

Hepatitis C virus seroprevalence and genotypes in patients with diffuse connective tissue diseases and spondyloarthropathies

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Abstract

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Many extrahepatic manifestations, including rheumatic diseases, have been reported to be associated with hepatitis C virus (HCV) infection. In order to investigate the prevalence of HCV infection among patients with rheumatic diseases, in the present study we interviewed 367 patients and tested their blood samples for HCV antibodies (anti-HCV) by an enzyme-linked immunosorbent assay. Anti-HCV-reactive samples were retested for confirmation by a line immunoassay and also for HCV RNA detection by the polymerase chain reaction. HCV RNA-positive samples were genotyped by INNO-LIPA. An overall HCV infection prevalence of 1.9% (7/367) was found. Of the 7 HCV-infected patients, 4 had systemic lupus erythematosus and 3 rheumatoid arthritis, resulting in positivity rates of 2.3 and 3.4%, respectively. HCV RNA genotyping revealed the presence of subtypes 1a (57.1%), 1b (28.6%) and 3a (14.3%). The clinical course was favorable for all HCV-infected patients, except one, who died due to renal insufficiency related to lupus nephritis. These results demonstrate a low HCV infection prevalence among the population studied. In the few positive cases, we observed no adverse influence of this infection on the clinical evolution of the rheumatic disease.

Key words

- Hepatitis C
- Rheumatic disease
- Arthritis
- INNO-LIPA

Hepatitis C virus (HCV) infection has become a major public health problem, with 170 million people considered to be infected worldwide. The disease progresses slowly and a chronic infection develops in 85% of the cases. Among patients with chronic hepatitis, 20 to 30% develop cirrhosis that, once established, carries a poor prognosis, with a high risk of developing hepatocarcinoma (1). Besides the seriousness of the liver disease,

several extrahepatic manifestations have been related to HCV infection. These manifestations can be dominant, while the hepatic disease can be quiescent or mild. Some studies have reported hematological, endocrine, renal, dermatological, pulmonary, neurological, ophthalmological, rheumatological, and autoimmune involvement (2).

The most frequent rheumatological manifestations in HCV-positive patients are: mus-

culoskeletal pain, mixed cryoglobulinemia, rheumatoid syndrome, Sjögren's syndrome (SS), vasculitis, glomerulonephritis, Raynaud's phenomenon, myopathies, autoantibodies, and other manifestations of diffuse connective tissue diseases (DCTD) (3). In addition, some reports have shown a high prevalence of this infection in rheumatic patients: 10% in polymyositis/dermatomyositis (4), 14% in SS (5), 1 to 11% in systemic lupus erythematosus (SLE) (6,7), 12% in psoriatic arthritis (8), and 0.65 to 5.4% in rheumatoid arthritis (RA) (8,9).

It is still unclear if HCV infection could be linked to the etiopathogenesis of autoimmune disease or if this association could be just coincidental. However, the question has been raised about the need to perform HCV serologic tests during investigation of rheumatic diseases on a routine basis. Accordingly, the clinical manifestations observed in patients with hepatitis C must be differentiated from those of DCTD. Patients with DCTD have an increased susceptibility to infections due to immunosuppression caused by the disease itself and by the drugs used in their treatment, in addition to their high exposure to the hospital environment for numerous invasive diagnostic or therapeutic procedures, including blood transfusions (10). In Brazil, investigations concerning the association between DCTD and HCV infection are still scarce (11,12). The objective of the present study was to determine the prevalence of HCV in patients with DCTD and spondyloarthritis in Central Brazil.

A total of 367 patients were seen at the Rheumatology Unit of Hospital das Clínicas, Faculdade de Medicina, Universidade Federal de Goiás (HC/FM-UFG), Goiânia, GO, Brazil, from March to August 2000, whose diagnoses (established according to internationally accepted criteria) were DCTD (N = 325) and spondyloarthritis (N = 42). The study was approved by the HC/FM-UFG Ethics Committee of Human and Animal Medical Research, and all patients gave writ-

ten informed consent to participate.

A standard form was used to collect socio-demographic data, diagnosis, time of evolution, hospital admissions, and use of immunosuppressive drugs and pulse therapy. The results of laboratory tests for antinuclear antibodies (by indirect immunofluorescence with HEP-2), rheumatoid factor and extractable antinuclear antibodies (by hemagglutination) were obtained from hospital records. After the interview, blood was collected (10 ml) from each patient and sera were stored at -20°C until tested.

Age ranged from 16 to 77 years (average: 38.3 years) and most patients were female (85%). The rheumatological diagnoses were: SLE (N = 175, 47.7%), RA (N = 89, 24.2%) and other illnesses (N = 103, 28.1%) including spondyloarthritis (N = 42), mixed connective tissue disease (MCTD; N = 17), vasculitis (N = 10), systemic sclerosis (N = 8), juvenile RA (N = 8), dermatomyositis/polymyositis (N = 7), SS (N = 5), rheumatic fever (N = 4), and primary antiphospholipid syndrome (N = 2).

All sera were tested for anti-HCV antibodies by a 3rd generation enzyme-linked immunosorbent assay (ELISA) (Innotest HCV Ab III, Innogenetics, Ghent, Belgium). Reactive samples were retested for confirmation using line immunoassay (INNO-LIA HCV Ab III, Innogenetics). Anti-HCV-positive samples were submitted to RNA extraction, reverse transcription and polymerase chain reaction with primers complementary to the 5' non-coding region of the HCV genome (13). Positive samples were genotyped by a line probe assay (INNO-LIPA HCV, Innogenetics).

Data from the interviews and the serological and molecular tests were analyzed with the Epi-Info program version 6.04a (Centers for Disease Control and Prevention, Atlanta, GA, USA). Prevalence and 95% confidence interval were calculated.

As shown in Table 1, 7 of the 367 patients were anti-HCV positive, corresponding to an

overall prevalence of 1.9% for hepatitis C in the study group. Positivity rates of 2.3 and 3.4% were found in SLE and RA patients, respectively.

All anti-HCV-positive samples (N = 7) presented viral RNA and were genotyped. Six (85.7%) individuals were infected with genotype 1, 4 of them (57.1%) with subtype 1a and 2 (28.6%) with subtype 1b. Only 1 (14.3%) patient presented genotype 3, subtype 3a (Table 2). Three patients had a history of blood transfusion, one patient reported a sexually transmitted disease, and all of them had a history of hospitalization.

Of the 7 HCV RNA-positive patients, 4 had a diagnosis of SLE and 3 of RA. All RA patients had used methotrexate and showed a stable course of disease without extra-articular manifestations. They were also rheumatoid factor positive, and although none of them had positive antinuclear antibodies, one had extractable antinuclear antibodies (anti-Ro and anti-Sm).

There were various lupus manifestations, but no visceral ones. None of the patients had used immunosuppressive drugs during the course of the disease, but all were taking chloroquine diphosphate and low doses of prednisone (<20 mg/day). They were positive for antinuclear antibodies, whose fluorescence patterns were homogeneous in two and thick-speckled in the remaining ones. Only one patient was positive for rheumatoid factor. Extractable antinuclear antibodies were found in only one lupus patient. After 6 months of attendance, one of the SLE patients died of nephritis-induced renal insufficiency.

An anti-HCV prevalence of 2.3% was observed in the SLE patients, which was higher than that reported in Israel (1%) (6), but lower than those obtained in Italy (6%) (14) and Spain (11%) (7). Regarding the patients with RA, our prevalence (3.4%) was higher than that found in France (0.65%) (9). These divergences may be due to the use of different techniques for HCV diagnosis, dis-

tinct populations, and even to the variability in prevalence of HCV infection observed in different geographical areas.

Although high anti-HCV rates have been observed in patients with polymyositis/dermatomyositis (10%) (4) and SS (14%) (5), in the present study, none of these patients was anti-HCV positive. Individuals with primary antiphospholipid syndrome, rheumatic fever, systemic sclerosis, juvenile rheumatoid arthritis, vasculitis, MCTD, and spondyloarthropathy were also all anti-HCV negative. However, no conclusion can be drawn from this finding due to the small number of patients studied with these diseases.

In studies conducted in Southern Brazil, an anti-HCV prevalence of 12.8% was found among 70 DCTD patients (RA, SLE, SS, systemic sclerosis, MCTD, polymyositis,

Table 1. Prevalence of hepatitis C virus (HCV) infection in patients with diffuse connective tissue disease and other arthropathies.

Base disease	HCV positive/total	95% CI
Systemic lupus erythematosus	4/175 (2.3%)	0.7-5.4
Rheumatoid arthritis	3/89 (3.4%)	0.8-8.9
Other types of arthritis	0/103 (0%)	-
Total	7/367 (1.9%)	0.8-4.0

CI = confidence interval.

Table 2. Hepatitis C virus (HCV) genotypes in seven anti-HCV-positive patients with diffuse connective tissue disease.

Patient	Disease	RNA-HCV	HCV genotype
67	RA	+	1a
114	SLE	+	1a
187	SLE	+	3a
188	SLE	+	1b
252	RA	+	1b
254	RA	+	1a
260	SLE	+	1a

RA = rheumatoid arthritis; SLE = systemic lupus erythematosus.

and Behçet's disease) (12) and a prevalence of 11% was detected in Raynaud's phenomenon patients (primary Raynaud phenomenon, systemic sclerosis, RA, systemic vasculitis, SS, essential cryoglobulinemia, MCTD, neoplasia, rheumatoid syndrome, and ankylosing spondylitis) (11). Both prevalence rates were higher than that observed in our study. However, the cited studies involved a smaller number of patients and the diagnosis of HCV infection was made only by ELISA. Moreover, they included diseases that are known to induce B lymphocyte polyclonal stimulation, with high autoantibody production, raising the possibility of cross-reactions, which can cause false-positive anti-HCV results.

If we consider only the female patients of our study (N = 312), the anti-HCV prevalence would be 2.2%, which is twice as high as that found in pregnant women in Goiânia (0.9%) (15). A possible explanation for this difference could be the fact that DCTD patients are more exposed to hospital procedures, as well as their higher susceptibility to infections caused by their own disease and by the immunosuppressive treatment. On the other hand, the overall prevalence found (1.9%) was similar to those observed in blood donors (1.4%) (16) and health care workers (2.0%) (17) from the same region.

In a previous study conducted on hemophilic patients from Goiânia, subtype 1a (41%) was also dominant, followed by subtypes 1b (25.6%) and 3a (20.5%) (18). Among blood donors, subtype 1a (54.6%) was also the most frequent, but subtype 3a (31.8%) was detected more frequently than subtype 1b (9.1%) (19). Data concerning HCV genotypes in patients with rheumatic diseases are still rare. In a study on 13 SLE anti-HCV-positive patients, 8 presented viral RNA, 1 (12.5%) being infected with genotype 1a, 4 with 1b (50%), and 3 other patients with genotype 3a (37.5%) (20). This genotype distribution, with type 1 predominance, was similar to that found in the present study.

It is possible that the prevalence of genotype 1 in this population can have clinical-therapeutic implications, because this genotype has been associated with a poor response to antiviral therapy.

There are few follow-up studies of rheumatic diseases in anti-HCV-positive patients (20). In a study of 13 SLE patients infected with HCV, Perlemuter et al. (20) observed a serious clinical picture with systemic involvement and concluded that corticoid therapy did not alter the course of chronic hepatitis. In this study, the 3 RA patients had used methotrexate and showed a stable clinical course (20). SLE patients showed only cutaneous and articular manifestations and had not used immunosuppressive drugs. It was not possible to determine the duration and the importance of HCV infection in these patients. However, the course of rheumatic disease apparently was not influenced by this infection, since most of the patients (85.7%) had a favorable clinical course.

Several therapeutic considerations should be kept in mind when dealing with the association of HCV infection and autoimmune diseases. The common use of corticoid and immunosuppressive drugs in autoimmune rheumatic diseases can increase HCV replication. In addition, the interferon used for the treatment of hepatitis C can precipitate and exacerbate a variety of autoimmune diseases and causes several side effects, such as arthralgia, leukopenia and thrombocytopenia (2), which are common during the active phases of some rheumatic diseases such as SLE, further impairing the evaluation of patients.

Although the overall prevalence of HCV infection found in the present study was similar to that observed previously in blood donors from the same geographical area (16), a higher positivity was found among SLE and RA patients. These data emphasize the importance of considering a differential diagnosis between DCTD and HCV infection and suggest the inclusion of an anti-HCV test

as part of the investigation process of rheumatic diseases. However, further studies will be necessary for a better understanding of the influence of HCV infection on the clinical course of rheumatic diseases and vice-versa.

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