Rheumatol Int (2011) 31:65–69
DOI 10.1007/s00296-009-1225-8

ORIGINAL ARTICLE

Anti-cyclic citrullinated peptide antibodies as a discriminating marker between rheumatoid arthritis and chronic hepatitis C-related polyarthropathy

Wafaa M. Ezzat · Hala M. Raslan · Azza A. Aly · Nahed A. Emara · Manal M. El Menyawi · Amr Edrees

Received: 19 August 2009 / Accepted: 7 October 2009 / Published online: 1 November 2009
© Springer-Verlag 2009

Abstract Articular involvement is a frequent extrahepatic manifestation of hepatitis C virus (HCV) infection. The distinction between HCV-related polyarthropathy and true RA may be very difficult, especially with recent onset RA before articular damage and erosions develop. The objective of the study is to assess the diagnostic utility of anti-CCP antibodies and compare it with that of rheumatoid factor (RF) in distinguishing between rheumatoid arthritis (RA) and HCV-related polyarthropathy. Anti-cyclic citrullinated peptide (CCP) antibodies and RF were determined in the sera of 30 patients with RA and 22 patients with HCV-related polyarthropathy. Anti-CCP antibodies were positive in 83.3% of patients with RA and in 4.5% in patients with HCV and polyarthropathy. RF was positive in 90% of RA patients and in 81.1% of HCV patients with polyarthropathy. The anti-CCP antibodies showed higher specificity for RA compared with RF (95.4 vs. 18.2%). However, the sensitivity of anti-CCP was comparable to that of RF (83.3 vs. 90%). In conclusions, anti-CCP antibodies are reliable laboratory markers to differentiate between RA and HCV-related polyarthropathy.

Keywords Rheumatoid arthritis · Anti-cyclic citrullinated peptide antibodies · Hepatitis C-related polyarthropathy

W. M. Ezzat · H. M. Raslan
Internal Medicine Department,
National Research Centre, Cairo, Egypt

A. A. Aly · N. A. Emara
Clinical and Chemical Pathology Department,
National Research Centre, Cairo, Egypt

M. M. El Menyawi
Internal Medicine Department,
Kasr El Eini Hospital, Cairo, Egypt

A. Edrees (✉)
Internal Medicine Department,
University of Missouri, Kansas City, USA
e-mail: edreesa@umkc.edu

Introduction

The presence of extrahepatic manifestations is a relatively common feature in patients with chronic hepatitis C virus (HCV) [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], ranging from polyarthralgia to monoarticular or oligoarticular intermittent arthritis and symmetric chronic polyarthritis mimicking rheumatoid arthritis (RA) [4]. RA-like HCV-related arthropathy could be clinically indistinguishable from recent onset RA, when articular damage and deformities have not yet occurred. Most patients with RA-like HCV-related polyarthritis fulfill the American College of Rheumatology (ACR) criteria for RA [5].

The detection of Ig M rheumatoid factor (RF) is of little utility as a diagnostic tool as it has low specificity for RA because it may be found in healthy elderly individuals, healthy immunized subjects, patients with other autoimmune diseases, or chronic infections [6]. A higher percentage of patients with chronic HCV infection display serum RF reactivity and the frequency of RF increase in patients with articular involvement [5, 7]. Thus, differentiating patients with HCV-related symmetric polyarthritis from patients with RA represents both a diagnostic and therapeutic challenge.

As a possible way of distinguishing between these two conditions, anti-keratin antibodies (AKA) have been

investigated. These share a high specificity for RA [8] and were found scarce among HCV-infected patients with arthropathy [9]. However, there are technical difficulties in standardizing AKA, and poor sensitivity for their detection in RA. Several studies have shown that anti-cyclic citrullinated peptide (anti-CCP) antibodies in RA have high sensitivity (41–70%) and specificity (91–98%) depending on the population under study and the generation of an enzyme-linked immunosorbent assay (ELISA) kit [10, 11].

Our aim in the present study is to investigate the diagnostic reliability of anti-CCP antibodies in distinguishing HCV-associated polyarthropathy from RA in Egyptian patients.

Materials and methods

The study included 30 patients with definite RA and 22 patients with chronic HCV and polyarthropathy. All patients were recruited from the rheumatology and hepatology clinic of the Medical Services Unit at National Research Centre.

All RA patients were fulfilling the ACR criteria for RA [12]; they were 3 males and 27 females their ages ranged between 31 and 63 years (mean 45.8 ± 9.6).

Patients with HCV infection were diagnosed on the basis of HCV antibodies and confirmed by the detection of viral RNA in serum. Patients were all complaining of polyarthropathy in the form of arthritis and/or arthralgia, they were 4 males and 18 females, their ages ranged between 39 and 65 years (mean 51.8 ± 6.9).

All patients were subjected to history recording, clinical examination including musculoskeletal examination, abdominal ultrasound and laboratory investigations in the form of routine laboratory tests and RF and anti-CCP antibodies. HCV antibodies and HCV-RNA by real-time PCR were done for diagnosis of patients with chronic HCV infection.

Laboratory methods

Ten milliliters venous blood was withdrawn from all patients, serum was separated immediately, and part of serum was used immediately for routine laboratory investigations and RF assay. The rest of serum samples were aliquoted, stored at −20°C, and thawed only once before doing anti-CCP antibodies, HCV antibodies and HCV-RNA.

1. Routine laboratory investigations: including liver function tests, kidney function tests were done using Hitachi, 912.
2. RF was assayed using HUMATEX RF test from HUMAN (made in Germany) cat. no. 40 050: this test is based on the agglutination reaction between RFs of a patient specimen or control serum and human immunoglobulin G (IgG) coated onto polystyrene latex particles.
3. Anti-CCP IgG (cyclic citrullinated peptide) antibodies were assayed by ELISA technique using INOVA QUANTA Lite™ CCP IgG kit (made in Germany) catalog no. 708790.

The sample can then be classified as negative, weak positive, moderate positive or strong positive according to the followings:

negative <20 units;
weak positive 20–39 units;
moderate positive 40–59 units;
strong positive ≥60 units.

4. Anti-HCV antibodies was detected by ELISA using Equipar Kits (made in Italy) catalog no. 2000CB-2000CBA.
5. HCV-RNA testing was done for those patients with positive anti-HCV antibodies to confirm the diagnosis of HCV infection. This test was done using real-time PCR.

Primers and probes

The primers used were NCR-s (5′-TGC GGA ACC GGT GAG TAC A; positions −193 to −175) and NCR-as (5′-CTT AAG GTT TAG GAT TCG TGC TCA T; positions 24–1) (nucleotide positions are relative to the sequence of Genbank accession no. M58335, first codon). The hybridization probes were HCV-NCR-LR (5′-LC Red640-TGC CTG ATA GGG TGC TTG CGA GT-p; positions −52 to −30) and HCV-NCR-FL (5′-GGT CGC GAA AGG CCT TGT GGT A-FL; −75 to −54). Primer and probe sequences were selected to optimal conservation between HCV isolates.

RNA extraction and preparation

Viral RNA was extracted from 140 µl of serum using the Qia Amp viral RNA kit (catalog no. 52904; Qiagen, Hilden, Germany); one tube RT and amplification. The RT-PCR was performed using LightCycler RNA amplification kit for tests with hybridization probes (catalog no. 2015145; Roche Diagnostics) with 6.5 Mm $MgCl_2$. The hybridization probes were added in unequal molarity with 8 pmol of the detection probe (HCV-NCR-LR) and 4 pmol of the anchor probe (HCV-NCR-FL). Adding 10 pmol each of primer (NCR-s and NCR-as) also results in an asymmetric concentration, since the same amount of primer NCR-as was already added for preincubation of the RNA extract. This results in an increased efficiency of amplification.

The RT-PCR conditions were 12 min at 55°C for RT, 30 s at 95°C for denaturation of the reverse transcriptase, followed by 45 cycles of 3 s at 95°C (slope 20°C/s) and 7 s at 64°C (first annealing temperature) to 54°C (touchdown second annealing temperature), with a decreasing step size of 1°C per cycle, and extension for 14 s at 72°C (slope 2°C/s). After the last cycle, a melting curve analysis was performed from 50 to 90°C. The fit point mode for Light Cycler was used for evaluation.

Statistical methods

Data were presented by mean ± SD and percentages. The compiled data were computerized and analyzed by SPSS PC+, version 12. "*t*" test between means was used to analyze mean difference, "*t*" test between percentages to analyze percent difference. χ^2 was used to study the pattern of distribution of different variables. Multiple correlation coefficients $\{r\}$ were used to determine the correlation of the studied parameters to each other. A level of significance with $P \leq 0.05$ was considered significant.

Results and analysis of the results

Patient characteristics

Thirty patients with definite RA and 22 patients with chronic HCV infection and polyarthropathy were included in this study. Their demographic, clinical and laboratory characteristics were presented in Tables 1 and 2.

Three of the RA patients were not receiving any medication; the other patients were receiving disease-modifying antirheumatic drugs in the form of methotrexate and/or hydroxychloroquine.

Table 1 Demographic, clinical and laboratory characteristics of RA patients

Variable	RA patients ($n = 30$)
Age, years (mean ± SD)	45.8 ± 9.6
Sex (males, females)	5, 25
Duration of disease, years (mean ± SD)	10.3 ± 7.2
Deformity, *n* (%)	16 (53.3)
Patients on DMARDs, *n* (%)	26 (86.7)
Patients on steroids, *n* (%)	10 (33.3)
ESR, mm/h (mean ± SD)	41.7 ± 21.4

RA rheumatoid arthritis, *DMARDs* disease-modifying antirheumatic drugs, *ESR* erythrocyte sedimentation rate

Table 2 Demographic, clinical and laboratory characteristics of HCV patients with polyarthropathy

Variable	HCV patients ($n = 22$)
Age, years (mean ± SD)	51.8 ± 6.9
Sex (males, females)	3, 19
Liver cirrhosis *n* (%)	3 (13.6)
Patients with swollen joints, *n* (%)	5 (22.7)
ALT, U/L (mean ± SD)	40.3 ± 15.1
AST, U/L (mean ± SD)	47.1 ± 25.1
ESR, mm/h (mean ± SD)	29.9 ± 11.4

HCV hepatitis C virus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ESR* erythrocyte sedimentation rate

All HCV patients had polyarthropathy in the form of polyarthralgia and five patients had polyarthritis and none of the patients had joint erosion nor deformity.

Results of anti-CCP antibodies and RF

In RA patients, the anti-CCP antibodies were negative in five patients (16.7%), weak positive in one patient (3.3%), moderate positive in two patients (6.6%) and strong positive in 22 patients (73.3%). In HCV patients with polyarthropathy, the anti-CCP antibody was weak positive in a single patient (4.5%) and it was marginally elevated, as it was 20.7 U/ml. The prevalence of anti-CCP antibodies and RF in RA patients and HCV patients with polyarthropathy are shown in Table 3.

The anti-CCP antibodies showed higher specificity for RA compared with RF (95.4 vs. 18.2%). However, the sensitivity of anti-CCP was comparable to that of RF (83.3 vs. 90%).

There was no significant correlation between anti-CCP antibodies and any of the demographic, clinical or laboratory data of the patients except with erythrocyte sedimentation rate a significant positive correlation was found in RA patients ($n = 0.397$, $P = 0.009$).

Table 3 Prevalence of anti-CCP antibodies and RF in RA patients and HCV patients with polyarthropathy

Variable	RA patients $n = 30$	HCV patients $n = 22$
Anti-CCP antibodies, *n* (%)		
Positive	25 (83.3)	1 (4.5)
Negative	5 (16.7)	21 (95.5)
RF		
Positive	27 (90)	18 (81.8)
Negative	3 (10)	4 (18.2)

CCP cyclic citrullinated peptide, *RF* rheumatoid factor

Discussion

Rheumatological manifestations are common during HCV infection and in some cases may mimic the onset of RA. In a prospective study on a large, cohort of HCV patients, articular involvement represented the most common extrahepatic manifestations, affecting nearly 20% of patients in a 1-year follow-up [13]. The differential diagnosis between HCV-related polyarthropathy and true RA may be very difficult especially with recent the onset RA before articular damage and erosions develop [5]. The detection of RF is not a reliable tool for differential diagnosis. Although antibodies can be detected in up to 80% of patients with RA, they are not very specific for RA as they may be detected in many non-rheumatic conditions such as HCV infection [14].

In the present study, RF was present in 90% of RA patients and in 81.8% of HCV patients with polyarthropathy. In two previous studies, the prevalence of RF in HCV-related polyarthropathy was 54 and 66.7% [15, 16]. The higher prevalence in our study indicates that RF cannot be used to differentiate RA from HCV-related polyarthropathy in Egyptian patients.

The currently available test anti-CCP2 for anti-cyclic citrullinated peptide antibodies has been shown to play a high specificity for RA accompanied by a reasonable high sensitivity [17, 18]. In the present study, anti-CCP antibodies were present in 83.3% of patients with definite RA, while in HCV-related polyarthropathy, a single patient (4.5%) showed marginally elevated anti-CCP antibodies. This makes the anti-CCP antibodies a reliable marker for differentiating RA from HCV-related polyarthropathy. Sène et al. [15] demonstrated the presence of anti-CCP antibodies in 5.7% of patients with HCV-related polyarthropathy and in 78% of patients with RA. In the study of Wener et al. [19] anti-CCP antibodies were present in 7% of patients with HCV-related polyarthropathy. In another study done on eight patients with HCV-associated polyarthropathy, none showed positive anti-CCP antibodies [16]. A single positive anti-CCP antibody in our patients may be a predictor for the development of RA in the future as anti-CCP antibodies are usually detected very early in RA patients and can be detected years before the disease becomes manifest. Rantapää-Dahlqvist et al. [20] analyzed blood samples from 83 blood donors who subsequently developed RA. They found that anti-CCP2 antibodies could be detected in some patients 10 years before the appearance of the first clinical symptoms. In a similar type of study, Nielen et al. [21] reported that anti-CCP positivity could be observed up to 14 years before the first clinical symptoms; also observations described by van Gaalen et al. show that anti-CCP positivity can be used to identify a subset of patients with undifferentiated arthritis that in 2–3 years will progress into RA [22].

The pathogenesis of anti-CCP antibodies in RA has been shown to be attributable to the body's humoral response to citrulline. Citrullination is the post-translational conversion of arginine to citrulline by an enzyme called peptidylarginine deaminase (PAD). PAD activation is assisted by calcium ions. PAD is normally present as an inactive intracellular enzymes. During programmed cell death (apoptosis) in the synovial joints of patients with RA, PAD may leak out of the dying cells. Once activated, PAD will cause citrullination of extracellular arginine. In the synovium, the citrulline acts as an antigenic stimulant to induce anti-citrullinated protein antibodies, locally produced by plasma cells [23]. In the present study, the anti-CCP antibodies showed 83.3% sensitivity and 95.5% specificity for RA, while RF showed 90% sensitivity and 18.2% specificity. The high specificity of anti-CCP antibodies is an evidence of its utility as a serological biomarker for differentiating between RA and HCV-related polyarthropathy, while the very low specificity of RF indicates that it is useless marker in this differentiation.

The distinction between HCV-associated arthropathy and RA is of great relevance in clinical practice, as HCV-associated arthropathy has a relatively benign course that is not associated with bony erosions and joint destruction. In contrast, RA usually lead to joint erosions and disability [24]. Thus, an early differential diagnosis is important to be able to target the use of potentially toxic and expensive drugs to RA patients where the benefits clearly outweigh the risks, and protect HCV patients from hepatotoxic effects of these drugs [25].

Conclusions

The present study showed that anti-CCP antibodies compared with RF had higher specificity for RA. In HCV-related polyarthropathy, RF is usually positive, however, anti-CCP antibodies are very rarely found and with low titer. These results demonstrate that anti-CCP antibodies are reliable laboratory markers to differentiate between RA and HCV-related polyarthropathy.

References

1. Cacoub P, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, Opolon P (1999) Extra-hepatic manifestations of chronic hepatitis C. MULTIVIRC Group (Multidepartment Virus C). Arthritis Rheum 42:2204–2212
2. Mariette X (1998) The hepatitis C virus and systemic diseases. Rev Rheum 65:737–740
3. Rivera J, Garcia-Monforte A, Pineda A, Millan Nunez-Cortes J (1999) Arthritis in patients with chronic hepatitis C virus infection. J Rheumatol 26:420–424

4. Buskila D, Shnaider A, Neumann L, Zilberman D, Hilzenrat N (1998) Musculoskeletal manifestations and autoantibodies profile in 90 hepatitis C virus infected Israeli patients. Semin Arthritis Rheum 28:107–113
5. Zuckerman E, Keren D, Rozenbaum M (2000) Hepatitis C virus related arthritis: characteristics and response to therapy with interferon alpha. Clin Exp Rheumatol 18:579–584
6. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R (2001) Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 47:1089–1093
7. Olivieri I, Palazzi C, Padula A (2003) Hepatitis C virus and arthritis. Rheum Dis Clin North Am 29:111–122
8. Aho K, Palusuo T, Kurki P (1994) Marker antibodies of rheumatoid arthritis: diagnostic and pathogenetic implications. Semin Arthritis Rheum 23:379–387
9. Kessel A, Rosner I, Zuckerman E, Golan TD, Toubi E (2000) Use of antikeratin antibodies to distinguish between rheumatoid arthritis and polyarthritis associated with hepatitis C infection. J Rheumatol 27:610–612
10. Bas S, Perneger TV, Seitz M, Tiercy JM, Roux-Lombard P, Guerne P (2002) Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies and IgM rheumatoid factors. Rheumatology (Oxford) 41:809–814
11. Vittecoq O, Incaurgarat B, Jouen-Beades F, Legoedec J, Ltourneur O, Rolland D (2004) Auto antibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis. Clin Exp Immunol 135:173–180
12. Arnett FC, Edworthy SM, Bloch DA, McShane D, Fries JJ, Cooper N (1998) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
13. Cacoub P, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC (2000) Extra hepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC (Groupe d'Étude et de Recherche en Médecine Interne et Maladies Infectieuses sur le Virus de l'Hépatite C). Medicine (Baltimore) 79:47–56
14. Palosuo T, Tilvis R, Strandberg T, Aho K (2003) Filaggrin related antibodies among the aged. Ann Rheum Dis 62:261–263
15. Sène D, Ghillani-Dalbin P, Limal N, Thibault V, van Boekel T, Piette J, Cacoub P (2006) Anti-cyclic citrullinated peptide antibodies in hepatitis C virus associated rheumatological manifestations and Sjögren's syndrome. Ann Rheum Dis 65:394–397
16. Bombardieri M, Alessandri C, Labbadia G, Lannuccelli C, Carlucci F, Riccieri V, Paoletti V, Valesini G (2004) Role of anti-cyclic citrullinated peptide antibodies in discriminating patients with rheumatoid arthritis from patients with chronic hepatitis C infection-associated polyarticular involvement. Arthritis Res Ther 6:R137–R141
17. Pinheiro GC, Scheinberg MA, Aparecida da Silva M, Maciel S (2003) Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. Ann Intern Med 139:234–235
18. Van Venrooij WJ, Hazes JM, Visser H (2002) Anti-citrullinated protein/ peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. Neth J Med 2002(60):383–388
19. Wener MH, Hutchinson K, Morishima C, Gretch DR (2004) Absence of Antibodies to cyclic citrullinated peptide in sera of patients with hepatitis C virus infection and cryoglobulinemia. Arthritis Rheum 50:2305–2308
20. Rantapää-Dahlqvist S, de Jong B, Berglin E (2003) Antibodies against citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 48:2741–2749
21. Nielen MM, van Schaardenburg D, Reesing H (2004) Specific auto antibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50:380–386
22. Van Gaalen F, Linn-Rasker S, van Venrooij W (2004) Autoantibidies to cyclic citrullinated peptides (CCP) predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum 50:709–715
23. Vossenaar ER, van Venrooij WJ (2004) Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. Arthritis Res 6(3):107–111
24. Lovy MR, Starkebaum G, Uberoi S (1996) Hepatitis C infection presenting with rheumatic manifestations: a mimic of rheumatoid arthritis. J Rheumatol 23:979–983
25. Mok MY, Ng WL, Yuen MF, Wong RW, Lau CS (2000) Safety of disease modifying anti-rheumatic agents in rheumatoid arthritis patients with chronic viral hepatitis. Clin Exp Rheumatol 18:363–368