

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

Sanne J.H. Van Erp^a, Marije K. Verheul^b, E.W. Nivine Levarht^b, Johan J. van der Reijden^a, Désirée van der Heijde^b, Floris A. van Gaalen^b, Daniel W. Hommes^{a,c}, Gary L. Norman^d, Zakera Shums^d, Michael Mahler^d, Hein W. Verspaget^a, Leendert A. Trouw^b and Andrea E. van der Meulen-de Jong^a

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

Patients and methods Sera from 155 IBD patients with and 99 IBD patients without arthropathies were analyzed for immunoglobulin (Ig) M rheumatoid factor (RF), IgA-RF, anti-cyclic citrullinated peptide 2, anti-cyclic citrullinated peptide 3.1, and anti-carbamylated protein antibody positivity using enzyme-linked immunosorbent assays. The prevalence of the autoantibodies in the IBD patients was compared with 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 positivity for IgM-RF or IgA-RF.

Conclusion Our findings suggest that there is no apparent clinical value in the detection of RA biomarkers in serum of IBD patients to help identify arthropathies. *Eur J Gastroenterol Hepatol* 29:345–348

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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 as 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 in 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 nonautoimmune 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 [5,6]. Anti-CCP antibodies are highly specific (up to 99%) for RA, but less sensitive compared with 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.* [8] as anti-CarP antibodies has been described as a disease marker in RA patients and targets carbamylated proteins rather than citrullinated proteins. Carbamylation involves a post-translational 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 anti-CCP-negative patients and may predict the development of RA, independent of anti-CCP antibodies [11–13].

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

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Departments of ^aGastroenterology and Hepatology, ^bRheumatology, Leiden University Medical Center, Leiden, The Netherlands, ^cCenter for Inflammatory Bowel Diseases, University of California Los Angeles, Los Angeles and ^dInova Diagnostics, San Diego, California, USA

Correspondence to Andrea E. Van der Meulen-de Jong, MD, PhD, Department of Hepatology and Gastroenterology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

Tel: +31 71 526 2915; fax: +31 71 524 8115; e-mail: ae.meulen@lumc.nl

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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.

Patients and 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, carried out at the Department of Gastroenterology and Hepatology of the Leiden University Medical Center, 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 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 and the C-reactive protein. HLA-B27 was only typed in patients with chronic back pain 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 classification criteria used most often 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 (F.v.G.).

For the current protocol, the presence of the different serological biomarkers in 147 RA patients from the early arthritis clinic was used as a comparison. This inception cohort includes patients with arthritis with a duration of the complaints of less than 2 years. After 1 year of follow-up, a final diagnosis was established and for this measurement, only baseline samples of patients who were diagnosed with RA and fulfilled the 1987 criteria were used [18]. The study was approved by the institutional medical ethical committee of the Leiden University Medical Center and patients signed a written informed consent before study enrollment, including the 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 an enzyme-linked immunosorbent assay; the cutoff value used

for anti-CCP2 was 25.0 AU/ml. For anti-CarP antibodies, the cutoff was established as the mean + 2 × SD of 200 healthy controls, as before [8]. Cutoff levels of IgM-RF, IgA-RF, and anti-CCP3.1 were 6.0, 6.0, and 20.0 AU/ml according to the manufacturer's (Inova Diagnostics Inc., San Diego, California, USA) recommendation. Positivity of IgM-RF, IgA-RF, anti-CCP2, anti-CCP3.1, and anti-CarP in IBD patients with arthropathies was compared with those without arthropathies and RA patients.

Statistical analysis

Statistical analysis was carried out using SPSS, version 23.0 software (IBM Corp., Armonk, New York, USA). χ^2 and Student's *t*-tests 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 a dependent variable, was carried out to assess variables associated with a positive biomarker. Univariate analyses were carried out for several variables including age, sex, type of IBD, IBD disease activity (Harvey Bradshaw Index or Simple Clinical Colitis Activity Index > 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*-value less than 0.1 in the univariate analysis were included in the multivariate analysis. A *P*-value less than or equal to 0.05 was considered 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 females 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 sex [odds ratio (OR) = 0.5, 95% confidence interval (CI) = 0.25–0.99, *P* = 0.05] and smoking (OR = 2.4, 95%

Table 1. Characteristics of the inflammatory bowel disease patients ($n = 254$)

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

CarP, carbamylated protein; CCP, cyclic citrullinated peptide; IBD, inflammatory bowel disease; Ig, immunoglobulin; RF, rheumatoid factor.

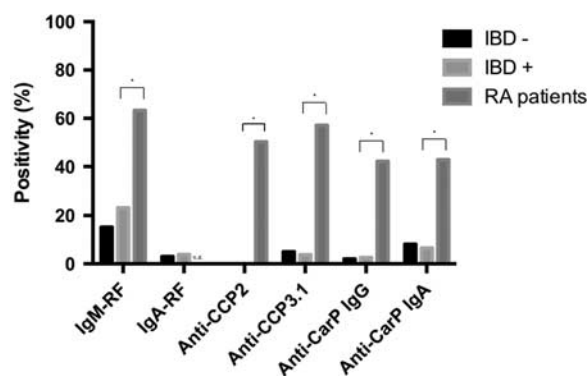


Fig. 1. Differences in biomarker positivity between IBD and RA patients. Comparisons of the positivity of IgA-RF, anti-CCP2, IgM-RF, anti-CCP3.1, anti-CarP IgG, and anti-CarP IgA in IBD patients with arthropathies (IBD+), IBD patients without arthropathies (IBD-) and RA patients. IgA-RF is not determined (n.d.) in the RA patients. * $P < 0.001$. CarP, carbamylated protein; CCP, cyclic citrullinated peptide; IBD, inflammatory bowel disease; Ig, immunoglobulin; RA, rheumatoid arthritis; RF, rheumatoid factor.

CI = 1.21–4.55, $P = 0.01$) were associated with a risk of having a positive IgM-RF antibody test. In the multivariate analysis, smoking (OR = 0.44, 95% CI = 0.22–0.85, $P = 0.02$) remained independently associated with a positive IgM-RF test. In the univariate analysis for IgA-RF, smoking turned out to be significantly associated (OR = 4.3, 95% CI = 1.12–6.60, $P = 0.03$) with a positivity for IgA-RF.

When a subdivision was performed within the group of IBD patients with arthropathies on the basis of CD and ulcerative colitis (UC), significantly more UC patients were positive in the 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 was compared with RA patients (Fig. 1). Approximately 40% of the IBD patients with arthropathies were classified with SpA and 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 (Fig. 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 with 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 with RA patients. In addition, as found 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 and IBD patients [21–24]. Haga *et al.* [22] 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 Papamichael and colleagues [23,24], no significant association was found between the prevalence of anti-CCP positivity and IBD-related arthropathies. This is in agreement with the present study; in none of the IBD patients was anti-CCP2 detected and in 11 (6/155 = 3.8% with arthropathies and 5/99 = 5% without) IBD patients, anti-CCP3.1 was present.

Although 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.* [8] reported that anti-CarP are the most sensitive antibodies that are present before RA becomes clinically apparent. In our study, anti-CarP was detected in only four (2.6%) IBD patients with arthropathies compared with 66 (42.6%) RA patients.

In the present study, smoking seems to be related to a positive IgM-RF and IgA-RF in IBD patients with arthropathies and supports the findings by Mikuls *et al.* [25] 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.

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 total of all 15 IBD patients diagnosed with arthritis was too small to make this difference.

Taken together, our data show 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 immunopathogenesis 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.

Acknowledgements

Conflicts of interest

G.L.N., Z.S., and M.M. are employees of Inova Diagnostics, San Diego, USA, and L.A.T. is listed as an inventor on a patent application for the detection of anti-CarP antibodies in rheumatoid arthritis. For the remaining authors there are no conflicts of interest.

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