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Determination of anticyclic citrullinated peptide antibodies in the sera of patients with liver diseases

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ABSTRACT

Objective. To determine the frequency of anti-cyclic citrullinated peptide (anti-CCP) antibodies in patients with HCV infection, primary biliary cirrhosis (PBC) and type-I autoimmune hepatitis (AIH) to assess the specificity of anti-CCP antibodies.

Methods. Rheumatoid factor (RF) and anti-CCP antibodies were measured in the sera from patients with HCV infection (n = 45), PBC (n = 73), AIH (n =55) and rheumatoid arthritis (n = 48), and also from the sera of healthy subjects (n = 23). Anti-CCP antibodies were measured using a second generation enzyme-linked immunosorbent assay (ELISA).

Results. No sera with elevated anti-CCP were found in the patients with HCV infection. Two PBC patients (2.7%) and six AIH patients (10.5%) had anti-CCP antibodies. The seropositivity for anti-CCP in these autoimmune disease patients was associated with a high frequency of RA association [PBC; 100% (2/2), AIH; 86.4% (5/6)].

Conclusions. Although anti-CCP antibodies may be present in patients with autoimmune liver diseases, almost seropositive patients had concomitant RA. As a result, the measurement of anti-CCP antibodies may therefore be helpful for accurately diagnosing RA in patients with these liver diseases.

Introduction

Arthralgia is one of the most common extra-hepatic manifestations in patients with hepatitis C virus (HCV) infection or HCV-related cryoglobulinemia (1, 2). In addition, associations of rheumatoid arthritis (RA) in autoimmune liver diseases, such as primary biliary cirrhosis (PBC), have also been observed (3). The serological test routinely used is the determination of the IgM rheumatoid factor (RF) which constitutes one of the classification criteria proposed by the American College of Rheumatology (ACR) (4).

Many autoantibodies, including RF, are common in HCV-infected patients (5). In the light of these features, the distinction between liver disease-associated arthropathy and the occurrence

of rheumatoid arthritis may be difficult. Therefore, the detection of classical RF is of little utility as a diagnostic tool because a high percentage of patients with HCV infection or autoimmune liver diseases have been shown to display serum RF reactivity.

Anti-cyclic citrullinated peptide (CCP) antibodies are antibodies against synthetic citrullinated peptides (6, 7). The currently available test, namely the socalled second generation test (CCP-2), uses highly reactive peptides which are identified from dedicated libraries of citrullinated peptides screened with RA sera and recent studies indicate that this anti-CCP2 test is extremely specific (91%), while also demonstrating a sensitivity of approximately 81% (8). Other studies indicate that anti-CCP antibodies are present early in the disease and their presence can accurately predict the development of RA (9). However, the presence of anti-CCP antibodies has been described in patients with systemic lupus erythematosus (SLE) and primarily Sjögren's syndrome (10). Similarly, it has been reported that anti-CCP antibodies can be detected in 9% of autoimmune hepatitis (AIH) patients, in the absence of any recognizable RA overlap (11). To our knowledge, there have so far been only a few studies investigating anti-CCP antibodies in patients with liver diseases. Therefore, the specificity of anti-CCP antibodies should be re-evaluated in a large population of patients with liver diseases. Our aim in the present study was to investigate the presence of anti-CCP antibodies in patients with liver diseases, and to determine the diagnostic reliability of such antibodies for the identification of RA association.

Materials and methods

Anti-CCP antibodies and RF detection Anti-CCP antibodies were detected using a commercial enzyme-liked immunosorbent assay kit (second generation test, anti-CCP2, Axis Shield, Dundee, Scotland) in accordance with the manufacturer's instruction. Anti-CCP antibodies were considered to be positive when the absorbance was higher than the cut-off value as determined for the kit (5U/ml). The concentration of anti-CCP antibodies was estimated by interpolation from a dose- response curve based on standards. IgM-rheumatoid factor (RF) was measured by a latex-enhanced immunonephelometric assay (Dede Behring, Marburg, Germany). The cut-off value for RF was 20 IU/ml.

Patient sera

Serum samples were obtained from patients with chromic liver disease (CLD) related to HCV infection (n = 45, male:16, female: 29, mean age: 61.3 ± 11.6 years), type-I AIH (n = 55, male: 9, female: 46, mean age: $59.5 \pm$ 13.8 years), PBC (n = 73, male: 9, female: 64, mean age: 62.8 ± 12.1 years), and RA (n = 48, male: 6, female: 42, mean age: 56.7 ± 12.7 years) and then were stored at -80°C. Of 45 patients with HCV infection, 28 had chronic hepatitis, 17 had liver cirrhosis. In addition, sera from healthy volunteers (n = 23, male: 7, female: 16, mean age: 37.0 ± 9.4 years) were used as control. The diagnosis was based on liver histology results in all chronic hepatitis patients, whereas the diagnosis of liver cirrhosis was made based on the findings of clinical analyses (biochemical, ultrasonographic, and endoscopic results). All patients were positive for anti-HCV antibodies, which were detected by a third generation enzyme immunoassay containing HCV antigens and they were positive for HCV-RNA in the serum as assessed by means of reverse transcription polymerase chain reaction. All patients with type I AIH were classified to have definite AIH based on the International Autoimmune Hepatitis Group score, (12) and all patients were sero-negative for antibodies to hepatitis A, B, C or any other hepatotropic viruses. As for the associations of autoimmune diseases, 5 of them had chronic thyroiditis, 1 had overlapping Sjögren's syndrome. All PBC patients were histologically diagnosed based on the internationally accepted criteria and were classified based on Scheuer's classification using specimens obtained by a needle liver biopsy. As for the associations of autoimmune diseases, 4 of them had



Fig. 1. IgM rheumatoid factor (RF) values in various patient populations and a reference population (healthy subjects). A cut-off value was set at 20 IU/ml.



Fig. 2. Anti-cyclic citrullinated peptide (anti-CCP) values in various patient populations and a reference population. A cut-off value was set at 5 U/ml. Values in all 23 healthy subjects and 45 patients with HCV infection were normal.

chronic thyroiditis, 1 had overlapping Sjögren's syndrome.

The patients with articular involvements were evaluated by rheumatologists and all RA patients were diagnosed as having definite RA, thus fulfilling the American College of Rheumatology (ACR) criteria for this disease (4).

Results

RF

RF was not detected in any of the 23 sera from the healthy subjects. In contrast, RF was more often positive in the patients with RA (81.3% 39/48) with markedly elevated values (Fig. 1). RF was also present in 22.2% of the patients

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Table I. The results of anti-CCP in relation to ANA or SMA.

AIH (n = 55)	Anti-CCP positive $(n = 6)$
ANA positive $(n = 40)$	4/40 (10.0%)
ANA negative $(n = 15)$	2/15 (13.3%)
SMA positive $(n = 16)$	1/16 (6.3%)
SMA negative $(n = 39)$	5/39 (12.8%)

ANA: anti-nuclear antibody; SMA: anti-smooth muscle antibody.



Fig. 3. Relationship between the titers of RF and anti-CCP antibodies in different patient groups with liver diseases. (open square: AIH; cross: PBC; open circle: CHC).

with HCV infection (8/45), however, the RF values tended to be low values (26~60 U/ml). In addition, RF was frequently present in PBC (13/65, 27%) and AIH patients (13/55, 23.6%) with positive values ranging from 21 IU/ml to 450 IU/ml and 60% demonstrated a high titer (> 100 IU/ml).

Anti-CCP antibodies

Anti-CCP antibodies were measured using the same sera (Fig. 2). Anti-CCP was not detected in any of the 23 sera from healthy subjects and 45 sera from patients with HCV infections. Anti-CCP antibodies were detected in 89.5% of RA patients (43/48) and the mean values of anti-CCP antibodies in RA were markedly elevated. The prevalence of anti-CCP antibodies in PBC was 2 of 73 patients with PBC (2.7%) and 6 out of 55 patients with AIH (10.9%). In all anti-CCP positive patients with PBC (n = 2), an association with definite RA has been previously confirmed. Regarding the patients with AIH, out of 6 anti-CCP positive patients, 5 were diagnosed to have definite RA. However, the one remaining anti-CCP positive patient had no documented rheumatic symptoms, including arthritis, and coexisting RA was ruled out. The prevalence of anti-CCP antibodies in AIH patients with anti-nuclear antibodies (ANA) or anti-smooth muscle antibodies (SMA) is shown in Table I. There was no statistically significant association between the presence of these autoantibodies and positivity for anti-CCP antibodies. Figure 3 indicated the relationship between the titers of RF and anti-CCP antibodies in the different patient groups. There was no correlation between RF and anti-CCP antibodies, however, anti-CCP-positive sera seem to contain RF with high titers.

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Discussion

RF can be detected in from 50~80% of RA patients, however, it is also detected in patients with either other autoimmune diseases or HCV infection, as well as even in normal healthy subjects. Extrahepatic manifestations are frequently observed in patients with HCV infection, and articular involvement represented the most common extra-hepatic manifestation in these patients (1-3). Many auto-antibodies are common in HCV-infected patients and autoimmune liver diseases, including RF and anti-nuclear antibodies (5). Therefore, making a differential diagnosis between liver disease-related polyarthritis and "true" RA is often very difficult because most these patients fulfill the ACR criteria for RA. Anticyclic citrullinated peptide (anti-CCP) antibodies are thus considered to be a useful marker for accurately diagnosing RA with a high specificity (8, 13). In this study we assessed the prevalence of anti-CCP antibodies and RF in patients with HCV infections, PBC and AIH. Our data indicated that anti-CCP antibodies were not detected in HCV-infected patients, in contrast to their frequent association with RF. Our results closely correlate with the previous reports demonstrating that anti-CCP was not observed in patients with HCV infection or HCV-related cryoglobulinemia (14, 15). In contrast to HCV-infected patients, we found that 2 of 73 PBC patients (2.7%) had anti-CCP antibodies. This is more than would normally be expected in view of the high specificity of anti-CCP antibodies. However, in all these anti-CCP positive PBC patients, the coexistence of RA had also been confirmed. Fusconi et al. reported that anti-CCP antibodies were found in two (4%) of 49 patients with PBC, and these two anti-CCP positive patients had no rheumatic manifestation (11). Discrepancies were observed between our results and these reported results, and further study using a large scale of PBC patients would be needed.

Regarding the prevalence of the anti-CCP antibodies in AIH, Fusconi *et al.* reported that anti-CCP antibodies were detected in 9% of type-1 AIH patients in the absence of any recognizable RA overlap, and in some cases with a high titer (11). Vannini et al. also demonstrated that anti-CCP antibodies can be detected in 9% of type 1 AIH patients in the absence of RA overlap with citrullinated-independent reactivity (16). We re-evaluated the prevalence of anti-CCP antibodies in type-1 AIH patients. We found anti-CCP antibodies in 6 of 55 AIH patients, while 5 of 6 anti-CCP positive AIH patients had coexisting definite RA and frequent RA association was observed in our AIH patients in accord to the nationwide survey of Japanese AIH patients (17). Therefore, the frequencies of anti-CCP antibodies (10.4%) in our type 1 AIH patients were quite similar to those of the previous investigations (11, 16). However, the prevalence of RA associations (5/6) in anti-CCP positive AIH patients differed from those reported by Vannini et al. (16).

The reason for this discrepancy remains unclear. It was demonstrated that shared epitope-containing DRBI alleles were associated with the presence of anti-CCP antibodies (18). These genetic backgrounds of the investigated populations could possibly contribute to the positivity for anti-CCP antibodies. More recently, Montano-Loza et al. demonstrated that anti-CCP antibodies were detected in 11% of patients with type-1 AIH and that seropositivity for anti-CCP antibody was associated with a high frequency of RA (19). These findings are consistent with our results. Further studies are therefore needed to clarify the precise prevalence or specificity of anti-CCP antibodies in AIH patients.

In conclusion, the distinction between liver disease-associated arthropathy and RA has great relevance for clinicians. Our results in a large group of patients with liver diseases showed that anti-CCP antibodies were rarely present in patients with HCV infection and autoimmune liver diseases and such antibodies may therefore be a reliable serological marker for RA in these patients.

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