Arthropathies in inflammatory bowel disease : Characteristics and impact on daily functioning

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CHAPTER 4

Absence of serological rheumatoid arthritis biomarkers in inflammatory bowel disease patients with arthropathies

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ABSTRACT

Background: Biomarkers that are associated with future progression to rheumatoid arthritis (RA) and joint destruction have previously been discovered in patients with arthralgia. The present study examined these RA biomarkers in inflammatory bowel disease (IBD) patients with arthropathies.

Methods: Sera from 155 IBD patients with and 99 IBD patients without arthropathies was analysed for IgM rheumatoid factor (IgM-RF), IgA-RF, anti-cyclic citrullinated peptide 2 (anti-CCP2), anti-cyclic citrullinated peptide 3.1 (anti-CCP3.1) and anti-carbamylated protein (anti-CarP) antibody positivity using enzyme-linked immunosorbent assays (ELISA). The prevalence of these autoantibodies in IBD patients was compared to the prevalence in RA patients.

Results: No differences were found in biomarker positivity between IBD patients with and without arthropathies. Significantly more biomarker positivity ($p<0.001$) was observed in RA patients compared with IBD patients with arthropathies. Also, smoking turned out to be significantly associated with IgM-RF and IgA-RF positivity.

Conclusion: Our findings suggest that there is no apparent clinical value to detect RA biomarkers in serum of IBD patients to help to identify arthropathies.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by destructive polyarthritis, which leads to disability and increased mortality.[1] Early diagnosis and initiation of treatment is important in RA, since a considerable number of patients develop irreversible joint damage shortly after disease onset.[2-3]

Serological biomarkers, including rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein antibodies (anti-CarP), have previously been reported to be important diagnostic markers and predictive factors for the development of RA at an early stage.[4-10] RF is an autoantibody directed against the Fc region of immunoglobulin (Ig)G and commonly detected in RA, but can also be positive in patients with other autoimmune and non-autoimmune diseases as well as in healthy individuals.[4] ACPA are often detected using assays based on cyclic citrullinated peptides (CCP), such as the CCP2 and CCP3 assays. Citrullination is the conversion of the amino acid Arginine into Citrulline, mediated by peptidylarginine deiminase (PAD).[5-6] Anti-CCP antibodies are highly specific (up to 99%) for RA, but less sensitive compared to RF. Testing for RF and ACPA simultaneously, has been suggested to improve sensitivity. Recently an anti-CCP3.1 assay was developed that detects both IgG and IgA anti-CCP antibodies to improve both sensitivity and specificity.[7] In addition, another autoantibody designated by Shi et al. as anti-CarP antibodies, has been described as a disease marker in RA patients and targets carbamylated proteins rather than citrullinated.[8] Carbamylation constitutes a posttranslational modification of lysine to homocitrulline under the influence of cyanate.[9] Increased carbamylation is related to chronic inflammatory conditions.[10] Anti-CarP antibodies are present in both anti-CCP positive and negative patients and may predict the development of RA, independently from anti-CCP antibodies.[11-13]

Inflammatory bowel disease (IBD) is associated with various extra-intestinal manifestations, including arthropathies with a prevalence of approximately 30%.[14] IBD-associated arthropathies can be subdivided into inflammatory (spondyloarthritis; SpA) based on the rheumatological ASAS criteria for axial and peripheral SpA and non-inflammatory (arthralgia) joint complaints.[15]

Although previous studies report a genetic link, with shared susceptibility genes between RA patients and arthropathies in IBD,[16] less is known about

the presence of serological RA biomarkers in IBD patients with arthropathies. Therefore, in the present study we examined the presence of RA biomarkers in IBD patients with arthropathies and compared biomarker positivity in these patients with IBD patients without arthropathies and RA patients.

METHODS

Study population

The inclusion procedure of IBD patients was as described previously.[15] Briefly, serum samples were collected from 254 IBD patients included in the JOINT study, a single-center prospective longitudinal study focused on IBD patients with and without arthropathies, performed at the department of Gastroenterology and Hepatology of the Leiden University Medical Center (LUMC), the Netherlands. Patients visiting the IBD outpatient clinic from July 2009 to February 2010, were asked to complete a questionnaire to assess the presence of joint complaints during the previous year. Patients with self-reported joint and/or back pain (n=155) were invited to attend the JOINT outpatient clinic. This clinic was established by the department of Gastroenterology and Hepatology and the department of Rheumatology to expand the knowledge of IBD joint complaints. All IBD patients without self-reported joint complaints served as controls (n=99). At the JOINT outpatient clinic, medical history and data on extra-intestinal manifestations (EIMs) were collected. In addition to routine physical examination, a rheumatologic examination was performed in all IBD patients, including a detailed assessment of the number of tender and swollen joints. Laboratory assessments included the erythrocyte sedimentation rate (ESR) and the C-Reactive Protein (CRP). HLA-B27 was only typed in patients with chronic back pain (CBP) and/or peripheral joint complaints. Of the 155 patients with self-reported arthropathies, 63 (40.6%) were classified according to the different SpA classification criteria as reported previously.[15] Of these patients, 19 (12.3%) patients fulfilled the ASAS criteria for axial and peripheral SpA, the most often used classification criteria in clinical trials and the most practical system with which to classify SpA.[15,17] Eventually in total 15 (9.6%) patients were diagnosed with arthritis by a rheumatologist (FvG).

For the current protocol, the presence of the different serological biomarkers in 147 RA patients from the early arthritis clinic (EAC) was used as a comparison. This inception cohort comprises patients with arthritis with a disease

duration of less than 2 years. After 1 year of follow-up a final diagnosis was established and for this measurement, only baseline samples were used of patients who were diagnosed with RA and fulfilled the 1987 criteria.[18] The study was approved by the institutional medical ethical committee of the LUMC and patients signed a written informed consent prior to study enrolment, including biobanking protocol.

Measurement of serologic biomarkers

Serum levels of IgM-RF, IgA-RF, anti-CCP2, anti-CCP3.1 and anti-CarP IgG and IgA were determined using enzyme-linked immunosorbent assay (ELISA); the cut-off values used for anti-CCP2 was 25.0 AU/ml. For anti-CarP antibodies the cut-off was established as the mean plus 2 x SD of 200 healthy controls, as before.[8] Cut-off levels of IgM-RF, IgA-RF and anti-CCP3.1 were 6.0 AU/ml, 6.0 AU/ml and 20.0 AU/ml according to the manufacturer's (Inova Diagnostics Inc., San Diego) recommendation. Positivity of IgM-RF, IgA-RF, anti-CCP2, anti-CCP3.1 and anti-CarP in IBD patients with arthropathies were compared with those without arthropathies and RA patients.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 software (IBM). Chi-square tests and Student's t-test for independent samples were used to compare the biomarker positivity in IBD patients with arthropathies with IBD patients without arthropathies and RA patients. Logistic regression analysis, with the different biomarkers as dependent variable, was performed to assess variables associated with a positive biomarker. Univariate analyses were performed for several variables including age, gender, type of IBD, IBD disease activity (Harvey Bradshaw Index (HBI) or Simple Clinical Colitis Activity Index (SCCAI) > 4), smoking, arthritis (diagnosed by the rheumatologist) or SpA, classified according to the different SpA classification criteria.[15] Variables with a statistical level of $p<0.1$ in the univariate analysis were included in the multivariate analysis. A p-value ≤ 0.05 was considered as statistically significant.

RESULTS

Characteristics of the 254 patients with and without arthropathies are presented in Table 1. IBD patients with arthropathies were significantly more often diagnosed with Crohn's Disease (CD), more frequently female and smokers. No differences in biomarker positivity were found between IBD patients with and without arthropathies. Univariate analysis in all IBD patients showed that female gender (p=0.05, OR=0.5, 95%CI 0.25-0.99) and smoking (p=0.01, OR=2.4, 95%CI 1.21-4.55) were associated with a risk of having a positive IgM-RF antibody test. In the multivariate analysis, smoking (p=0.02, OR=0.44, 95%CI 0.22-0.85) remained independently associated with a positive IgM-RF test. In the univariate analysis for IgA-RF, smoking turned out to be significantly associated (p=0.03, OR=4.3, 95%CI 1.12-6.60) with a positivity for IgA-RF.

When a subdivision was made within the group of IBD patients with arthropathies based on CD and Ulcerative Colitis (UC), significantly more UC patients had a positive anti-CarP IgG antibody test (CD: 1 (0.8%) vs UC: 3 (8.8%), p=0.009). In addition, positivity of IgM-RF, IgA-RF, anti-CCP2, anti-CCP3.1, anti-CarP IgG and anti-CarP IgA in IBD patients with arthropathies were compared with RA patients. IBD patients with arthropathies were significantly less frequently positive for IgM-RF, anti-CCP2, anti-CCP3.1, anti-CarP IgA and anti-CarP IgG antibodies biomarkers (p<0.001) compared with the RA patients (Figure 1). When the 15 IBD patients with arthritis were compared with the 239 IBD patients without arthritis, none of the biomarkers were significantly more prevalent in the group of patients with both IBD and arthritis.

DISCUSSION

In the present study, RA biomarkers were assessed in IBD patients with and without arthropathies and the frequency of biomarker positivity was compared to RA patients. The RA markers were infrequently present in the IBD patients with no significant differences in positivity between IBD patients with and without arthropathies. A striking difference in autoantibody positivity was observed when comparing IBD patients with arthropathies to RA patients. In addition, as seen in RA, smoking seems to be related to IgM-RF and IgA-RF positivity in IBD.[19-20]

Although anti-CCP is highly specific for RA, previous studies have shown the occurrence of positive anti-CCP antibodies in other arthropathies such as psoriatic arthritis (PsA) and IBD patients.[21-24] Haga et al. concluded that the prevalence of anti-CCP IgA antibodies in IBD patients is low (1.2%), but significantly associated with arthritis and IgM-RF positivity. However, in studies of Papamichaels and Koutroubakis no significant association between the prevalence of anti-CCP positivity and IBD related arthropathies was found.[22-24] This is in accordance with the present study; in none of the IBD patients anti-CCP2 was detected and in 11 (6/155=3.8% with arthropathies and 5/99=5% without) IBD patients anti-CCP3.1 was present.

While the presence of anti-CCP2 and anti-CCP3.1 in IBD patients has been examined previously,[22-24] positivity for other possible arthropathy-related biomarkers has not been reported in IBD patients before. In the present study, besides the presence of anti-CCP2 and anti-CCP3.1, IgM-RF, IgA-RF and anti-CarP antibodies were assessed in the sera of IBD patients with and without arthropathies. In contrast to the increased sensitivity in RA patients achieved by combinations of these biomarkers, they did not add clinical value. Recently, Shi et al. reported that anti-CarP antibodies are the most sensitive antibodies that are present before RA becomes clinically apparent.[8] In our study anti-CarP was detected in only 4 (2.6%) IBD patients with arthropathies compared to 66 (42.6%) RA patients.

In the present study, smoking seems to be related with a positive IgM-RF and IgA-RF in IBD patients with arthropathies and supports the findings by Mikuls et al. in which current smokers were approximately twice as likely as never smokers to have increased IgA-RF concentrations. This association was most pronounced in the patients with more than 20 pack-years of exposure.[25]

An important strength of this study is the well-defined study cohort with all IBD patients classified thoroughly with or without SpA. Second, all different biomarkers known in RA patients were evaluated in this study design in IBD patients and compared with RA patients. A limitation of this study is the limited number of 15 patients with proven arthritis after rheumatologic examination although a total of 63 patients fulfilled one of the clinical SpA criteria. No difference in biomarker positivity was found between these 15 IBD patients with and 239 IBD patients without arthritis. Probably the number of in total 15 IBD patients diagnosed with arthritis was too small to make this difference.

Taken together, our data reveal that the presence of arthropathies in IBD is not accompanied by the presence of different RA serological biomarkers. The differences between positivity in IBD patients with arthropathies and RA patients suggest that the immuno-pathogenesis of arthropathies in IBD may differ from mechanisms in RA patients. More studies are required to investigate these differences. Furthermore, this study implies that there is no apparent clinical value in detecting these RA biomarkers in the serum of IBD patients with arthropathies.

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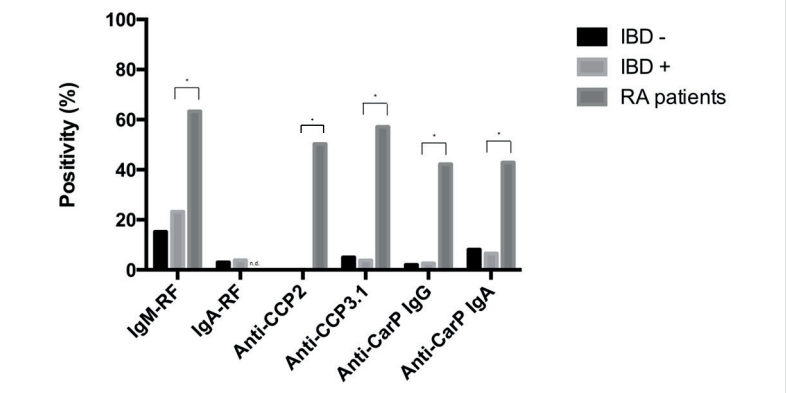
TABLES AND FIGURES

Table 1. Characteristics of the IBD patients (n=254).

	IBD patients with arthropathies (n=155)	IBD patients without arthropathies (n=99)	P-value
Type of IBD, n (%)			0.04
Crohn's Disease	121 (78.1)	66 (66.7)	
Ulcerative Colitis	34 (21.9)	33 (33.3)	
Male, n (%)	46 (29.7)	50 (50.5)	0.001
Age at inclusion (years), mean ± SD	43.4 ± 13.6	42.7 ± 13.6	0.70
Age of IBD onset (years), mean ± SD	27.5 ± 11.3	25.9 ± 10.1	0.26
IBD disease duration (years), mean ± SD	15.4 ± 11.8	16.3 ± 11.1	0.54
Smoker, n (%)	47 (30.0)	13 (13.1)	0.001
Positive IgM-RF, n (%)	36 (23.2)	15 (15.2)	0.12
Positive IgA-RF, n (%)	6 (3.9)	3 (3.0)	0.73
Positive anti-CCP2, n (%)	0 (0.0)	0 (0.0)	-
Positive anti-CCP3.1, n (%)	6 (3.8)	5 (5.0)	0.71
Positive anti-CarP IgG, n (%)	4 (2.6)	2 (2.0)	0.77
Positive anti-CarP IgA, n (%)	10 (6.5)	8 (8.1)	0.62

Figure 1. Differences in biomarker positivity between IBD and RA patients.



Comparisons of the positivity's of IgA-RF, anti-CCP2, IgM-RF, anti-CCP3.1, anti-CarP IgG and anti-CarP IgA in IBD patients with arthropathies (IBD +), IBD patients without arthropathies (IBD -) and RA patients. IgA-RF is not determined (n.d.) in the RA patients.
* p<0.001