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Novel Biomarker of Collagen Degradation Can Identify Patients Affected With Both Axial Spondyloarthritis and Crohn Disease

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ABSTRACT. ***Objective.*** Chronic inflammatory arthritis is a hallmark of axial spondyloarthritis (axSpA), where coexistence of Crohn disease (CD) is prominent. We investigated the association between biomarkers of collagen degradation in healthy controls (HCs) and in patients with axSpA, CD, and CD and axSpA overlap (CD-axSpA), with the aim to investigate the ability of the biomarkers to identify patients with CD-axSpA.

Methods. Patients with axSpA who fulfilled Assessment of Spondyloarthritis international Society criteria (n = 13), had biopsy-proven CD (n = 14), had CD-axSpA (n = 10), and HCs (n = 11) undergoing standard-of-care colonoscopies were included in the study. The collagen biomarkers measuring type III, IV, VI and X collagen (C3M, C4M, C6M, and C10C, respectively) were measured in plasma samples from all subject groups. Statistical analysis was performed using an ANCOVA adjusted for age, an area under the receiver-operating characteristic (AUROC) curve analysis, and Spearman correlation.

Results. C4M was significantly higher in patients with CD-axSpA overlap compared to axSpA, CD, and HCs (all $P < 0.001$). In an AUROC analysis, C4M showed a complete separation between the patients with CD-axSpA overlap compared to HC, axSpA and CD with an area under the curve (AUC) = 1.00 ($P < 0.001$). No differences were found between the patient groups for C3M, C6M, and C10C. No correlations were found between the collagen biomarkers and C-reactive protein, Bath Ankylosing Spondylitis Disease Activity Index, Simple Clinical Colitis Activity Index, or Harvey-Bradshaw Index scores.

Conclusion. Degradation of type IV collagen quantified by C4M showed a complete separation of patients with CD-axSpA overlap, compared to axSpA, CD, and HCs, and indicates excessive collagen degradation and epithelial turnover. This biomarker could potentially be used to identify patients affected by both manifestations and to guide treatment decisions.

Key Indexing Terms: ankylosing spondylitis, biomarkers, collagen, inflammatory bowel disease, spondyloarthritis

Chronic inflammatory arthritis is a hallmark of axial spondylitis (axSpA), where the coexistence of inflammatory bowel disease (IBD), such as Crohn disease (CD), is prominent.[1] The clinical overlap of axSpA and CD (CD-axSpA) has raised the hypothesis that these conditions may have similar pathophysiological mechanisms. However, the overlap of the 2 conditions results in a unique challenge when diagnosing and treating individuals with both diseases. Clinically, 6% to 14% of patients with axSpA have evident IBD, while silent microscopic gut inflammation is found in 60% of all patients with axSpA.[2,3] Of those patients with silent inflammation, 5% to 20% develop CD within 5 years.[4] In general, axSpA is more commonly found in patients with CD compared to those with ulcerative colitis.[5] Despite the prevalent overlap of IBD and axSpA, most biological treatments are effective in only one of the diseases, with no or worsening effect for the other indication. Examples of these are the interleukin (IL)-17A inhibitors secukinumab and ixekizumab, which are approved by the US Food and Drug Administration (FDA) for axSpA, but cause worsening in CD[1,6]; and the IL-12/IL-23 inhibitor ustekinumab, which is FDA-approved and effective for CD, but shows no clinically meaningful improvement over placebo for axSpA, and in addition, a proportion of the patients with axSpA experienced adverse events.[7] Despite the differences in pathologies between axSpA and IBD, some studies have

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indicated that both bacterial antigens and reactive T cell clones are activated and lead to activation of both gut and joint inflammation. Nevertheless, the immunological mechanisms linking these 2 organs are not fully understood.[8] Novel biological treatments are therefore being developed, but there is a lack of tools to help guide treatment decisions in IBD-associated axSpA.

Biomarkers measuring the pathological processes of the individual diseases could help identify patients with overlapping disease, and also potentially be used for prediction of treatment response. For example, fecal calprotectin is often used to identify and monitor patients with IBD[9]; however, this biomarker is influenced by nonsteroidal antiinflammatory drugs (NSAIDs), which are commonly used by patients with axSpA.[10,11] Further, axSpA lacks a reliable diagnostic and disease-monitoring biomarker. Therefore, there is still a need to identify not only biomarkers for the individual diseases but also biomarkers for the overlapping disease.

Both axSpA and CD are characterized by an altered extracellular matrix turnover, where particularly collagens are remodeled.[12,13] Collagens are prone to degradation of matrix metalloproteinases (MMPs), resulting in protease-specific collagen fragments. These fragments are produced locally upon inflammation and are released into the circulation where they serve as blood-based biomarkers reflecting local pathogenic processes.[13] Type III, IV, VI, and X collagen are present in different joint tissues (connective tissues and bone), while type III and IV collagen are highly expressed in the intestines. C3M, a fragment of MMP-mediated type III collagen, is found in the interstitial matrix and has previously been found to be upregulated in both axSpA and CD.[12,13] C4M, a fragment of MMP-mediated type IV collagen, found in the basement membrane, has been shown to be upregulated in axSpA and to have potential as a biomarker of treatment response in CD.[13,14] C6M, a fragment of MMP-mediated type VI collagen, found in the interface between the interstitial matrix and basement membrane, has shown to be associated with treatment response and Ankylosing Spondylitis Disease Activity Score (ASDAS) in axSpA,[15] whereas C10C, a cathepsin-mediated type X collagen biomarker, has shown to be upregulated in SpA.[16] These 4 blood-based biomarkers have shown potential in either axSpA or CD, but not in patients with overlapping disease. We investigated the association between biomarkers of collagen degradation in healthy controls (HCs) and patients with axSpA, CD, and CD-axSpA, with the aim to investigate the biomarkers' ability to identify patients with CD-axSpA.

METHODS

Study population. Study participants were recruited at the University of Colorado Hospital between November 2017 and November 2018. A detailed description of the sampling can be found elsewhere.[17] Briefly, the subjects with axSpA who fulfilled Assessment of Spondyloarthritis international Society criteria (n = 13), had biopsy-proven CD (n = 14), had CD-axSpA (n = 10), and HCs (n = 11) undergoing standard-of-care colonoscopies for routine colon cancer screening were included in the study. Exclusion criteria included presence of bowel disease other than CD in the CD groups, rheumatic disease except axSpA in the axSpA groups, pregnancy, use of antibiotics 2 weeks prior to study entry, cancer or cancer history, inability to stop aspirin or NSAIDs for 7 days before and after endoscopy, use of anticoagulation, HIV, and clostridium difficile infection within the past 3 months. Questionnaires regarding demographics, Harvey-Bradshaw Index (HBI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Simple Clinical Colitis Activity Index (SCCAI) were collected at the time of colonoscopy. Ethylenediamine tetraacetic acid (EDTA) plasma samples were collected according to predefined standard operating procedures and stored at −80 °C until analysis. Informed consent was obtained for all study participants recruited from the single site of the University of Colorado Hospital as approved by the Colorado Multiple Institutional Review Board (protocol 14-1507).

Biomarker measurements. The collagen degradation biomarkers measuring MMP-mediated degradation of type III, IV, and VI collagen (C3M, C4M, and C6M, respectively) and cathepsin K–mediated degradation of type X collagen (C10C) were developed and validated previously.[13,16] All 4 biomarkers are competitive ELISAs, which are validated for sample measurements in EDTA plasma. The inter- and intraassay coefficients of variation are > 15% and 10%, respectively for all assays. Samples below the lower limit of quantification (LLOQ) were assigned the value of LLOQ.

Statistical analysis. Study participant demographics are described as means with SDs for continuous variables, and percentages for categorical variables. Statistical differences between the clinical and participant demographics are calculated by a Kruskal-Wallis test (nonparametric). Correlations of the biomarkers with clinical scores were performed by Spearman correlation. An ANCOVA analysis adjusted for age was used to calculate the differences between the groups of patients. The diagnostic power of the biomarkers was investigated by the area under the receiver-operating characteristics (AUROC) curve, calculated by the method of DeLong et al.[18] Optimal cut-offs were estimated by Youden index. For all statistical analyses performed, a P value < 0.05 was considered significant. Statistical analysis and graphs were performed using GraphPad Prism version 9 (GraphPad Software, Inc).

RESULTS

Patient demographics and associations between biomarkers and clinical scores. Participant demographics and clinical characteristics are summarized (Table). EDTA plasma samples were available for a total of 13 patients with axSpA, 14 patients with CD, 10 patients with CD-axSpA, and 11 HCs. The mean age for patients with axSpA was 47.9 years, the mean age for patients with CD was 32.9 years, the mean age for patients with CD-axSpA was 53.5 years, whereas the mean age for HCs was 50.9 years (P < 0.001). Most of the participants were female (85%), with a BMI of 27.7 (SD 7.2), and a disease duration of 10 years in the axSpA, CD, and CD-axSpA groups. All patients were on treatment, where tumor necrosis factor-α inhibitors (TNFi) were the most common treatment across the 3 disease groups. None of the biomarkers correlated with any clinical disease score (BASDAI, HBI, or SCCAI), but C3M showed a mild correlation to C-reactive protein and BMI (r = −0.29, P = 0.04 and r = 0.35, P = 0.02, respectively).

Biomarkers of collagen degradation. The collagen biomarker C4M, indicating type IV collagen degradation, was significantly higher in patients with CD-axSpA compared to axSpA, CD, and HCs (all P < 0.001; Figure 1A). The levels of C4M in patients with axSpA were significantly lower than HC (P < 0.001), whereas CD also showed a lower level compared to HCs though not significant (P = 0.079). No difference was found between axSpA and CD alone. Neither C3M, measuring type III collagen degradation, C6M, measuring type VI collagen

Table. Patient demographics.

	axSpA, n = 13	CD-axSpA, n = 10	CD, n = 14	Healthy Controls, n = 11	*P*
Age, yrs, mean (SD)	47.9 (12.3)	53.5 (12.0)	32.9 (8.3)	50.9 (12.4)	**< 0.001**
Sex, male, n (%)	4 (31)	2 (20)	1 (7)	0 (0)	0.14
BMI, kg/m^2, mean (SD)	29.1 (6.4)	28.8 (7.3)	27.0 (9.8)	25.9 (5.5)	0.35
Disease duration, months, mean (SD)	121.9 (134.5)	138.0 (100.3)	104.4 (102.7)	NA	0.40
CRP, mg/dL, mean (SD)	3.6 (8.1)	13.9 (25.3)	1.8 (3.0)	NA	0.02
HBI total	3.0 (1.7)	3.5 (1.4)	2.7 (3.0)	1.0 (1.3)	**0.01**
SCCAI total	3.2 (1.6)	3.8 (1.7)	2.6 (1.9)	1.3 (1.4)	**0.004**
BASDAI	5.1 (2.5)	5.1 (2.4)	2.2 (2.3)	1.7 (1.8)	**0.001**
HLA-B27 positive, n (%)	9 (69)	5 (50)	7 (50)	1 (9)	0.12
WBC	7.2 (1.7)	7.1 (1.5)	7.4 (2.7)	6.4 (1.3)	0.28
Medication, n (%)					
Azathioprine	0 (0)	0 (0)	6 (46)	0 (0)	
Sulfasalazine	1 (7.7)	0 (0)	0 (0)	0 (0)	
TNFi	12 (91)	6 (60)	13 (93)	0 (0)	
NSAID	7 (54)	5 (50)	4 (29)	0 (0)	
Methotrexate	3 (23)	3 (30)	1 (7)	0 (0)	
Budesonide	0 (0)	2 (20)	1 (7)	0 (0)	
Ustekinumab	0 (0)	3 (30)	1 (7)	0 (0)	

Values in bold are statistically significant. AxSpA: axial spondyloarthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CD: Crohn disease; CRP: C-reactive protein; HBI: Harvey-Bradshaw Index; NA: not applicable; NSAID: nonsteroidal antiinflammatory drug; SCCAI: Simple Clinical Colitis Activity Index, TNFi: tumor necrosis factor inhibitor; WBC: white blood count.

degradation, or C10C, measuring type X collagen degradation, were able to separate the different patient groups ($P = 0.44$, 0.56, and 0.70, respectively).

Diagnostic potential of C4M to identify patients with CD-axSpA. AUROC was performed to evaluate the diagnostic power of C4M for identification of CD-axSpA from axSpA alone, CD alone, or HCs. As shown in Figure 2, C4M showed a complete separation between the patients with CD-axSpA compared to HCs, with an area under the curve (AUC) of 1.00 (95% CI 0.84-1.00, sensitivity of 100.00%, specificity of 100.00%, Youden index of 1.00, associated criterion > 24.41, $P < 0.001$), CD with an AUC of 1.000 (95% CI 0.86-1.00, sensitivity of 100.00%, specificity of 100.00%, Youden index of 1.00, associated criterion > 21.85, $P < 0.001$), and axSpA with an AUC of 1.00 (95% CI 0.85-1.00, sensitivity of 100.00%, specificity of 100.00%, Youden index of 1.00, associated criterion > 21.57, $P < 0.001$). C3M, C6M, and C10C were not able to differentiate CD-axSpA from the other groups (P range = 0.10 to 0.95) and showed a nonclinical or diagnostically useful AUROC (AUROC range = 0.51 to 0.72).

DISCUSSION

In the present study, we evaluated the 4 collagen degradation biomarkers—C3M, C4M, C6M, and C10C—as potential biomarkers to identify patients with CD-axSpA. The main finding was the ability of C4M to separate patients with CD-axSpA from patients with axSpA alone, CD alone, and HCs, with high diagnostic accuracy. No differences between the patient groups were found for the other 3 biomarkers, C3M, C6M, and C10C.

Despite the clinical connection between patients with axSpA and IBD, there is a lack of studies comparing the relationship between both inflammatory conditions. To our knowledge, this is the first time collagen biomarkers have been investigated in patients with CD-axSpA, but not the first time they were investigated in CD alone or in axSpA alone. Type III, IV, VI, and X collagens are 4 extracellular matrix proteins known to be expressed in the joint,[13,16] whereas type III, IV, and VI are also expressed in the intestines.[14,19] In the joint, the biomarkers C3M and C4M have both shown to be upregulated in axSpA and are associated with quality of life, functional index, and ASDAS, indicating that they may be biomarkers of disease activity in axSpA.[13,20] C6M was previously shown to be a biomarker predictive of treatment response to adalimumab (ADA), and correlated to total Spondyloarthritis Research Consortium of Canada (SPARCC) score measured by magnetic resonance imaging,[15] whereas C10C was shown to be upregulated in patients with axSpA.[16] In the intestines, C3M and C4M have both been investigated for CD, and were shown to be biomarkers of treatment response to both ADA and infliximab.[14] There were no differences between the CD, axSpA, CD-axSpA, and HCs for C3M, C6M, and C10C; this may be explained by the fact that 60% to 92% of all the patients with CD, CD-axSpA, and axSpA were on TNFi therapies, which have previously shown to lower the levels of the biomarkers in clinical studies.[14,15] On the other hand, in our study, levels of C4M in patients with CD-axSpA were not suppressed by TNFi treatment, despite previous findings where patients with either rheumatoid arthritis, IBD, or axSpA showed a decrease in C4M levels with TNFi therapy.[14,21] The C4M levels in CD and axSpA alone may be suppressed by TNFi therapy as shown previously; nonetheless, this was not seen in patients with CD-axSpA. Type IV collagen is a basement membrane protein found in the synovial membrane of the joint and below the epithelial cells in the intestines. This specific fragment of type

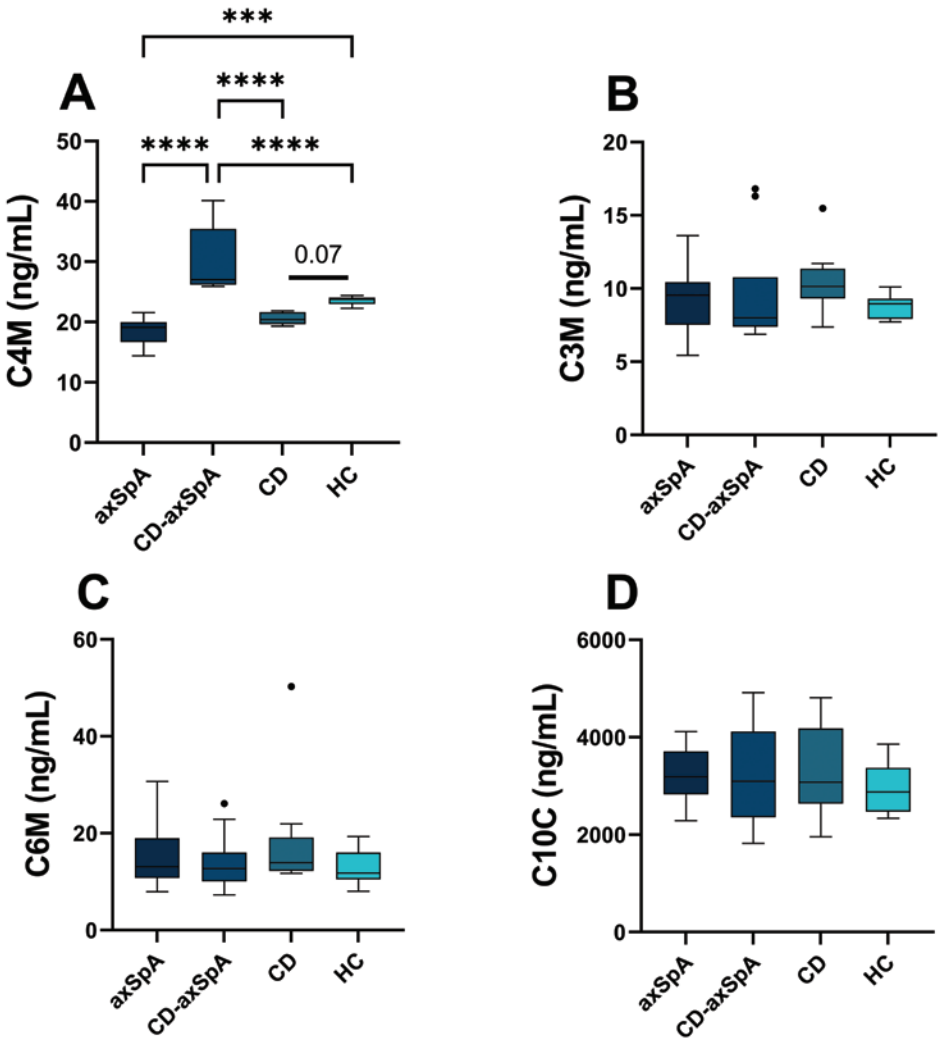

Figure 1: Levels of (A) C4M, (B) C3M, (C) C6M, and (D) C10C in EDTA plasma from patients diagnosed with axSpA (n = 13), CD (n = 14), CD-axSpA overlap (n = 10), and HCs (n = 11). Graphs are presented as Tukey box plots. An ANCOVA analysis adjusted for age was applied. ***$P < 0.001$, ****$P < 0.0001$. AxSpA: axial spondyloarthritis; C3M: type III collagen; C4M: type IV collagen; C6M: type VI collagen; C10C: type IX collagen; CD: Crohn disease; EDTA: ethylenediamine tetraacetic acid; HC: healthy control.

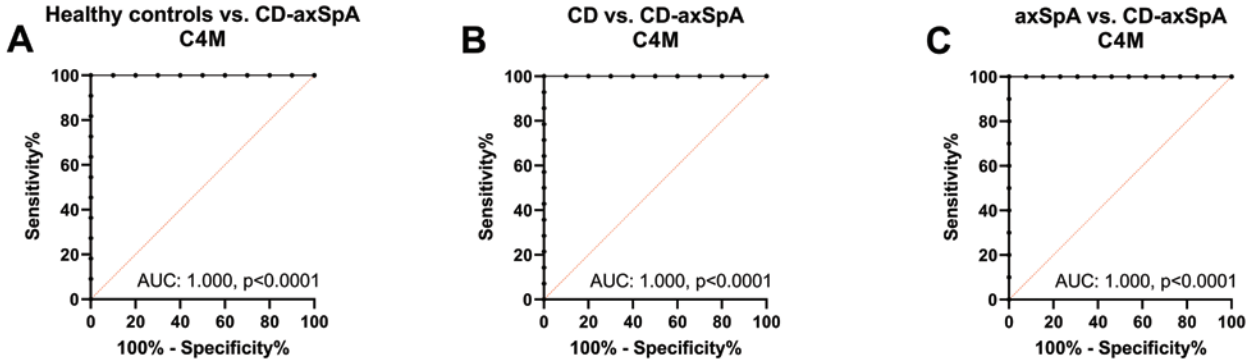

Figure 2. Receiver-operating characteristic curve analysis was used to evaluate the ability of C4M to discriminate between (A) healthy controls vs CD-axSpA; (B) CD vs CD-axSpA; and (C) axSpA vs CD-axSpA. Analyses were adjusted for age. AUC: area under the curve; axSpA: axial spondyloarthritis; C4M: type IV collagen; CD: Crohn disease.

IV collagen C4M is generated by MMPs, which are highly expressed during inflammation.[22] Nonetheless, healthy individuals also have quantifiable levels of C4M, since type IV collagen is degraded as a part of intact tissue homeostasis.[23] The results of an AUROC = 1 for separating patients with CD-axSpA from the other investigated patient groups should be further investigated, since such clear separation is rarely found in biological biomarker studies. Understanding the relationship and mechanisms of inflammation-induced collagen turnover in CD-axSpA may be useful to identify patients with both diseases.

Although IBD and axSpA share common immunologic features, including involvement of the IL-17 pathways and treatment response to TNFi therapy, suggesting some common molecular pathways, clinical trials therapeutically targeting IL-17 and IL-23 revealed critical divergences in the immunopathology. Namely, use of IL-17 inhibition in CD worsened disease activity scores, particularly in those patients with higher fecal calprotectin levels upon entering the study.[24] Yet IL-17 inhibition is a growing class of biologic therapies for the treatment of axSpA.[1] Conversely, IL-23 inhibition alone or in combination with IL-12 inhibition is effective for IBD but not axSpA.[7] In these studies, the overlapping condition of CD-axSpA was not considered. In a recent study evaluating peripheral blood mononuclear cells in a clinically well-defined cohort of individuals with pure CD, pure axSpA, and overlapping CD-axSpA, the immunophenotype of those with CD-axSpA diverged from either CD or axSpA alone and was notable for an expansion of CD4+ cytotoxic T cells and interferon signaling.[17] Although these findings have not yet been associated with treatment responses, the data suggest that treatment needs in this CD-axSpA population may be unique. Having biomarkers to aid in differentiating the CD-axSpA population will aid further research to identify treatment needs.

Some limitations should be considered during the interpretation of these results. Importantly, the present study had a low sample size and was not evenly distributed on age and sex between the groups. Indeed, axSpA has significant differences in clinical presentations between men and women that necessitate the evaluation of disease based on sex differences.[25] Further, the effect of treatment in the groups with CD, CD-axSpA, and axSpA may confound comparisons, particularly to the control group, and may dampen biomarker generation. The mean BASDAI scores in each of the axSpA groups was ~5, suggesting active disease, whereas the low HBI scores < 5 in the CD groups indicate remission. With a low overall sample size, adjustments for these factors are not possible. Nevertheless, the strength of this study should be emphasized: as colonoscopy was performed for all patients, the presence of bowel inflammation in axSpA could be fully assessed. Degradation of type IV collagen quantified by C4M showed a complete separation of patients with CD-axSpA overlap, compared to axSpA, CD, and HCs, indicating excessive collagen degradation and epithelial turnover. This biomarker could potentially be used to identify patients affected by both conditions and to guide treatment decisions.

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