

## Absence of Antibodies to Cyclic Citrullinated Peptide in Sera of Patients With Hepatitis C Virus Infection and Cryoglobulinemia

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**Objective.** To determine if antibodies to cyclic citrullinated peptide (anti-CCP) are found in chronic hepatitis C virus (HCV) infection.

**Methods.** Rheumatoid factor (RF) and anti-CCP were measured in sera from 50 patients with HCV infection but without cryoglobulinemia, sera from 29 patients with mixed cryoglobulinemia (including 13 with rheumatic symptoms and 5 with arthritis), and sera from 20 normal blood donors. Anti-CCP was measured by second-generation enzyme-linked immunosorbent assay (ELISA).

**Results.** No sera with elevated anti-CCP were found in patients with HCV infection without cryoglobulinemia, and in that population, the maximum anti-CCP was 10 units, well below the positive cutoff of 20 units. Positive findings on RF testing >13 IU/ml were present in 22 (44%) of the HCV patients, with RF >50 IU/ml in 8 (16%) and a maximum RF of 526 IU/ml. Of the cryoglobulinemia patients, 22 (76%) had positive results on tests for RF, including 18 (62%) with RF >50 IU/ml and a maximum RF of 5,540 IU/ml. Two (6.9%) of the cryoglobulinemia patients had borderline-positive findings on tests for anti-CCP (25 units and 37 units), which were false-positive results caused by nonspecific binding in the ELISA. No association between the RF and the anti-CCP concentrations was found.

**Conclusion.** Whereas RF was frequent in patients with HCV infection with and without cryoglobulinemia, anti-CCP was not observed in patients with uncomplicated HCV infection. Borderline-positive anti-CCP results were observed infrequently in patients with mixed

cryoglobulinemia and were caused by nonspecific binding to plastic. Measurement of anti-CCP may help in diagnosing RA in patients with chronic HCV infection.

Patients with chronic hepatitis C virus (HCV) infection may have a variety of rheumatic symptoms and signs, including arthralgias and arthritis (1,2). Furthermore, the sera of patients with chronic HCV infection may contain rheumatoid factor (RF) in 50–70% of the cases (3), with an even higher prevalence in patients with HCV-related cryoglobulinemia. These features can lead to challenges in making or excluding a diagnosis of rheumatoid arthritis (RA) in patients infected with HCV (2,4). For distinguishing between true RA and HCV-associated rheumatic symptoms, the presence of RF is of little help and can be confusing or misleading.

Antibodies to citrullinated peptides, detected using cyclic citrullinated peptide (CCP) as the target antigen, are associated with RA and have a diagnostic usefulness that equals or exceeds the utility of RF for RA (5–7). Approximately 80–90% of patients with RF+ RA also have antibodies to CCP (7,8). We sought to determine if antibodies to CCP are found in chronic HCV infection. If anti-CCP is not present in the setting of RF+ HCV, measurement of anti-CCP could help distinguish between RF+ RA coexistent with HCV infection and HCV-associated arthritis. Furthermore, information on the association of anti-CCP with RF+ chronic HCV infection could help determine whether the presence of anti-CCP was linked primarily to the diagnosis of RA or to the presence of RF. To our knowledge, the prevalence of anti-CCP in HCV infection has not previously been investigated systematically.

### MATERIALS AND METHODS

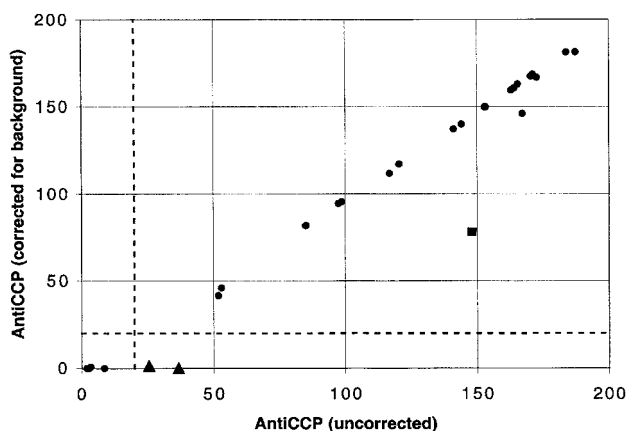
**Autoantibodies.** RF was measured using the Behring BN II nephelometer (Dade Behring, Deerfield, IL). This

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**Figure 2.** Comparison of anti-cyclic citrullinated peptide (anti-CCP) results obtained after correction for nonspecific binding to enzyme-linked immunosorbent assay plates and using the conventional test without correction for nonspecific binding. Broken lines show the upper limit of the normal reference range. The 2 specimens from cryoglobulinemia patients with positive anti-CCP results (see Figure 1) (▲) demonstrate that the positive results were eliminated when nonspecific binding was subtracted. One of the positive sera (■) had a significant reduction in the measured level of anti-CCP, from 148 to 78 units, after correcting for background binding, but the result remained elevated even after this correction. For most of the sera tested (●), the background correction caused no significant change in the calculated anti-CCP value.

the RF assay in the cryoglobulinemia sera. The RF was weakly positive (20 IU/ml) in 1 of the 2 anti-CCP-positive cryoglobulinemia patients and negative (<12 IU/ml) in the other.

Among the total of 29 cryoglobulinemia patients, 13 (45%) had documented rheumatic symptoms, including 5 with arthritis. Clinicians caring for 3 of these patients had listed RA as a diagnostic consideration at some time during the course of the disease. One of the 2 cryoglobulinemia patients with a positive test for anti-CCP had arthralgias, but neither had documented arthritis nor was either of them clinically considered to have RA.

Cryoglobulins and other immune complexes can contribute to nonspecific binding of Ig to plastic wells used in ELISAs and, thus, can contribute to false-positive results in ELISAs (13). We therefore tested for nonspecific binding of sera by using plastic microtiter plates that had been blocked using the techniques for blocking the CCP-coated wells, but without the CCP antigen, using the same serum dilution and diluent used in the standard anti-CCP assay. Both of the cryoglobulin-containing sera that had been positive for the anti-CCP test as well as 28 sera from representative RA patients with a range of anti-CCP were tested for the

presence of nonspecific binding and retested in parallel using the standard anti-CCP assay. Tests of the 2 cryoglobulin-containing sera demonstrated that the elevated absorbance in the anti-CCP test could be attributed entirely to nonspecific binding. Thus, while the prevalence of a positive anti-CCP test result in cryoglobulinemic patients was 6.9%, the frequency of true antibodies to CCP in cryoglobulinemia sera was 0% (95% CI 0–11.9). In contrast to the importance of nonspecific binding in the cryoglobulinemia sera, none of the positive anti-CCP results in the RA sera tested was due entirely to nonspecific binding, although 1 of the positive specimens had a significant reduction in the level of anti-CCP, from 148 to 78 units (Figure 2).

## DISCUSSION

Antibodies to citrullinated peptides are a family of antibodies with specificities directed against a variety of citrullinated peptides (14) and are most commonly detected using CCP as the antigen (6). Antibodies to CCP are present in the sera of a majority of patients with RA, only rarely in the sera of patients with other diagnoses, and are usually present in the sera of RA patients with RF as well as in a minority of RA patients in whom serum RF is not present. The presence of anti-CCP antibodies has been shown in many studies to be a useful marker for RA (5–7).

Patients with chronic HCV infection may have a variety of rheumatic symptoms, and their sera often contain high titers of RF (2). Since the prevalence of HCV in the general population (1–2% in the US) is similar to the prevalence of RA, and since RF is detected at similar frequencies in HCV patients and RA patients, the presence of RF is generally not helpful in establishing a diagnosis of RA in patients with concurrent HCV infection. We sought to determine if anti-CCP was also associated with HCV infection. The data presented here clearly indicate that anti-CCP rarely is found in the sera of unselected patients with HCV. We confirmed that, in contrast, RF is common in the sera of those patients. No cases of cryoglobulinemia with true elevations in anti-CCP levels were found, although nonspecific binding led to borderline-positive results in 2 of 29 cryoglobulinemia sera.

Our observations demonstrate that HCV and cryoglobulinemia are not associated with anti-CCP, in contrast to their frequent association with RF. In evaluating patients with arthralgias or arthritis, positive RF tests, and chronic HCV infection, the presence of anti-CCP in the serum at high levels would provide strong

support that the patient has true concurrent RA. In contrast, the absence of anti-CCP in such a patient would suggest that the patient is less likely to have erosive, deforming RA and is more likely to have HCV-associated rheumatic disease. Previous studies of patients with other infections have also shown that anti-CCP is rarely seen in the sera of patients with chronic infection, whereas RF is occasionally seen (6).

By demonstrating the lack of association between positive findings on tests for RF and anti-CCP in a population with a high prevalence of RF but without RA, our data contribute to the evidence that the factors leading to the production of RF are distinct from those that lead to the development of anti-CCP. Citrullinated synovial proteins are present in RA, suggesting a plausible explanation for the origin of anti-CCP and the potential for a pathogenic role (15,16), although further research is necessary to clarify the role of anti-CCP.

Nonspecific binding of Ig to plastic microtiter plates can cause false-positive ELISA results (13). Nonspecific binding may be enhanced by the presence of Ig aggregates or immune complexes, such as cryoglobulins. If elevated values on antibody tests are observed in a clinical setting in which the results are unlikely, the possibility of false-positive test results should be considered. Testing serum that was allowed to bind to blocked but antigen-uncoated wells, the approach used in these studies, is one effective way to test and correct for nonspecific binding. Our data indicate that nonspecific binding can cause borderline or weakly false-positive results, and such a mechanism should be considered in evaluating the specificity of diagnostic blood tests. Furthermore, manufacturers of diagnostic reagents, laboratories performing these tests, and clinicians using the findings of these tests should be aware of the potential for nonspecific binding.

Our study confirms previous data indicating that RF is frequently present in the sera of patients with HCV, and therefore, RF testing is of little, if any, value in assessing the possibility of RA in such patients. In contrast, we have shown that the presence of high levels of anti-CCP in a patient with HCV and arthritis is unlikely to be related to HCV infection. Testing HCV-infected patients for the presence of anti-CCP is likely to be helpful in diagnosing early RA in these cases.

**Addendum.** In a recent electronic publication, it was reported that anti-CCP is not present in the serum of patients with chronic HCV infection (17).

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