Type I and III collagen turnover is increased in axial spondyloarthritis and psoriatic arthritis. Associations with disease activity and diagnostic capacity

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Abstract

Objective

To investigate the turnover of type I and III collagen by neo-epitope markers in patients with axial spondyloarthritis (axSpA) and psoriatic arthritis (PsA).

Methods

Patients with PsA (n=101) or axSpA (n=110) and healthy subjects (n=120) were included. Demographic and clinical data were recorded. Markers of type I and III collagen were quantified by RIA (ICTP) or ELISA (C1M and C3M). Non-parametric statistics were applied for intergroup comparisons and correlation studies. The diagnostic potential of these marker molecules was assessed by ROC analysis.

Results

C1M and C3M, which originate from soft connective tissues, were significantly higher in axSpA and PsA as compared with healthy control subjects. CIM and C3M correlated with ASDAS and DAS28. Overall, ICTP, which arises from bone degradation, did not differ between disease versus healthy. However, ICTP was lower in HLA-B27 positive than in HLA-B27 negative patients with axSpA. There was no association between bone and soft connective tissue collagen I markers (ICTP and C1M), while C1M and C3M were highly correlated ($p<0.0001$). C1M discriminated between healthy and diseased with AUCs of 0.83 for PsA and 0.79 for axSpA. C3M AUCs were 0.77 for PsA and 0.78 for axSpA.

Conclusion

Type I and III collagen remodelling in soft connective tissue is increased in axSpA and PsA and associates with disease activity. Bone collagen degradation is lower in HLA-B27 positive compared with HLA-B27 negative axSpA, which may represent an aspect of enhanced enthesopathic bone proliferation in HLA-B27 carriers. C1M and C3M distinguish well between healthy and diseased individuals.

Key words

spondyloarthropathies, axial spondyloarthritis, psoriatic arthritis, biomarkers, collagens

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Introduction

Axial spondyloarthritis (axSpA) and psoriatic arhtritis (PsA) are core members of the spondyloarthritis (SpA) complex. Synovitis, osteitis and enthesitis, with enhanced inflammation driven remodelling of bone and soft connective tissue of peripheral joints, are cardinal characteristics shared by the two pathologies. The aetiology of both diseases is largely unknown, but genetic and environmental triggers are implicated (1, 2). Early diagnosis and availability of reliable tools for assessment of disease activity and identification of patient subsets in particular need for intensive management are crucial for improving long-term prognosis. In the present era of increasingly targeted biologic therapies, fine mapping of the current metabolic state of joint structural elements may be a useful adjunct to conventional measures for precision diagnosis and disease surveillance (3).

Seromarkers of type I and III collagen turnover based on quantification of matrix metalloproteinase (MMP) generated protein fragments using ELISA or RIA have previously been applied to joint diseases including osteoarthritis (4, 5), rheumatoid arthritis (6-8) and ankylosing spondylitis (9-11). The seromarkers were associated with disease progression and treatment response. A common feature of these diseases is uncoupling of extracellular matrix metabolism with overweight of anabolic or catabolic pathways. This often leads to syndesmophyte and spur formation like in spondyloarthritis (12, 13) or destructive joint lesions as seen in rheumatoid arthritis and psoriatic arthritis (14, 15). Type I collagen is the most abundant structural element in soft connective tissues and bone, where it constitutes around 80% of the total protein content (16). During homeostatic turnover of bone, type I collagen degradation is mainly mediated by MMPs leading to release of a crosslinked C-telopeptide (ICTP) (17, 18). ICTP is used as disease marker in various bone pathologies including ankylosing spondylitis (AS) and rheumatoid arthritis (19). By contrast the C1M fragment is considered to be a marker of soft tissue type I collagen turnover because it is degraded by cathepsin K mediated collagenolysis (20). The C3M fragment arises from MMP degradation of type III collagen (21) and both C1M and C3M are released from the synovial membrane (22).

Based on these observations we hypothesised that remodelling of type I and type III collagen is increased in axSpA and PsA. We therefore aimed to study soluble type I and III collagen neoepitope patterns in the systemic circulation of patients with axSpA or PsA in order to assess their clinical utility as disease process markers and their diagnostic capacity against a healthy reference population.

Materials and methods

Study populations

The study population has previously been described by Munk *et al.* (23). The inclusion criteria of the study were x-ray or MRI verified axial SpA (n=110) according to the ASAS criteria (24, 25) or PsA (n=101) fulfilling the CASPAR criteria. Patients fulfilling both criteria were categorised as PsA. Exclusion criteria were age <18 and >75 years, heart failure, past or present malignancy, elevated serum creatinine or ALAT, hepatitis B, C or other inflammatory diseases. Demographics and disease parameters obtained at baseline included sex, age, BMI, smoking status, treatment and HLA-B27 status (Table I). In PsA IgM-rheumatoid factor and anti-CCP were recorded as well. Additional disease characteristics included: swollen and tender joint counts (68 joints), Spondyloarthritis Research Consortium of Canada (SPARCC) enthesitis index (27), Health Assessment Questionnaire (HAQ score), visual analogue scale (VAS pain, fatigue, patient and physician global assesment), Bath Ankylosing Spondylitis (BAS) indices (BASDAI, BASFI, BASMI) and disease Activity Scores (DAS28 and ASDAS-CRP). X-ray or MRI recordings of sacroiliac joints in patients with SpA and in PsA with axial involvement were available from the time of diagnosis, but were not repeated for this study. All blood samples were collected as non-fasting between 9am–4pm, followed by clotting for 30–120 min. and

centrifugation for 12 min. at 2.200 rpm. Samples were frozen at -80°C until analysis. As healthy subjects 120 blood donors aged 20–65 years with an equal number of men and women in each decade were recruited. The study was approved by the Regional Scientific Ethics Committee of Southern Denmark (j.nr S-20110086) and conducted in accordance with the Helsinki II Declaration. Consent to participate in the study was obtained from all patients.

Biomarker measurements

Three neo-epitope markers were measured in serum: MMP degraded type I collagen (C1M[20] and ICTP[17]) and type III collagen (C3M[21]). The analyses were carried out in duplicate and the measurements were performed on blinded samples. ICTP was measured by radioimmunoassay (RIA) (Orion Diagnostica, Finland). The assay employs polyclonal antibodies raised in rabbits against ICTP, which consists of three peptides from 2 type I collagen molecules and a mature trivalent crosslink (17). Intra- and inter-assay coefficients of variation were around 5% and 7%, respectively. C1M (20) and C3M (28) (Nordic Bioscience, Herlev, Denmark) were measured using competitive enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies raised in mouse. Intra- and inter- assay coefficients of variation were below 12% and 14% respectively for both C1M and C3M. All analyses were performed according the manual provided by the manufacturer.

Statistics

All analyses were performed in MedCalc Statistical Software v. 14.8.1 (MedCalc Software bvba, Ostend, Belgium; 2014). Means were calculated for variables with a normal distribution and medians for variables, which were not normally distributed. Intergroup comparisons were made by Kruskal Wallis and Mann-Whitney tests. Correlation analyses were performed by Spearman's rank test. Bonferroni corrections were applied to correct for multiplicity. Statistical significance was considered when $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. The discriminative power of the neo-epitope markers between healthy and diseased was calculated by receiver operator characteristics (ROC) and expressed by the area under the curve (AUC). Youden index was applied to determine the optimum sensitivity and specificity and the corresponding cut-off values.

Table I. Patient characteristics, disease activity measures and serum level of ICTP, C1M and C3M in axSpA, PsA and healthy subjects.

	axSpA (n=110)	PsA (n=101)
Sex (% male)	72%	59%
Age (y)	36.6 (35.3-38.0)	37.0 (35.6-38.5)
BMI	25.5 (24.8-26.3)	27.4 (26.2-28.5)
Smoker (%)	36%	35%
HLA-B27 (%)	87%	20%
Disease duration (y)	6.4 (5.4-7.5)	6.7 (5.6-7.8)
Patient global VAS	34 (29-39)	39 (34-45)
Patient pain VAS	32 (27-37)	32 (27-37)
Patient fatigue VAS	40 (34-45)	43 (37-49)
Physician global VAS	4 (1;16)	3 (1;14)
BASDAI	31 (26-35)	36 (31-41)
BASFI	23 (19-27)	27 (22-32)
BASMI	10 (0;20)	10 (0;10)
ASDAS(CRP)	2.0 (1.8-2.3)	2.1 (1.9-2.4)
Swollen joint (%)	9%	38%
hs-CRP (mg/l)	3 (1;7)	3 (1;9)
Conventional DMARDs naïve (%)	73%	20%
Biologic DMARDS naïve (%)	39%	54%
ICTP (ug/l) healthy subjects 3.60 (3.4-3.7)	3.50 (3.30-3.90)	3.35 (3.20-3.50)
C1M (ng/ml) healthy subjects 48.3 (37.2-64.2) ng/ml	77.6 (58.8-114.2)	85.6 (64.1-127.4)
C3M (ng/ml) healthy subjects 20.9 (17.9-24.1) ng/ml	27.7 (23.0-34.2)	28.7 (23.2-35.8)

Means were calculated for variables with a normal distribution (age, BMI, disease duration, global VAS, pain VAS, fatigue VAS, BASDAI, BASFI, ASDAS) and medians for variables, which were not normally distributed (physician global VAS, BASMI, CRP, ICTP, C1M, C3M). Numbers in parentheses show 95% confidence intervals (CI) and 25-75 percentiles, respectively.

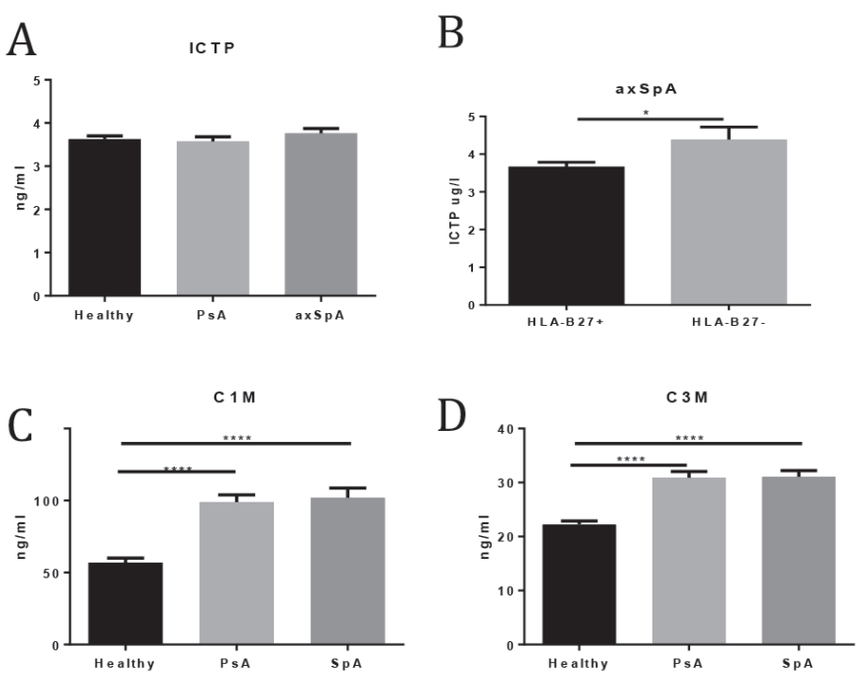

Fig. 1. Biomarker levels in axSpA, PsA and healthy controls. A) ICTP in SpA, PsA and healthy subjects. B) ICTP in HLA-B27 positive vs. HLA-B27 negative axSpA. C) C1M in axSpA and PsA compared to healthy subjects. D) C3M in axSpA and psoriatic arthritis (PsA) compared to healthy subjects. Data are shown as mean + SEM. $^{*}p<0.05$, $^{****}p<0.0001$.

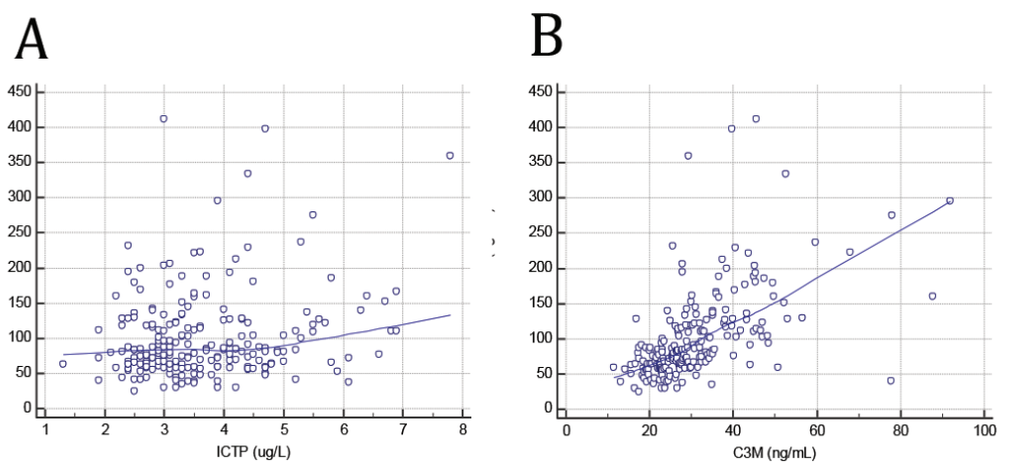

Fig. 2. The associations between the neo-epitope markers:
A) ICTP *vs*. C1M,
B) C3M *vs*. C1M.

Results

Patient demographics and clinical characteristics

Patient demographics and disease characteristics are presented in Table I. Clinical characteristics are listed for patients, but were not available from the healthy subjects. The two disease groups were similar in terms of most characteristics (with the exception of sex, HLA-B27 positivity and swollen joint count).

Neo-epitope marker levels in axSpA and PsA

There was no significant difference between diseased and healthy subjects when evaluating ICTP (Fig. 1A). However, HLA-B27 positive axSpA had significantly lower levels of ICTP compared to HLA-B27 negative axSpA (p=0.029) (Fig. 1B). There were no statistically significant differences in ICTP levels when patients were separated according to smoking status, male/female, current treatment (neither biologics nor synthetic disease-modifying anti-rheumatic drugs (DMARD)) or SJC68 in patients with axSpA. In PsA males had significantly higher ICTP levels compared to females (p=0.006) (data not shown).

Both C1M and C3M were significantly higher in axSpA and PsA compared to healthy subjects (Fig. 1C-D). C1M and C3M did not differ according to HLA-B27, male/female, treatment or any other clinical parameters in any of the two diseases. There were no differences between axSpA and PsA in any of the evaluated neo-epitope markers.

Additionally, there was no association between ICTP and C1M when combining both disease (Fig. 2A), or when separated by disease population or gender. In contrast C1M and C3M were highly correlated (p<0.0001 in all groups) (Fig. 2B).

Associations between neo-epitope markers and clinical parameters

The levels of ICTP were associated with age in axSpA (r= -0.30, p=0.0015). C1M levels were significantly associated with ASDAS in both axSpA and PsA (r=0.32, p=0.0008 and r=0.32, p=0.001 respectively). C3M levels were also associated with ASDAS in both axSpA and PsA (r=0.28, p=0.003 and r=0.34, p=0.0006 respectively). C1M and C3M levels were both associated with DAS28 in axSpA (r=0.40, p<0.0001 and r=0.39, p<0.0001 respectively). Associations between the biomarkers and the demographic data and disease parameters in the total patient population are listed in Table II.

The discriminative power of C1M and C3M

The discriminative power of the neo-epitope markers C1M and C3M were calculated by ROC (Fig. 3). The AUC

Table II. Correlations between collagen neo-epitope markers and disease variables in axSpA and PsA Spearman's rank levels of the different patient groups and clinical parameters.

	ICTP		C1M		C3M	
	axSpA (n=110)	PsA (n=101)	axSpA (n=110)	PsA (n=101)	axSpA (n=110)	PsA (n=101)
Age	r=-0.30, p=0.0015	ns	ns	ns	ns	ns
BMI	ns	ns	ns	ns	ns	ns
Disease duration	ns	ns	ns	ns	ns	ns
ASDAS	ns	ns	r=0.32, p=0.0008	r=0.32, p=0.001	r=0.28, p=0.003	r=0.34, p=0.0006
DAS28	ns	ns	R=0.40 p<0.0001	ns	R=0.39, p<0.0001	ns
HAQ	ns	ns	ns	ns	ns	ns
BASDAI	ns	ns	ns	ns	ns	ns
BASFI	ns	ns	ns	ns	ns	ns
SJC68	ns	ns	ns	ns	ns	ns
TJC68	ns	ns	ns	ns	ns	ns

Statistical significance was considered if p<0.0045 after correction for multiple testing.

Table III. The AUC, sensitivity and specificity of ROC-analysis of the ability of C1M and C3M to differentiate between healthy *vs*. psoriatic arthritis (PsA) and axial spondyloarthritis (axSpA).

The AUC, sensitivity and specificity of each ROC-analysis

	AUC (CI95%)	Sensitivity	Specificity	Criterion	Relative risk	OR
C1M						
Healthy *vs*. PsA	0.82 (0.76-0.86)	70.0	87.4	≤56.2	2.83	15.5
Healthy *vs*. axSpA	0.79 (0.73-0.84)	68.3	82.7	≤55.5	2.55	9.5
C3M						
Healthy *vs*. PsA	0.77 (0.71-0.82)	79.2	68.9	≤22.6	3.18	8.0
Healthy *vs*. axSpA	0.78 (0.72-0.83)	67.5	80.0	≤25.0	2.4	8.0

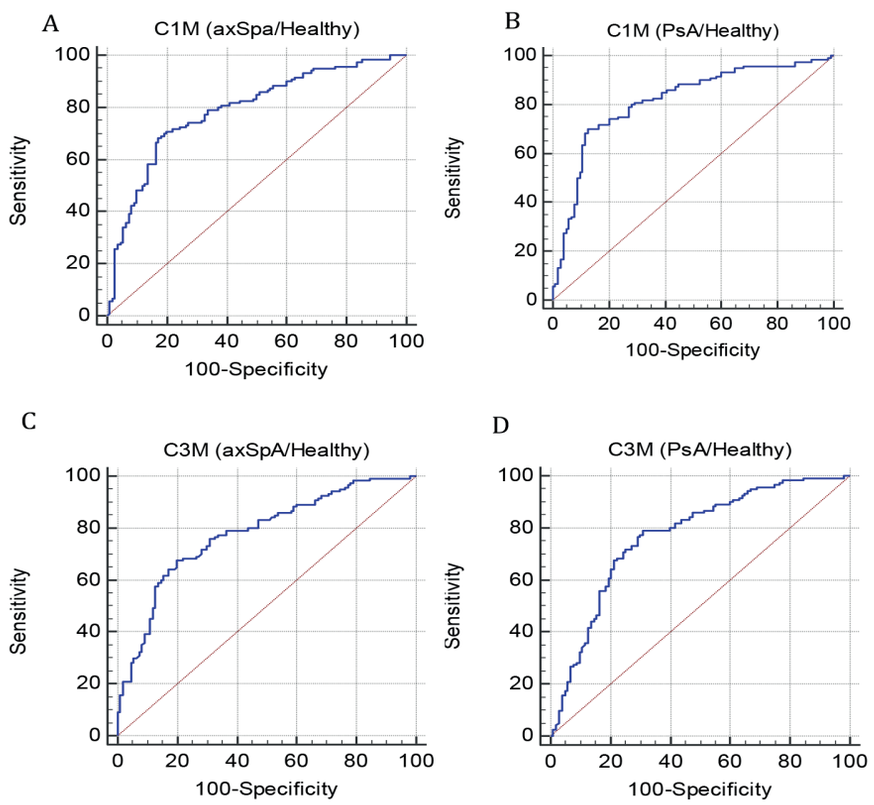

Fig. 3. Receiver-operator characteristics of **A**) C1M levels in axSpA *vs*. healthy, and **B**) C1M in PsA *vs*. healthy, **C**) C3M levels in axSpA *vs*. healthy, and **D**) C3M in PsA *vs*. healthy.

of healthy *versus* disease were, for C1M and C3M, approximately the same for the two diseases. For C1M healthy *versus* axSpA had a value of 0.79 (CI 0.73–0.84) and healthy *versus* PsA 0.82 (CI 0.76–0.86). With a cut-off value of 55.5ng/ml to discriminate between healthy subjects and axSpA, the relative risk of SpA was 2.55 and the odds ratio 9.9. For PsA *versus* healthy the relative risk was 2.83 and the odds ratio 15.5 for values exceeding the cut off 56.25ng/ml. For C3M the AUC was close to those of C1M. Healthy *versus* axSpA had a value of 0.78 (0.72–0.83) and healthy *versus* PsA 0.77 (0.71–0.82). With a cut-off value of 22.6ng/ml to discriminate between healthy subjects and axSpA, the relative risk of SpA was 2.4 and the odds ratio 8.0. For PsA *versus* healthy the relative risk was 3.18 and the odds ratio 8.0 for values exceeding the cut off 22.6 ng/ml. C1M and C3M combined did not increase the AUC compared to C1M alone.

Discussion

Chronic joint inflammation like in axSpA and PsA is associated with enhanced turnover of articular matrix constituents. This may lead to loss of structural integrity and dysfunction due to disruption of the normal tissue architecture by fibrotic and destructive lesions (29). In this study we report, that the neo-epitope markers C1M and C3M were significantly elevated in patients with axSpA and PsA as compared with healthy control subjects. Both C1M and C3M are mainly reflecting soft connective tissue turnover like in the synovial membrane and entheses. Additionally, these seromarkers correlated positively with composite disease activity measures. By contrast, ICTP which is a well-established marker of bone collagen degradation (17), was in the normal range in either disease subset and did not associate with disease activity. However, when stratifying axSpA patients according to HLA-B27 genotype, ICTP was significantly lower in HLA-B27 positives *versus* HLA-B27 negatives. C1M and C3M discriminated well between disease subset and healthy subjects.

Current guidelines for treatment of axSpA and PsA rely mainly on clinical and imaging assessment of disease activity and progression (30, 31). Biologically plausible seromarkers may provide additional process specific information regarding diagnosis and disease activity (32, 33). In general, the clinical utility of serum levels of proinflammatory cytokines for these purposes have turned out to be limited due to their short half-lives, analytic variability and redundancy in signaling pathways (34). The present study shows that the serum levels of soluble neo-epitope markers arising from connective tissue inflammation in axSpA and PsA are increased. This accords with previous reports on C1M and C3M in patients with related inflammatory joint diseases, *e.g*. ankylosing spondylitis (11). Another study reported that C1M decreased significantly during tumour necrosis factor inhibitor (TNFi) treatment in ankylosing spondylitis (AS) (35), while in the present cross-sectional study neither C1M nor C3M appeared to be suppressed in patients receiving biologic agents as compared to TNFi naïve patients. This discrepancy may be attributable to differences related

to study design and stage of disease. Although axSpA and AS have overlapping features, it should be considered that the amplitude and dynamics of the soft connective tissue response to TNFi treatment may differ between these disease entities. Furthermore, it cannot be excluded that the apparent no-response of C1M to TNFi medication in the present study reflects that C1M had actually declined since the onset of TNFi treatment.

Increased ICTP levels have previously been reported in patients with AS compared with healthy subjects (36). We therefore anticipated that the axSpA subpopulation would have higher serum levels of ICTP because axSpA and AS are closely interrelated (37) and patients with axSpA may progress to AS (38). However, our observation of unaltered ICTP accords well with a study by Pedersen *et al*. using CTX-I, a different degradation marker of bone collagen type I. These authors did not find differences between patients with AS and a healthy reference population (39). However, when stratifying the present axSpA cohort according to HLA-B27 status, HLA-B27+ patients had significantly lower ICTP but not C1M levels as compared with HLA-B27 negatives. HLA-B27 is an established risk factor for structural damage progression in the axial skeleton in axSpA (40). Thus, this finding may reflect that the normally well balanced bone collagen formation and degradation is skewed in favor of increased osteogenesis in axSpA, reflecting, *e.g*. outgrowth of syndesmophytes and spur formation. This is supported by previous reports on altered chondro-proliferative activity in HLA-B27 positive patients with axSpA (23, 41). The potential regulatory role of HLA-B27 on type I and II collagen metabolism is uncertain at this time.

ICTP and CIM levels did not correlate supporting the concept that these collagen fragments are generated by distinctive degradative pathways or originate from different tissues. Of note, the neo-epitope recognised by the ICTP assay is, in contrast to both C1M and C3M, cross-linked and thereby detects degraded fragments of collagen fibrils (17). C1M and C3M assays do not differentiate between epitopes released from mature and newly synthesised soluble collagen. This may account for the absence of an association between ICTP and C1M.

This study has some limitations. Type I and III collagens are expressed in most tissues including skin (42), lung (43), liver (44) and joints (22). Thus, a variety of tissues may contribute to the level in the systemic circulation, thereby potentially blunting differences between diseased and healthy neo-epitope patterns. Although, ICTP mainly originates from bone degradation (6, 45) additional sources should also be considered in some pathologies (46, 47). Future studies based on patients with newly diagnosed, DMARD naïve patients only are needed to validate the present seromarker findings in further detail. Strengths of this study are inclusion of two large and clinically well characterised patient populations with axSpA and PsA. To our knowledge this study is the first to evaluate the turnover of the major striated collagen constituents of soft connective tissue and bone collagen simultaneously in axSpA and PsA.

In conclusion, the present study indicates that type I and III collagen remodelling of soft connective tissue is enhanced in axSpA and PsA. Bone collagen turnover is skewed towards increased osteoproliferation in HLA-B27 positive patients with axSpA compared with HLA-B27 negatives. Circulating neo-epitopes arising from degradation of type I and III collagen hold promise as markers of disease pathways and diagnosis in axSpA and PsA.

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