C2, PRO-C3, PRO-C4, C1M, C2M, C3M, C4M, and CRPMlow/high)) and baseline clinical characteristics (age, gender, race, BMI and NSAID-use). The *P*-values are not corrected for multiplicity.

Symptomatic improvement[a] vs. stable/progressive disease		Biomarkers and clinical variates	Biomarkers and clinical variates Patients with marked symptomatic disease[c]
WOMAC pain	n (%)[b]	134 (26.1)	76 (40.8)
	Predictors	C2M, BMI	C4M, BMI, Age
	AUC [95%-CI]	0.68 [0.59–0.76]	0.67 [0.56–0.78]
	P	0.0023	0.016
WOMAC stiffness	n (%)[b]	134 (16.4)	80 (42.5)
	Predictors	C2M	C2M
	AUC [95%-CI]	0.64 [0.55–0.72]	0.61 [0.50–0.72]
	P	0.0085	0.057
WOMAC function	n (%)[b]	134 (16.4)	68 (27.9)
	Predictors	C1M, C2M, C3M, $CRPM_{low/high}$	C1M, Age, NSAID-use
	AUC [95%-CI]	0.74 [0.65–0.81]	0.75 [0.63–0.85]
	P	0.0015	0.044
WOMAC total	n (%)[b]	134 (19.4)	73 (32.9)
	Predictors	PRO-C2, C1M, C2M, NSAID-use, Age	C1M, Age, NSAID-use
	AUC [95%-CI]	0.78 [0.70–0.85]	0.61 [0.49–0.73]
	P	0.0011	0.013

[a] Symptomatic improvement was defined as a 20 mm or more decrease in WOMAC pain, stiffness, or function and a 60 mm or more reduction in WOMAC total over two years.
[b] Rate of regression in percentage in each of the sub-populations.
[c] More than 30 and 90 mm at baseline for the individual and total scores, respectively.

Table 5
Prediction of symptomatic regressive OA. Multiple variate logistic regression LN-transformed biomarkers (PRO-C1, PRO-C2, PRO-C3, PRO-C4, C1M, C2M, C3M, C4M, and CRPMlow/high), and baseline clinical characteristics (age, gender, race, BMI and NSAID-use). The *P*-values are not corrected for multiplicity.

Progressive[a] vs. stable/regressive disease		Biomarkers and clinical variates	Biomarkers and clinical variates Patients with mild symptomatic disease[c]
WOMAC pain	n (%)[b]	134 (18.7)	58 (24.1)
	Predictors	Race	C1M
	AUC [95%-CI]	0.59 [0.50–0.67]	0.61 [0.47–0.73]
	P	0.037	0.056
WOMAC stiffness	n (%)[b]	134 (20.9)	54 (27.8)
	Predictors	Age	PRO-C3, C1M, C3M, C4M
	AUC [95%-CI]	0.67 [0.58–0.75]	0.61 [0.50–0.72]
	P	0.0036	0.011
WOMAC function	n (%)[b]	134 (17.9)	66 (18.2)
	Predictors	PRO-C2, C2M, C3M, C4M, Race	C3M, C4M, Race
	AUC [95%-CI]	0.77 [0.69–0.84]	0.84 [0.73–0.92]
	P	0.0019	0.0017
WOMAC total	n (%)[b]	134 (17.9)	61 (23.0)
	Predictors	C3M, C4M, Age	C1M, C2M, BMI, Race
	AUC [95%-CI]	0.67 [0.58–0.75]	0.80 [0.68–0.90]
	P	0.091	0.012

[a] Progressive disease was defined as a 10 mm or more decrease in WOMAC pain, stiffness, or function and a 30 mm or more decrease in WOMAC total over two years.
[b] Rate of regression in percentage in each of the sub-populations.
[c] Less than 30 and 90 mm at baseline for the individual and total scores, respectively.

together with age and NSAID use, was predictive of function improvement (AUC = 0.75, $p < 0.05$) and of total score improvement (AUC = 0.61, $p < 0.05$).

3.6. Prediction of symptomatic worsening

Next, we investigated whether any markers were predictors of symptomatic worsening (Table 5). Race was the only independent predictor of a 10-mm increase in WOMAC pain with an AUC of 0.59 ($p < 0.05$), while age alone was an independent predictor of WOMAC stiffness worsening (AUC = 0.67, $p < 0.01$). The combination of PRO-C2, C2M, C3M, C4M, and race was predictive of function worsening (AUC = 0.77, $p < 0.01$). No significant predictors were found for the total WOMAC increase.

Patients with mild symptomatic disease are statistically more likely to progress than those with marked symptomatic disease. Thus, we also tested the ability of the markers to predict symptomatic worsening in patients with mild symptomatic disease (Table 5, last column). No significant predictors were found for WOMAC pain increase. Together, PRO-C3, C1M, C3M, and C4M were predictors of stiffness worsening (AUC = 0.61, $p < 0.05$). C1M, C4M, and race were predictors of function worsening (AUC = 0.84, $p < 0.01$). C1M, C2M, race and BMI were predictors of total WOMAC worsening (AUC = 0.80, $p < 0.05$).

4. Discussion

In this study, we found significant upregulation of Types I, II, III, and VIα4 collagens in OA cartilage compared to healthy samples. In OA synovium, Type Iα1, Type II, and Type IVα3 collagens were upregulated, while Type IVα6 was downregulated, indicating ongoing tissue remodeling. Among all participants, only Type II collagen degradation (C2M) was negatively associated with WOMAC scores, suggesting that higher levels of C2M were associated with less pain. These findings contrast with previous publications linking Type II collagen fragments like CTX-II to pain [15]. Our results may suggest that different Type II collagen fragments provide distinct pathological information about remodeling in the presence or absence of inflammation leading to varying associations with patient-reported outcomes (PROs). In addition, there may be confounding factors which we presently are not aware off. Similar trends were observed for C3M and C4M, although the significance was lower.

The low tissue inflammation population MMP-mediated tissue destruction of Type III collagen (C3M) was positively correlated with pain and function scores. Type III collagen is a main collagen of the interstitial ECM, including synovium, and degraded by MMPs sequestered in the tissue by inflammatory cells during tissue inflammation [36]. C3M is likewise pharmacodynamically modulated by anti-inflammatory treatments in OA and rheumatoid arthritis [37,38]. We speculate that patients with low CRPM levels display noninflammatory endotype where it is possible to assess local changes to joint tissue degradation (C3M), which are associated with PROs recorded on that joint (in our case, the knee). In the high CRPM group, there was a limited but negative association. Conversely, cartilage destruction quantified by Type II collagen degradation by MMPs (C2M) was negatively associated with the WOMAC scores, though to a lesser extent than that of the ALL population and the CRPM-high population. Thus, for the CRPM-low population, there seems to be a weaker association between cartilage degradation and PROs.

The high CRPM population displayed the most significant associations between PROs and tissue remodeling markers. MMP-mediated tissue destruction of Type I collagen (C1M) and Type IV collagen remodeling (PRO-C4) were positively correlated with pain and function scores: the higher the marker, the higher the level of PROs recorded. This association coincides with a highly significant negative correlation between C2M and PROs. These data suggest that in the inflammatory situation there is a relationship between how the patients function and feel

with the level of interstitial and basement membrane matrix turnover. This suggests that the repair processes of the basement membrane (PRO-C4) and MMP-mediated tissue destruction caused by tissue inflammation are clearly associated to pain in those patients with chronic inflammation. Through the predictive analyses, we showed that collagen degradation and formation could be independent predictors of symptomatic progression and regression.

These results indicate that collagen degradation and formation are associated with symptomatic OA and that those associations differ depending on the inflammatory subgroup to which the patients belong. This also suggests that tissue turnover is important for how patients function and feel. In direct alignment, C1M and C4M are shown to predict the progression of joint damage in rheumatoid arthritis [34,39]. In OA, C1M was associated with neuropathic pain in patients scheduled for a total joint replacement [31]. Moreover, markers of cartilage degradation and formation (e.g., urinary CTX-II, PRO-C2, and PIIANP) are predictive of radiographic progression and symptomatic progression [15,28,40,41].

Our findings of a negative association between pain and the Type III collagen marker C3M do not stand alone. In a recent publication by Yang et al., a negative association between pain and C3M was found [42]. This suggests that collagen remodeling is essential for disease activity, which is correlated to the velocity of the progression of diseases. In fact, all bone treatments either prove efficacious by preventing bone resorption or stimulating bone formation, quantified by a change in CTX-I or PINP [14], respectively. This suggests that the collagen tissue turnover biomarkers need to be affected to demonstrate efficacy for diseases in which the collagen is vital. While healthy and osteoporotic individuals do not feel the physiological bone remodeling [13], bone cancer is associated with high bone erosion, ECM remodeling, and pain [43,44], in which bone resorption molecular biomarker (CTX-I) levels are well outside the healthy reference range [45]. This is a well-documented but underappreciated area, suggesting that pathological remodeling of tissues generates different signals, which cause pain, in contrast to healthy remodeling. The exact reason for this phenomenon is still being investigated. Most likely, the cause is a combination of remodeling areas, including functional nerves, accelerated remodeling with aggressive and overactive cells, disorganized organ composition, and altered molecular components.

Recently, much attention has been directed to how specific danger-associated molecular patterns activate toll-like receptors (TLRs). The relation of protein fragments during tissue remodeling, such as the fragments of aggrecan [3] has been shown to stimulate TLRs directly [46], induce interleukin-6 expression by almost 100-fold, and cause pain [47]. This may be a causal link between ECM remodeling and pain [46].

In continuation of the relationship between disease activity, progression, and PROs to that of ECM biomarkers, a range of ECM biomarkers associated with patients performing the 6-min walk test [48], demonstrating a clear link between how patients function and feel and the level of ECM remodeling. Although many components affect a simple 6-min walk test and many tissues are involved, from muscle to the skeletal systems and lung and heart capacity, this suggests that we need to understand ECM remodeling in more detail.

This study has several limitations. The analyses only suggest associations, as the study was explorative and the hypothesis was formulated after the study's completion and the study was not specifically designed for this investigation. Power analysis was not conducted due to the exploratory nature of the study. To confirm predictive value, future test and validation studies with prospective hypotheses are required. For determining progression and regression, a cutoff of 20% and 10% increase and decrease, corresponding to 20-mm and 10-mm changes, respectively, was chosen based on previous publications assessing clinically meaningful differences (ranging from 7 mm to 19 mm depending on the score assessed) [21–24]. The selected cutoffs should be more carefully determined, and sample size estimations in a prospective study should be conducted. Our work does not provide insight into how general turnover (e.g., degradation, posttranslational modification) are associated with gene regulation.

In conclusion, assessment of collagen formation and degradation in blood reflecting joint tissue turnover may provide a link between how patients function and feel and structural changes.

Author contribution

ACBJ, MA and MAK have designed, analyses and written up the manuscript. JA, CST and SAA have provided advise on the design, analysis and have reviewed the manuscript. MA, JA and SAA have been responsible for the biospeciments, while ACBJ, CST and MAK have been responsible for the biomarker measurement and validation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocarto.2023.100379.

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