Absence of Cyclic Citrullinated Peptide Antibody in Nonarthritic Patients with Chronic Hepatitis C Infection

DOUGLAS LIENESCH, ROBERT MORRIS, ALLAN METZGER, PAIGE DEBUYS, and KENNETH SHERMAN

ABSTRACT. ***Objective.*** The increased prevalence of rheumatoid factor (RF) in patients with chronic hepatitis C virus (HCV) infection markedly diminishes the diagnostic specificity of serum rheumatoid factor (RF) for rheumatoid arthritis (RA) in patients with HCV. Cyclic citrullinated peptide (CCP) antibody, a highly specific biomarker for RA in the general population, may have better diagnostic utility for RA in the HCV population. To investigate if CCP antibody retains its specificity for RA in HCV infection, we determined the prevalence of CCP antibodies and examined the relationship between RF production and CCP antibody levels in a population of nonarthritic patients with chronic HCV infection.

Methods. CCP antibody and IgM, IgG, and IgA RF isotypes were determined by ELISA in serum from nonarthritic patients with chronic HCV infection.

Results. In a series of 50 HCV patients, IgG-RF, IgM-RF, and IgA-RF were detectable in 52%, 26%, and 14%, respectively, with a total seropositivity rate of 54%. Marginally elevated CCP antibody was detected in a single patient (2%). By regression analysis, serum levels of CCP antibodies did not correlate with RF levels.

Conclusion. In contrast to RF, CCP antibody is not increased in HCV infection. CCP antibody may have improved utility for the diagnosis of RA in this patient population. (J Rheumatol 2005;32:489–93)

Key Indexing Terms:
HEPATITIS C VIRUS CYCLIC CITRULLINATED PEPTIDE RHEUMATOID FACTOR

Rheumatoid arthritis (RA) and hepatitis C virus (HCV) infection are 2 distinct chronic diseases that share several intriguing similarities. Each illness has a prevalence in the general population of about 1% and is associated with immune system activation, autoantibody and cryoglobulin production, secondary vasculitis and Sjögren's syndrome, and an increased risk of B cell lymphoma[1,2]. An inflammatory polyarthritis indistinguishable from the synovitis typical of RA has been reported in HCV infected patients[3,4]. Also, an increased rate of HCV seropositivity has been reported in patients diagnosed with RA[5]. However, this association has recently been challenged[6].

HCV infected patients manifesting arthralgia and synovitis may satisfy the 1988 American Rheumatism Association (ARA) criteria for the classification of RA[4,7]. Differentiating those patients whose symptoms are an extrahepatic manifestation of HCV from patients who have concomitant RA is essential for appropriate management. Patients with virus induced symptoms require antiviral therapy; patients with RA benefit from disease modifying antirheumatic drugs (DMARD)[4,8]. The traditional serological test for RA, rheumatoid factor (RF), has markedly diminished specificity and diagnostic utility for RA in patients with chronic HCV. Detectable in less than 5% of the general population and present in 70–80% of patients with RA[9], RF is also elevated in 19–80% of nonarthritic HCV infected patients[8,10-13]. This high rate of RF seropositivity leads to diagnostic uncertainty in the HCV population with inflammatory arthropathy[4].

Non-RF autoantibody production is common in RA patients. Due to poor sensitivity or specificity, most of these autoantibodies have limited diagnostic or prognostic utility compared to RF[14]. However, cyclic citrullinated peptide (CCP) antibody is a commercially available serological marker for RA that is reported to have comparable sensitivity and improved specificity[15-22]. To our knowledge, CCP antibody has not been detected in patient populations with chronic infection. We hypothesized that CCP antibody, in contrast to RF, would not be present in patients with chronic HCV, and therefore might be a candidate biomarker for concurrent RA in this specific patient population. To investigate this possibility, we measured CCP antibodies and RF in the serum of nonarthritic HCV patients to determine if,

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similar to RF, CCP antibody production is a frequent nonspecific manifestation of chronic HCV infection.

MATERIALS AND METHODS

Patients. From January 2001 until December 2003, patients with chronic hepatitis C infection and under evaluation at the University of Cincinnati and affiliated clinical sites consented to participate in serum collection and database protocol. Clinical information and serum and, in most cases, liver biopsy data were obtained. Informed consent was obtained from all patients under the auspices of an approved protocol. The first 50 patients who satisfied the inclusion/exclusion criteria were selected in the reverse order of enrollment in the database. Patients were included if there was a history of HCV seropositivity and evidence of persistent infection by either liver biopsy or abnormal transaminases. The exclusion criteria included human immunodeficiency virus infection, hepatitis B surface antigenemia, known RA or other inflammatory polyarthropathy, or current use of antiviral therapy.

Laboratory methods. Serum samples were tested for anti-hepatitis C antibody by commercial ELISA testing and were confirmed in most cases by HCV RNA polymerase chain reaction amplification.

Testing for autoantibodies was performed at RDL Reference Laboratories (Los Angeles, CA, USA) using Quanta-Lite™ ELISA kits (Inova Diagnostics, San Diego, CA, USA) for IgG anti-CCP, IgG-RF, IgM-RF, and IgA-RF. All tests were performed according to the manufacturer's recommendations. For CCP antibody determination, a second generation synthetic CCP with a manufacturer reported sensitivity of 76% and specificity 98–99% for RA was bound to microwell plates. For RF determination, microwells were coated with rabbit IgG, as rabbit material has been shown to be more specific for diagnosing RA than when human IgG is used on the solid phase. Prediluted control and diluted patient sera are added to the microwell plates coated with the antigen. Unbound sample was washed away and an enzyme labeled anti-human IgG, IgM, or IgA was added to each well to detect IgG-RF, IgM-RF, or IgA-RF, respectively, or enzyme labeled anti-human IgG for CCP antibody detection. After washing away any unbound enzyme labeled anti-human immunoglobulin, the remaining enzyme activity was measured spectrophotometrically. The results of CCP ELISA were considered in the normal range if < 20 U, low positive if 21–39 U, moderately positive if 40–59 U, and strongly positive if > 60 U. For RF, patients were considered to be seropositive if serum RF was > 6 IU for all isotypes.

In our laboratory, the CCP antibody ELISA had a sensitivity of 93% and 46% for RF-positive and RF-negative RA patients, respectively, and a specificity of 97% and 99% for disease and healthy controls, respectively.

Analysis. Differences of categorical data between samples seropositive for RF and CCP antibodies were assessed using the chi-square test. Correlation of CCP antibody and RF concentrations in serum was determined by linear regression analysis.

RESULTS

Demographic characteristics of the group of 50 HCV patients in the study are presented in Table 1. Subjects had a mean age of 47 years (SEM 1.0); there were 39 men and 11 women, 44 Caucasians and 6 African-Americans. The mean estimated duration of disease was 24.1 (SEM 1.9) years, and the most common risk factors were intravenous drug use (44%) and blood transfusion (22%). No patients were receiving antiviral therapy. Fifty percent of patients were treatment-naive and 44% were treatment failures (previous treatment history was unavailable in 3 patients).

RF was elevated in the serum of 54% of the patients. Surprisingly, IgG-RF was detected most frequently (52%), followed by IgM-RF (26%), and IgA-RF (14%). More than one isotype was elevated in 26% of patients. CCP antibody was weakly positive (29 U) in a single patient, and no patient had moderate (40–59 U) or strong positive (> 59 U) test result (Figure 1). IgG-RF and IgM-RF were present in the CCP seropositive patient. When compared to CCP antibody seropositivity, there was a statistically significant increase in the number of patients with elevations of IgA, IgG, and IgM RF.

Table 1. Hepatitis C patient characteristics.

Age, yrs (SEM)	47 (1.0)
Race, %	
Caucasian	88
African American	12
Female, %	22
Male, %	78
Risk factor, %	
IV drug use	46
Blood product transfusion	22
Occupational needle stick	6
Nasal cocaine	6
Tattoo	6
Unknown	18
Treatment history, %	
Naive	50
Treatment failure	44
Unknown	6

Correlation of serum RF with serum CCP levels was examined by linear regression (Figure 2). In this group of patients, there was no statistically significant association between CCP antibody level and serum IgG-RF ($R^2 = 0.12$), IgM-RF ($R^2 = 0.06$), or IgA-RF ($R^2 = 0.21$). Age, race, sex, and estimated duration of HCV infection were also not associated with the serum levels of RF or anti-CCP antibody (data not shown).

DISCUSSION

Chronic HCV infection is associated with extrahepatic immune mediated conditions including vasculitis, glomerulonephritis, thyroiditis, and sialoadenitis[8,12]. Arthralgias are common, and oligo or polyarthritis have been reported[3-5,7]. Fadda, *et al* examined 302 HCV patients for arthritis[23]. In patients with mixed cryoglobulins, a nonerosive oligoarthritis was detected in 8%. Surprisingly, 15% of patients without mixed cryoglobulins had polyarthritis, with erosions noted in 30% of affected individuals. In some of these patients, treatment with interferon-α appeared to trigger or exacerbate arthritis. Others have reported increased HCV antibody rates in patients diagnosed with RA, although this relationship has been challenged[6]. The true properties of the relationship between HCV infection and RA remain controversial. However, increased autoantibody production in chronic HCV infection is well established. The autoantibodies include antinuclear and antiphospholipid antibodies, other anti-tissue antibodies, cryoglobulins, and RF[11,12,24].

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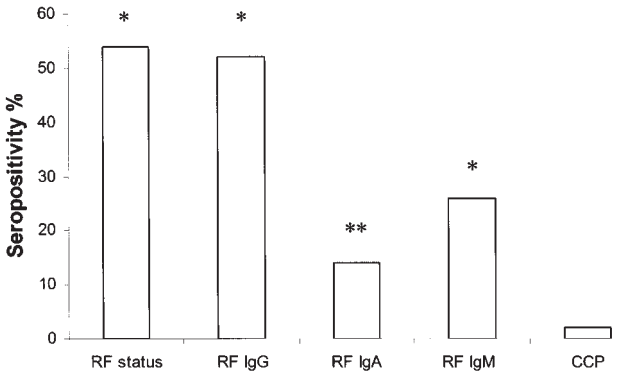

Figure 1. Autoantibodies in HCV patients. *p < 0.001 compared to CCP. **p = 0.02 compared to CCP.

RF can be highly prevalent, reaching 70–80% in some HCV populations[8,12,13,24]. In our population, slightly fewer were positive. It is possible that IgM-RF activity is underestimated due to precipitation of IgM-containing cryoglobulins during storage of the serum, or that that level decreased in some patients as a result of their previous antiviral therapy[25]. Indeed, fewer previously treated patients were RF seropositive (37%) compared to treatment-naive patients (65%), although comparison by Fisher's exact test did not quite meet statistical significance (p = 0.08).

This high rate of RF seropositivity in patients with chronic HCV infection presents diagnostic and therapeutic difficulties for the clinician. RF seropositivity, a component of the 1988 revised ARA criteria for the classification of RA, has markedly diminished positive predictive value when applied to populations with high RF prevalence in nonarthritic individuals. It is critical to identify patients with concomitant RA and HCV infection so that appropriate therapy can be initiated. Arthritis associated with mixed cryoglobulins or secondary to immune complex deposition related to the chronic viral infection might respond to interferon-α[4]. However, exacerbation of arthritis with interferon therapy in some HCV patients has been noted[4,23]. These patients might respond better to therapy directed specifically to RA. However, immunosuppressants are generally contraindicated in chronic HCV infection due to potential exacerbation of viral replication or direct hepatotoxicity. Recently, tumor necrosis factor-α inhibitors have been employed in HCV patients with polyarthritis. The early experience in select patients suggests these agents appear to be safe and effective[26].

The recent development of serological tests for antibodies to CCP in patients with RA has provided a new diagnostic tool for clinicians. Many patients with RA produce antibodies that recognize peptides containing the unusual amino acid citrulline. This discovery led to the development and subsequent US Food and Drug Administration approval of an ELISA that employs a synthetic cyclic peptide containing citrulline residues. This assay has a reported sensitivity

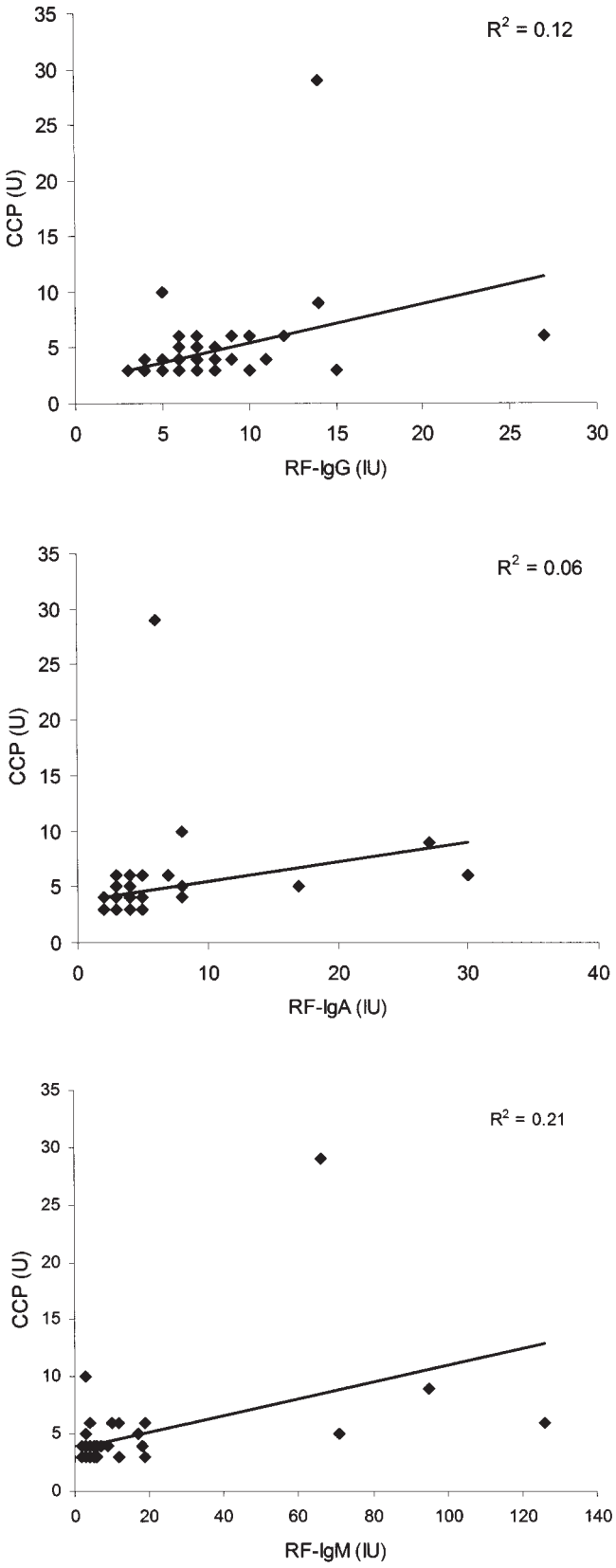

Figure 2. Correlation of serum CCP with RF-IgG, RF-IgM, RF-IgA.

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ranging from 41% to 68%, with a specificity of 96% to 98% in cohorts of patients with established RA[15-22]. In comparison, the sensitivity and specificity of RF for RA in patients with polyarthralgia/arthritis is 66%–80% and specificity 70%–85%, respectively[9]. In patients with new or early onset polyarthritis, the CCP antibody test had slightly less sensitivity than IgM-RF, but was significantly more specific for the diagnosis of RA[17,18,21]. Several studies have also suggested that anti-CCP antibodies identify patients at risk for more severe joint damage and functional disability[15,20,27-29].

In this study, we demonstrate that CCP antibody levels were not elevated in nonarthritic patients with chronic hepatitis C. In the single patient with CCP antibody above the upper limit of normal, the level was in the mild positive range and well below the cutoff level of 50 IU used to define the diagnostic characteristics of the CCP ELISA. Importantly, there was no correlation between the serum concentration of CCP and RF levels, suggesting that the HCV associated B cell activity responsible for RF production does not result in an increased production of antibodies against CCP. We have identified several HCV patients with an erosive polyarthritis meeting the diagnostic criteria for RA who have significantly elevated CCP antibodies (unpublished data). Considering these data together, a possible interpretation of the presence of CCP antibodies in HCV patients with arthritis is that the synovitis is secondary to concomitant RA. Supporting this hypothesis is the lack of CCP antibody production in other conditions associated with inflammatory arthropathy. Alternatively, CCP seropositivity in HCV patients with synovitis could occur as a result of antibody production in response to the local citrullination of peptides generated in the inflamed synovium. One limitation of this study is that our data cannot address this question. Ideally, patients with HCV related synovitis without concurrent RA would need to be studied to determine the prevalence of CCP antibodies. Studies designed for this objective will likely be significantly hampered by the lack of a gold standard by which to differentiate these patients from those with RA.

Interestingly, IgA-RF concentrations were elevated in a significant percentage of our HCV patients. IgA-RF is more specific for RA than IgG or IgM rheumatoid factors in the general population[28,30-32]. Both IgG-RF and IgM-RF have been described in HCV patients, often associated with mixed cryoglobulins[33,34], but to our knowledge elevated IgA-RF has not previously been reported. The significantly increased prevalence of IgA-RF in our HCV patients likely limits it as a diagnostic tool for RA in this population.

We did not include HCV patients with synovitis in this study, preventing us estimating the sensitivity of CCP for arthritis in this population. Also, we do not have data on the frequency of mixed cryoglobulins in these patients. Studies will be needed to clarify the CCP seropositivity rates in HCV patients with synovitis and the predictive value of CCP for erosions, and to examine the relationship of this antibody with mixed cryoglobulins. However, our data suggest that, unlike RF, CCP antibody is not nonspecifically produced in response to HCV infection, and may therefore prove to be a useful tool for the diagnosis of RA in this population.

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