Differentiating patients with psoriasis from psoriatic arthritis using collagen biomarkers

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Abstract

Objective

Around 30% of patients diagnosed with cutaneous psoriasis (PsC) will go on to develop psoriatic arthritis (PsA) which includes inflammation of the joints. Collagens are core proteins in all tissues, which are involved in the inflammatory process in both PsC and PsA. The aim of this study is to investigate collagen biomarkers and their potential use in separating the three patient groupings: PsC, PsA and healthy donors.

Methods

Healthy donors (n=41), patients with PsC (n=30) and patients with PsA (n=30) were recruited. Clinical disease parameters were recorded. Collagen remodelling was measured using ELISA immunoassays which detect the serological anabolic biomarkers quantifying formation of type I, III and IV collagen (PRO-C1, PRO-C3 and PRO-C4 respectively), and the catabolic biomarkers measuring degradation of type I, II, III, IV and X collagen (C1M, C2M, C3M, C4M and C10C respectively).

Results

Patients with PsC and PsA presented lower levels of PRO-C1 and C3M compared to healthy controls ($p<0.05$-$p<0.0001$), C1M was higher in PsA compared to healthy controls ($p<0.0001$) and C2M was all elevated in PsC and PsA compared to healthy controls ($p=0.0002$ and $p=0.0004$ respectively), reflecting alterations in the tissues. In addition, C1M was able to separate between PsC and PsA patients with an AUROC=0.664, indicating that this biomarker may be a biomarker of joint involvement.

Conclusion

This work provides evidence that serum collagen biomarkers are dysregulated in PsC and PsA, as compared to healthy controls. C1M was able to differentiate patients with PsC from PsA and could be a potential biomarker of inflammatory systemic musculoskeletal involvement.

Key words
psoriasis, psoriatic arthritis, collagens, biomarkers

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Introduction

Cutaneous psoriasis (PsC) affects 2–3% of the total world population. It is a chronic immune-mediated skin disease with erythematous, scaly patches – plaques – in the skin, resulting from abnormal proliferation and differentiation of epidermal keratinocytes, combined with immune cell infiltration of the dermis, and increased dermal capillary density (1). Up to 30% of individuals with Pso progress to developing Psoriatic Arthritis (PsA), which is a disease within the group of spondyloarthropathies.

PsA is characterized by joint involvement, enthesitis, dactylitis and spondylitis, and a large majority of PsA patients have skin and/or nail PsC (2). The incidence of PsA increases with time from PsC diagnosis. Nail disease in PsC is a strong predictor for the development of PsA, as are enthesitis, inverse Pso and severity of the cutaneous disease (3). Furthermore, patients with PsC who are positive for anti-citrullinated protein antibodies (ACPA) are at a higher risk of developing PsA, although, overall, only a small percentage of patients are seropositive (4). In addition, other known risk factors for developing PsA include obesity, smoking, family history of autoimmune disease, previous infection, and certain polymorphisms in the HLA and MICA loci (5, 6). However, despite some risk factors being known, there is currently no established way to determine which patient with PsC will develop PsA over time. Thus, the development of tools, such as biomarkers, that will allow the separation of patients with PsC from PsA, and potentially identify PsC patients who will subsequently develop PsA represents an important current unmet medical need (7). Identification and understanding of the 'risk factors' for development of PsA is an essential first step required to define the optimal point where disease prevention studies should be targeted.

Collagens are core proteins in all tissues, including skin, bone, cartilage and other connective tissues, which are involved in the inflammatory process in both PsC and PsA. Serological fragments of type I, II, III, IV or X collagen are released from the extracellular matrix upon disease-associated injury to the tissue and may be useful as biomarkers of PsA (8-11). All these collagens are present in the joint tissue (10, 12). Type I and III collagen are the most abundant collagens in the soft tissues. Type I collagen is the most abundant protein in the human body, and acts as a major structural component of both skin and bone. Type III collagen is found extensively in connective tissues, such as skin and the vascular system, and a key player in wound healing together with type I collagen (13-15). Type II collagen is a major component of articular cartilage, where it provides tissue integrity and resiliency to stress (16). Type IV collagen is the main component of the basement membrane functioning as a barrier between tissue compartments (17). Type X collagen is mainly expressed in hypertrophic chondrocytes in cartilage. Although it constitutes only around 1% of total collagen in cartilage in healthy adults, extracellular deposition and expression of type X collagen are increased in joint pathologies (8). Degradation metabolites of these collagens have previously been found to be elevated in both skin and joint disorders, such as PsA, rheumatoid arthritis (RA) and Systemic Sclerosis (SSc). One example is in an earlier publication from 2021, where biomarkers of type I collagen degradation (C1M), type IV collagen degradation (C4M), type IV collagen turnover (PRO-C4) and type IV collagen degradation (C4M) was increased in PsA patients compared to healthy controls (11). In RA, serum C1M and C4M are associated with bone and synovium turnover. Formation of collagen can be quantified by the formation biomarkers PRO-C1 (type I collagen, N-terminal propeptide of type I procollagen [PINP]) and PRO-C3 (type III collagen) which assess the release of pro-peptides from newly synthesised collagen. PRO-C1 has shown to be a biomarker of bone formation, while PRO-C3 is closely associated to fibrogenesis. PRO-C3 has been shown to be elevated in patients with SSc and associated with progressive disease (18). Type II collagen degradation (C2M) and type X collagen degradation (C10C) was

originally published as biomarkers to assess articular cartilage turnover and chondrocyte differentiation by various growth factors, which are also involved in PsA pathology (19, 20). The biomarker CRPM, a metabolite of C-reactive protein (CRP), is released into the circulation upon inflammation and has previously been identified as a biomarker for early identification of osteoarthritis patients with an inflammatory phenotype (21).

There is a critical need to identify patients with Pso that will subsequently develop PsA. With this in mind, serum samples were collected from patients with PsC and no clinical evidence of underlying inflammatory musculoskeletal disease, from patients with established PsA and from a subset of healthy individuals with the aim of investigating collagen biomarkers and their potential use in separating these three patient groupings.

Methods

Study design and population

Three patient groupings were included in this study; PsC, PsA and healthy individuals.

Firstly, PsC patients were recruited from dermatology clinics at St Vincent's University Hospital (SVUH), Dublin. The study was approved by the local ethical committee (Ethics and Medical Research Committee, Elm Park, Dublin 4). If no previous diagnosis of PsA, these PsC patients underwent a full rheumatologic assessment to exclude any evidence of a current, underlying inflammatory musculoskeletal process. A serum sample was obtained at the same time by venipuncture. Patients with symptoms suggestive of possible synovitis, enthesitis or inflammatory back pain were excluded.

Secondly, patients with an established diagnosis of PsA meeting the CASPAR criteria were recruited from rheumatology clinics in SVUH (22). If patients were agreeable to taking part in the study, they were asked to give fully informed written consent. Patients were brought back to the clinical research centre in SVUH where they completed a detailed assessment of their skin and musculoskeletal system, including the collection of a blood sample. The blood samples were collected and spun down in a centrifuge for 15 minutes at 1600 rpm. Serum was collected, and transferred into a separate vial, and stored at -80°C until analysis. To compare the biomarker levels to healthy controls, a third group of 41 age-, sex- and race-matched samples were obtained from the commercial vendor Discovery Life Science (AL, USA). Serum samples from the healthy controls were collected in 2018 and processed immediately after collecting according to standard operating procedures and stored at -80 °C until analysis. The study was conducted in accordance with the Declaration of Helsinki.

Table I. Overview biomarkers representing bone and tissue turnover measured.

Biomarker	Measures	Reference
Anabolic biomarkers		
PRO-C1	Propeptide of collagen fragment from synthesis of the type I collagen	(23)
PRO-C3	Propeptide of collagen fragment from synthesis of the type III collagen	(24)
PRO-C4	Internal fragment of type IV collagen 7S-domain	(25)
Catabolic biomarkers		
C1M	MMP-2, -9, -13 mediated degradation of type I collagen (alpha 1 chain)	(26)
C2M	MMP-9 mediated degradation of type II collagen	(27)
C3M	MMP-9 mediated degradation of type III collagen	(28)
C4M	MMP-2, -9, -12 mediated degradation of type IV collagen (alpha 1 chain)	(29)
C10C	Cathepsin-K mediated release of the NC-domain of type X collagen	(30)
CRPM	MMP-1 and -8 mediated degradation of C-reactive protein	(31)

MMP: matrix-metalloproteinase

Table II. Clinical characteristics of healthy controls, cutaneous psoriasis patients and psoriatic arthritis patients.

Variable	Healthy controls n=41	PsC n=30	PsA n=30	*p*-value
Mean age (SD)	45.9 (7.3)	41.1 (14.9)	50.3 (10.8)	0.002*
Gender, male (%)	20 (49%)	20 (67%)	21 (70%)	0.138*
PsA/Pso duration, years	NA	6.5 (2.9)	14.8 (10.3)	NA
BMI (kg/m^2)	NA	28.0 (5.5)	28.5 (5.4)	0.020
Smoking, n (%)	NA			
- Non-smoker		10 (33.3%)	14 (46.7)	
- Former smoker		9 (30%)	14 (46.7)	
- Current smoker		11 (36.7)	2 (6.6)	0.019
PASI	NA	8.1 (3.8)	2.8 (2.8)	<0.0001
SJC	NA	NA	1.2 (3.0)	NA
TJC	NA	NA	2.6 (2.6)	NA
LEI	NA	NA	0.5 (0.8)	NA
CRP (mg/L)	NA	3.5 (2.4)	5.5 (4.9)	<0.0001
NSAID use n (%)	NA	11 (36.7%)	26 (86.7%)	0.0001
DMARD use n (%)	NA	4 (13.3%)	27 (90.0%)	<0.0001
Current biologic use n (%)	NA	1 (3.3%)	18 (60.0%)	<0.0001

SD: standard deviation; BMI: Body Mass Index; PASI: Psoriasis Area Severity Index; SJC: swollen joint count; TJC: tender joint count; LEI: Leeds Enthesitis Index; CRP: C-reactive protein; NSAID: non-steroidal anti-inflammatory drug; DMARD: disease-modifying anti-rheumatic drug. *Comparison of healthy controls, PsC and PsA.

Biomarker analysis

The bone and tissue turnover were examined by measurement of serological immunoassays to assess the formation of collagen type I, III and IV (PRO-C1, -C3, -C4) and degradation of collagen type I, II, III, IV and X including C-reactive protein (C1M, C2M, C3M, C4M, C10C, CRPM), respectively (Table I). The biomarkers were quantified using competitive ELISA assays produced at Nordic Bioscience, Herlev, Denmark. Biomarkers were measured in serum samples from patients with PsC, PsA and from healthy individuals.

Briefly, the biomarker ELISA assays were performed as follows: firstly, streptavidin-coated microtitre plates

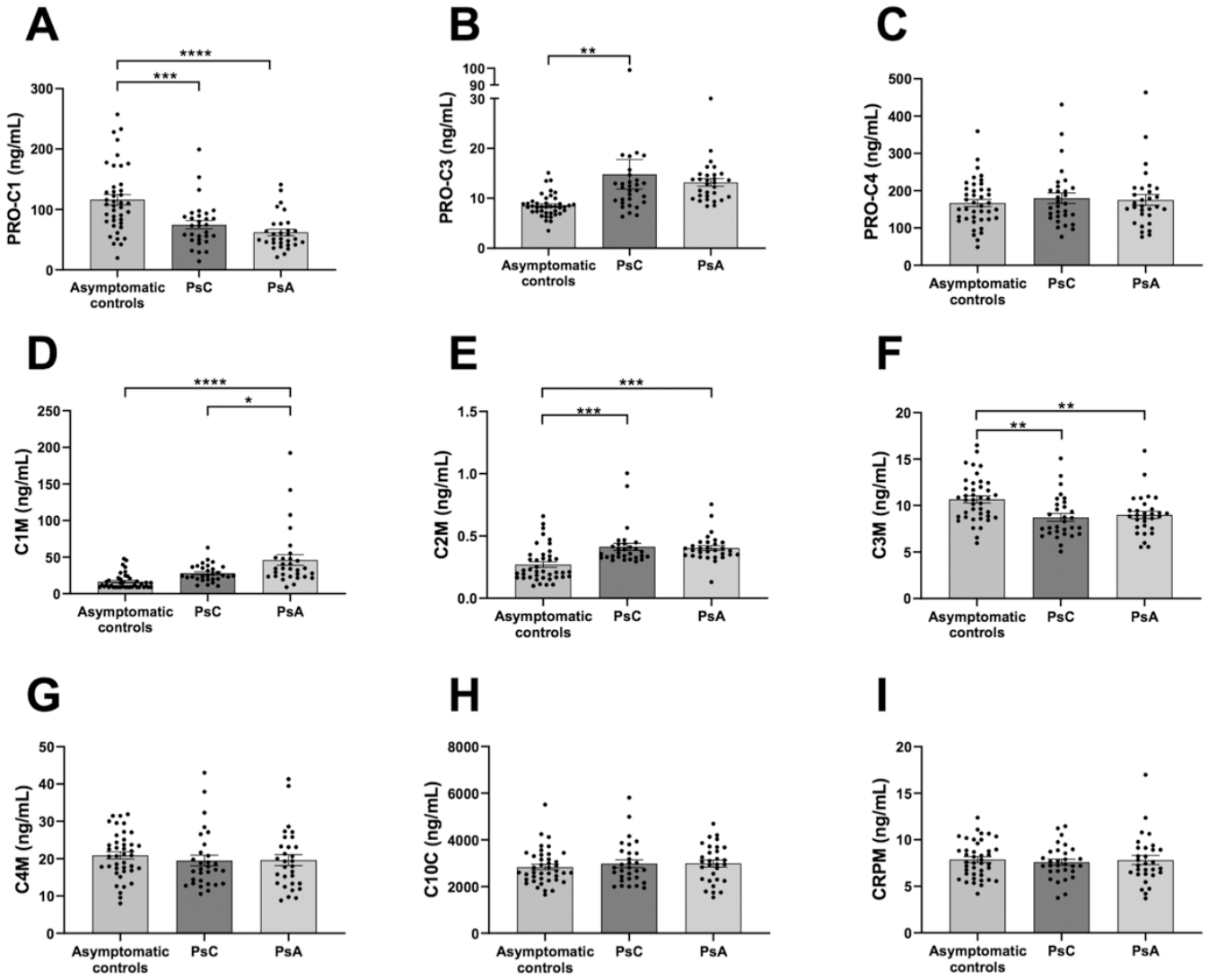

Fig. 1. Levels of collagen and inflammation biomarkers measured in serum from healthy controls (n=41), cutaneous psoriasis (PsC, n=30) and psoriatic arthritis (PsA, n=30) patients.

A-C: Serum levels of the anabolic biomarkers measuring type I collagen (PRO-C1), type III collagen (PRO-C3) and type IV collagen (PRO-C4).

D-I: Catabolic biomarkers of type I collagen (C1M), type II collagen (C2M), type III collagen (C3M), type IV collagen (C4M), type X collagen (C10C) and MMP-degraded C-reactive protein (CRPM).

Statistical differences were calculated using an ANVOCA corrected for age. Significance threshold was set at $p<0.05$, and data are presented as scatterplots with bars mean±standard error of mean. Significance levels: *$p<0.05$, **$p=0.001$, ***$p\leq0.001$, ****$p<0.0001$.

were incubated with biotinylated peptide for 30 min at 20°C. Unbound biotinylated-peptide was washed off five times with washing buffer (20 mM TRIS, 50 mM NaCl, pH 7.2). Next, standard peptide, serum, and control samples were added to the plate and instantly, peroxidase-labelled monoclonal antibody was added and incubated for 1 h at 20°C (PRO-C4, C3M, C4M, and CRPM), 3 h at 4°C (PRO-C1), or 20 h at 4°C (PRO-C3, C1M, and C2M). In the C10C ELISA assay, the standard peptide, serum and control samples were incubated with a primary capturing monoclonal antibody for 20 h at 4°C, washed five times, and further incubated with secondary peroxidase-labelled antibody for 1 h at 20°C. Subsequently for all ELISA assays, after being incubated with peroxidase-labelled antibody, plates were washed five times with washing buffer and incubated with 3,3',5,5'-Tetramethylbenzidine (TMB) for 15 min at 20 °C in the dark. The colorimetric enzyme reaction was stopped with 0.1% sulfuric acid and measured on an ELISA plate reader at 450 nm absorbance with 650 nm as reference.

Statistical analysis

This is an explorative analysis. Data are presented as mean ± standard deviation (SD) if not otherwise specified. Analysis was performed in MedCalc (version 14.8.1) and GraphPad Prism (version 8) and a *p*-value below 0.05 was considered significant. Baseline characteristics of healthy controls, PsC only patients and PsA patients were presented as mean ± standard deviation (SD) for continuous variables and number (frequency) for categorical variables. For normally distributed data with numerical variables parametric tests (ANOVA, t-test) were used. Non-parametric tests (Mann-Whitney U-test, Kruskal-Wallis test and Spearman correlation) were

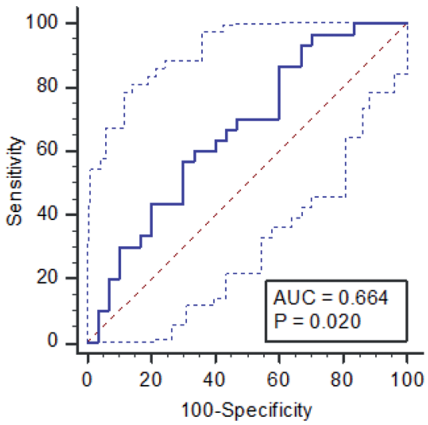

Fig. 2. ROC curve analysis of the C1M biomarker for distinguishing subjects with PsC from PsA. AUC=0.664, 95% CI 0.531–0.781, p=0.020, Youden index J=0.267, sensitivity 56.7, specificity 70.0, criterion ≤24.66.

Table III. Associations of C1M with clinical assessments for PsC and PsA patients. Spearman's correlations.

Variable	r	p-value
PsC patients		
Age	0.307	0.099
BMI	0.270	0.149
CRP	**0.574**	**0.004**
LEI	-	-
PASI	0.018	0.927
SJC	-	-
TJC	-	-
PsA duration	-	-
PsC duration	-0.273	0.145
PsA patients		
Age	0.008	0.967
BMI	**0.396**	**0.030**
CRP	**0.589**	**0.003**
LEI	-0.325	0.080
PASI	-0.041	0.831
SJC	0.041	0.829
TJC	0.082	0.666
PsA duration	**0.512**	**0.004**
PsC duration	0.300	0.108

used for non-normally distributed data. An ANCOVA corrected for age was applied to test the difference between biomarker levels across patient groups.

Results

Patient demographics and clinical characteristics are summarised in Table II. Age and Gender were compared between all three groups, while disease duration, Body Mass Index (BMI), smoking status, Psoriasis Area Severity Index (PASI), C-reactive protein (CRP), non-steroidal anti-inflammatory drug (NSAID) use, disease-modifying anti-rheumatic drug (DMARD) use and biologics use were compared between PsC and PsA patients. Musculoskeletal features (swollen joint count (SJC), tender joint count (TJC), Leeds Enthesitis Index (LEI)) were only positive in the patients with PsA. In general, patients with PsA were older than both the patients with PsC and the healthy controls. PsA patients also had significantly higher levels of CRP and were more likely to be treated with NSAIDS, DMARDs and biologics.

Accelerated turnover of collagen and inflammation biomarkers

Levels of collagen and inflammatory biomarkers released into blood, are shown in Figure 1. The biomarker PRO-C1, a measure of type I collagen formation, was decreased in PsC and PsA compared to healthy controls (p=0.0006 and p<0.0001, respectively; Fig. 1A). PRO-C3, measuring the formation of type III collagen, was significantly increased in patients with PsC compared to healthy controls (p=0.0057, Fig. 1B). No difference was found for the PRO-C4 biomarker which measures type IV collagen formation between the groups (Fig. 1C). The collagen type I biomarker C1M which provides a measure of the degradation of type I collagen was significantly increased in PsA compared to healthy controls (p<0.0001) and was also the only biomarker able to distinguish PsC from PsA (p=0.049, Fig. 1D). C2M, measuring type II collagen degradation was increased in both Pso and PsA compared to healthy controls (p=0.0002 and p=0.0004, respectively, Fig. 1E), while C3M, measuring type III collagen degradation, was decreased in PsC and PsA compared to healthy controls (both p=0.006). No significant differences were found between the groups for type IV collagen degradation, C4M, type X collagen degradation, C10C, or the MMP-mediated degradation of C-reactive protein, CRPM (Fig. 1 G-I).

C1M as a biomarker of joint involvement

The biomarker C1M was the only biomarker able to separate patients with PsC from PsA. The diagnostic power (AUROC) of C1M for separating a patient with PsA from PsC was 0.664 (95% CI 0.531-0.781, p=0.020, Fig. 2). C1M was moderately correlated to CRP in PsC patients (r=0.574, p=0.004, Table III), but not associated to the PASI score or duration of PsC. In PsA, C1M is significantly associated with BMI, CRP and PsA duration (BMI: r=0.396, p=0.030; CRP: r=0.589, p=0.003, PsA duration: 0.512, p=0.004, Table III), but no significant correlation was found with SJC and TJC. However, there is a trend towards a negative correlation between C1M and LEI (r=-0.396, p=0.080).

Discussion

More than 90% of all patients with PsA develop their arthritis on background of established Pso. PsA is a progressive and disabling disease, thus confirming the medical need to study Pso patients with the aim to identify early events in PsA (23). Clinically, nail pitting, psoriasis severity and psoriasis location provide an indication towards progression to PsA, as well as BMI, genetic factors such as HLA-B27 and family history (24, 25). Nevertheless, there are no sensitive or specific measures which separate these two groups of patients and potentially identify patients developing arthritis.

In the present study we measured biomarkers of collagen and inflammation in healthy individuals, PsC or PsA, with the aim of identifying patients with inflammatory systemic musculoskeletal involvement. The main findings were; 1) The biomarkers of type I collagen formation (PRO-C1) and type III collagen degradation (C3M) were decreased in both PsC and PsA compared to healthy controls, while type III collagen formation (PRO-C3) was upregulated in PsC compared to healthy controls, type I collagen degradation (C1M) was upregulated in PsA compared to healthy controls and type II collagen degradation (C2M) was upregulated in PsC and PsA compared to healthy controls, 2) C1M was the only biomarker able to distinguish between PsC and PsA with an AUROC 0.664, 3) C1M was positively correlated to PsA duration.

All collagen biomarkers measured in this study reflect the individual tissue

remodelling occurring in healthy tissues, skin and joint diseases. In healthy tissues there is a finely tuned balance between formation and degradation of the individual collagens, but these are altered upon a chronic insult as found in PsC and PsA. Collagens are core proteins in the tissues involved in PsC and PsA. Type I collagen is the most abundant protein in the human body, and present in both bone and connective tissue. Schett *et al*. recently observed C1M was significantly reduced in PsA patients treated with Guselkumab, an interleukin-23p19-subunit monoclonal antibody, at 24 weeks in the DISCOVER-2 trial indicating its potential role as a biomarker related to treatment response in PsA. In addition, C1M was also shown to be associated with ACR20 responders, compared to non-responders, highlighting the potential of C1M as a biomarker that tracks joint response (26). This may support the findings in this study indicating C1M as a biomarker to distinguish between PsC and PsA as a biomarker for inflammatory systemic musculoskeletal involvement. In addition to C1M, we also measured PRO-C1 (also known as PINP) which has mainly been associated with bone formation. In this study, we observed PRO-C1 being lowered in PsA compared to healthy subjects, which may be due to less general bone formation, but more bone resorption and bone erosions.

It is of interest that, C2M, quantifying articular cartilage degradation, was also upregulated in both PsC and PsA indicating an ongoing damage of the cartilage (27). C2M, C10C and CRPM have previously been shown to be upregulated in ankylosing spondylitis, but not associated with disease scores, CRP or radiographic progression (10, 28, 29). Type III collagen formation, PRO-C3, and degradation, C3M, are collagens found mainly in connective tissues and have previously been shown to be upregulated in patients affected by systemic sclerosis (SSc). PRO-C3 in particular has been shown to be upregulated in patients with diffuse SSc compared to limited SSc (30, 31). This suggests that these pro-peptides could potentially be used as biomarkers to separate out patients affected by diseases with skin involvement.

This was an exploratory study aiming to identify collagen biomarkers able to separate the three patient groups PsC, PsA and healthy controls. Whether the biomarkers can identify patients with PsC in risk of developing PsA needs to be tested in a prospective, longitudinal study designed specifically for this purpose. Such a study may help dermatologists to identify which patients are in risk of developing PsA, and opening up the possibility of disease prevention (32, 33). C1M could be used as a monitoring tool if it is shown to be able to identify PsC patients in transition to PsA, where again appropriate treatment might halt or slow down disease progression. The limitations of this study include the small sample size and the use of bDMARDS in 60% of the PsA patients. The use of biological treatments have previously been shown to lower the levels of collagen biomarkers (34-36).

Conclusion

This work provides evidence that serum collagen biomarkers are dysregulated in PsC and PsA, as compared to healthy controls. C1M was the only collagen biomarker able to differentiate patients with PsC from PsA and could be a potential biomarker of inflammatory systemic musculoskeletal involvement. Such biomarkers could aid the early identification of Pso patients developing arthritis and potentially be combined with clinical features (nail disease and psoriasis severity) for better prediction of disease.

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