Research article

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Role of anti-cyclic citrullinated peptide antibodies in discriminating patients with rheumatoid arthritis from patients with chronic hepatitis C infection-associated polyarticular involvement

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Abstract

This study was performed to assess the utility of anti-cyclic citrullinated peptide (anti-CCP) antibodies in distinguishing between patients with rheumatoid arthritis (RA) and patients with polyarticular involvement associated with chronic hepatitis C virus (HCV) infection. Serum anti-CCP antibodies and rheumatoid factor (RF) were evaluated in 30 patients with RA, 8 patients with chronic HCV infection and associated articular involvement and 31 patients with chronic HCV infection without any joint involvement. In addition, we retrospectively analysed sera collected at the time of first visit in 10 patients originally presenting with symmetric polyarthritis and HCV and subsequently developing well-established RA. Anti-CCP antibodies and RF were detected by commercial second-generation anti-CCP2 enzyme-linked immunosorbent assay and immunonephelometry respectively. Anti-CCP antibodies were detected in 23 of 30 (76.6%) patients with RA but not in patients with chronic HCV infection irrespective of the presence of articular involvement. Conversely, RF was detected in 27 of 30 (90%) patients with RA, 3 of 8 (37.5%) patients with HCV-related arthropathy and 3 of 31 (9.7%) patients with HCV infection without joint involvement. Finally, anti-CCP antibodies were retrospectively detected in 6 of 10 (60%) patients with RA and HCV. This indicates that anti-CCP antibodies can be useful in discriminating patients with RA from patients with HCV-associated arthropathy.

Keywords: anti-cyclic citrullinated peptide antibodies, hepatitis C virus, rheumatoid arthritis, rheumatoid factor

Introduction

The presence of extrahepatic manifestations is a relatively common feature in patients with chronic hepatitis C virus (HCV) infection [1,2]. Among the different clinical disorders associated with HCV infection, articular involvement is a frequent complication, and the clinical picture of HCV-related arthropathy varies widely [3,4], ranging from polyarthralgia to monoarticular or oligoarticular intermittent arthritis and symmetric chronic polyarthritis. In particular, monoarticular or oligoarticular involvement affects larger joints and is typically associated with mixed cryoglobulinemia, whereas symmetric polyarthritis associated with HCV infection frequently displays a rheumatoid arthritis (RA)-like clinical picture [3,4]. RA-like HCV-related arthropathy can be clinically indistinguishable from RA itself, and most patients with RA-like HCV-related polyarthritis fulfil the American College of Rheumatology (ACR) criteria for RA [5,6]. Thus, differentiating patients with HCV-related symmetric polyarthritis from patients with RA represents both a diagnostic and a therapeutic challenge.

ACR = American College of Rheumatology; AKA = anti-keratin antibodies; anti-CCP = anti-cyclic citrullinated peptide; HCV = hepatitis C virus; RA = rheumatoid arthritis; RF = rheumatoid factor.

Because the classic clinical picture of RA is not entirely helpful in differential diagnosis, other diagnostic tools, such as the detection of serologic abnormalities in sera of patients with RA, could be helpful in differentiating between these disorders. In this regard, however, the detection of classic IgM rheumatoid factor (RF) is of little utility as a diagnostic tool because a high percentage of patients with chronic HCV infection display serum RF reactivity, and the frequency of RF increases in patients with articular involvement [4,5].

In contrast, the currently available test – anti-CCP2 – for anti-cyclic citrullinated peptide (anti-CCP) antibodies has been shown to display a high specificity for RA accompanied by a reasonable high sensitivity [7–9]. Moreover, detection of anti-CCP antibodies is a useful diagnostic tool, particularly in the early stages of the disease, and a predictive factor in terms of disease progression and radiological damage [10–13]. However, so far no study has focused on the possible utility of anti-CCP antibodies in differentiating RA from HCV-related arthropathy.

The aim of this study was to evaluate, in a cohort of consecutive patients with chronic HCV infection, whether anti-CCP antibodies are useful in distinguishing between patients with HCV-related arthropathy and patients with RA.

Materials and methods

Patient sera

All the patients enrolled in this study were referred to the Department 'Clinica e Terapia Medica Applicata' of the University of Rome 'La Sapienza'.

To identify HCV patients with HCV-related arthropathy we enrolled 39 consecutive in-patients (16 females, 23 males; mean age 59 years, range 37–79) affected by chronic HCV infection that had been diagnosed on the basis of the presence of anti-HCV antibodies and confirmed by the detection of viral RNA in serum and who were undergoing hepatic biopsy. All the patients were subjected to careful historical interview and rheumatologic examination. On the basis of the presence of HCV-related arthropathy we identify two groups of HCV patients: group 1, including patients with articular involvement (8 patients), and group 2, comprising patients without articular involvement (31 patients).

To compare the prevalence of anti-CCP antibodies in HCV patients with that in patients affected by RA we enrolled 30 consecutive in-patients fulfilling the ACR criteria for RA (21 females, 9 males; mean age 60 years, range 35–75). Bleeding was performed after informed consent had been obtained; serum was recovered and then stored at –20°C until assayed.

To establish whether anti-CCP antibodies could help in the early diagnosis of RA in patients with HCV infection, in which the diagnostic role of RF is limited, we retrospectively analysed 10 patients (all females; mean age 55 years, range 44–73), initially referred to our department, presenting with symmetric polyarticular involvement and chronic HCV infection and subsequently developing an erosive pattern with a definite diagnosis of RA. Five of these patients were positive for RF. In this case, autoantibody detection was performed on sera collected at the time of first visit.

Anti-CCP antibodies and RF assays

Anti-CCP antibodies were detected with commercial enzyme-linked immunosorbent assay kits (DIASTAT™ anti-CCP; Axis Shield, Dundee, Scotland) in accordance with the manufacturer's instructions. In brief, microtitre plates were incubated for 60 min at 22°C with serum samples diluted 1:100 in phosphate-buffered saline. Pre-diluted anti-CCP standards and positive and negative controls were added to each plate. All assays were performed in duplicate. After three washes, plates were incubated for 30 min at 22°C with alkaline phosphatase-labelled murine monoclonal antibody against human IgG. After three washes, the enzyme reaction was developed for 30 min and stopped with sodium hydroxide–EDTA–carbonate buffer, and plates were read (SPECTRA II; SLT Labinstruments, Grödig, Austria) at 550 nm. Anti-CCP antibodies were considered to be positive when the absorbance was higher than the cut-off of the kit (5 U/ml). The concentration of anti-CCP antibodies was estimated by interpolation from a dose–response curve based on standards.

RF was assayed with a quantitative immunonephelometry test (Behring, Marburg, Germany). RF was considered to be positive when the concentration was higher than the cut-off value of the kit (15 IU/ml).

All serum samples with a high concentration of RF or anti-CCP antibodies were further quantified after further dilution.

Statistical analysis

The χ^2 test or Fisher's exact test was used as appropriate to compare qualitative variables in the different groups; $P < 0.05$ was considered statistically significant.

Ethics

Informed consent was obtained from each patient and the local ethics committee approved the study protocol.

Results

The main demographic and clinical characteristics of RA and HCV patients are summarised in Tables 1 and 2 respectively. All patients affected by RA received treatment with various disease-modifying antirheumatic drugs.

Articular involvement was observed in 20.5% (8 of 39) of HCV patients. Three of the eight (37.5%) HCV patients

Table 1

Clinical and demographic characteristics of patients with RA

Variable	RA ($n = 30$)	RA–HCV ($n = 10$)
Age (years), mean (range)	60 (35–75)	55 (44–73)
Disease duration (years), median (interquartile range)	10 (2.5–13.5)	0.5 (0.5–11)
ESR (mm/h), mean ± SD	42 ± 24	50 ± 35
CRP (mg/l), mean ± SD	24 ± 22	34 ± 23

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; interquartile range, 25th–75th; RA, rheumatoid arthritis; RA–HCV, hepatitis C virus-related RA-like polyarthritis.

Table 2

Clinical and demographic characteristics of patients with HCV

Variable	HCV without articular involvement ($n = 31$)	HCV with articular involvement ($n = 8$)
Age (years), mean (range)	58 (35–75)	67 (50–79)
Polyarthritis, no. (%)	0 (0)	3 (37.5)
Polyarthralgias, no. (%)	0 (0)	5 (62.5)
Cryoglobulinemia, no. (%)	5 (16)	2 (25)
Transaminase U/L, mean ± SD		
ALT	74 ± 41	72 ± 29
AST	119 ± 79	102 ± 47
Liver biopsy*		
Slight activity hepatitis	12	3
Minimum activity hepatitis	4	0
Moderate activity hepatitis	8	1
Severe activity hepatitis	5	2
HCV genotype†		
1a	2	0
2a	2	2
3a	2	0
2a/2c	4	0
1b	17	4
4c/4d	2	0

*Four biopsies lacking. †Four genotypes lacking.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; U/L, unit/liter.

Table 3

Prevalence of anti-CCP antibodies and RF in the serum of patients enrolled in this study

Variable	RA ($n = 30$)	HCV without articular involvement ($n = 31$)	HCV with articular involvement ($n = 8$)
Anti-CCP, no. (%)	23 (76.6)	0 (0)	0 (0)
RF, no. (%)	27 (90)	3 (9.7)	3 (37.5)

Anti-CCP, anti-cyclic citrullinated peptide; HCV, hepatitis C virus; RA, rheumatoid arthritis; RF, rheumatoid factor.

with articular involvement showed clinical evidence of a RA-like pattern characterised by a nonerosive symmetric polyarthritis that affected the small diarthrodial joints of the hands, whereas the remaining five patients were affected by diffuse polyarthralgia. In patients with HCV, histological examination of hepatic biopsies showed a grading of activity from minimum to severe, with no difference between the two groups.

The prevalence of anti-CCP antibodies and RF in each group of patients is shown in Table 3: 23 patients with RA (76.6%) were positive for anti-CCP antibodies and 27 (90%) for RF, whereas no patient with HCV was positive for anti-CCP antibodies and 15.4% were positive for RF ($P < 0.0001$ in both cases). Interestingly, the prevalence of RF-positive patients increased to 37.5% in patients affected by HCV presenting with articular involvement (3 of 8), and 66.7% in patients with RA-like polyarthritis (2 of 3).

When we retrospectively analysed sera collected from 10 HCV patients with RA at the time of presentation we detected anti-CCP antibodies in 60% of them.

Discussion

Extrahepatic manifestations are frequently observed in patients with chronic HCV infection, with a prevalence of more than 74% [1]. In a prospective study on a large cohort of HCV patients, articular involvement represented the most common extrahepatic manifestation, affecting nearly 20% of patients in a 1-year follow-up [14].

Although it is not possible to identify a specific pattern of HCV-related arthropathy, two different clinical subsets of arthritis have mainly been described (reviewed in [4]): a monoarticular–oligoarticular intermittent arthritis affecting large and medium-sized joints and almost invariably associated with the presence of mixed cryoglobulinaemia [3,15] and a polyarticular symmetrical arthritis closely resembling RA [3,5,16]. Differential diagnosis between HCV-related polyarthritis and 'true' RA is often very difficult because most patients with HCV-related polyarthritis fulfil the ACR criteria for RA [4,5]. Thus, because of the lack of reliable clinical parameters able to differentiate between the two diseases, other markers are needed for the differential diagnosis.

Together with the classical clinical features of the disease, serological abnormalities, such as the presence of RF, are usually useful in the diagnosis of RA, and RF is included in the ACR criteria for RA. However, in cases of HCV-related arthropathy, RF detection is not very useful because it is often observed in sera of patients with HCV. In particular, classic IgM RF can be detected in more than 60% of patients with HCV-related arthropathy [5]. Moreover, even IgA RF, which has been suggested to be useful in distinguishing between RA and HCV-related polyarthritis [4], failed to demonstrate any specificity for RA compared with HCV-related arthropathy [17].

Recently, anti-keratin antibodies (AKA) have been shown to be useful in distinguishing between RA and HCV-related arthropathy [18]. However, although characterised by a high specificity, AKA display a rather low sensitivity for RA [19]; moreover, the detection of AKA is laborious, difficult to standardise and of limited clinical utility. The limits displayed by AKA and later by the tests for detection of anti-filaggrin antibodies [19] were largely overcome after the discovery of citrulline residues as the main antigenic target of AKA and anti-filaggrin antibodies and the subsequent development of an immunoenzymatic test using cyclic citrullinated peptide to detect anti-CCP antibodies [20]. The currently available so-called second-generation test, anti-CCP2, has been shown to retain a high specificity for RA accompanied by a reasonable (about 80%) sensitivity [7,9]. In addition, anti-CCP antibodies have been showed to be useful diagnostic tools even in the early stages of the disease and predictive factors both in terms of disease progression and radiological damage [10–13].

In this study we demonstrated that anti-CCP antibodies are able to differentiate patients with HCV-related arthropathy from patients with RA. In our population of consecutive chronic HCV patients we identified eight patients with HCV-related articular involvement. Three patients presented chronic polyarthritis that was clinically indistinguishable from RA. Remarkably, 37.5% (three of eight) of patients with HCV-related articular involvement and 66.7% (two of three) of patients with RA-like polyarthritis were positive for RF, whereas no patients displayed anti-CCP reactivity. In contrast, we confirmed the increased sensitivity of the anti-CCP2 test with a prevalence of 76.6% in our patients with RA, comparable with that obtained in recent studies [7,8].

In addition, when we retrospectively analysed the prevalence of anti-CCP antibodies in patients presenting with symmetric polyarthritis and HCV infection who subsequently developed a well-established erosive RA, we demonstrated that anti-CCP antibodies were present in 60% of patients at the time of first visit. Although confirmation is needed in a larger cohort of patients with HCV-related RA-like polyarthritis, these results suggest that anti-CCP antibodies can be useful in differential diagnosis with RA.

Differentiating between patients with RA and those with HCV-related arthropathy has great relevance in clinical practice. In fact, in contrast with RA, RA-like HCV-related arthropathy usually shows a relatively benign course that is not associated with bony erosions and joint deformation [4,5,16]. Thus, management of HCV-related arthropathy usually does not require the use of heavy immunosuppressive treatment [4,21], which is associated with potential hepatotoxicity as demonstrated in patients with RA with concomitant chronic HCV infection [22] or may worsen the evolution of liver damage in HCV-affected patients. Thus, an early differential diagnosis could be of great importance in establishing the correct treatment to prevent joint erosions in patients with 'true' RA as well as chronic HCV infection and reducing the risk of immunosuppressive therapy in patients with HCV-related arthropathy.

Conclusion

In this study we provide evidence that anti-CCP antibodies are absent from the serum of patients with chronic HCV infection and associated articular involvement, and we confirm the high specificity and good sensitivity of anti-CCP2 for RA. In particular, the demonstration that the test for anti-CCP antibodies is negative in patients with HCV-related RA-like arthropathy who are seropositive for RF indicates that anti-CCP antibodies can be useful in discriminating patients with RA from patients with HCV-associated arthropathy.

Competing interests

None declared.

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