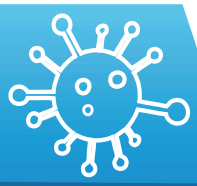
Case Report



INFECTIOUS DISEASESS

Long-term antiviral hepatitis C treatment associated with Rods and Ring Cytoplasmic antibodies

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Abstract

Aim. Clinical description of a patient diagnosed with chronic hepatitis C virus infection, which associated a rare anti-cytoplasmic pattern, known as "Rods and Ring".

Method. Clinical case report.

Results. A 76-year old female patient with chronic hepatitis C virus infection under treatment for several months with pegylated Interferon-Ribavirin (started eight months ago) presented for clinical and biological evaluation of the therapeutic response.

Conclusion. This is the first reported clinical case of a patient with cytoplasmic filamentous rods and rings autoantibodies associated with chronic hepatis C from the Clinical Hospital IRGH Prof. Dr. O. Fodor Cluj-Napoca, Romania. The presence of these antibodies appears to be triggered by antiviral therapy. Although these are newly identified antibodies, they could be used as serological markers for detecting patients at risk of developing associated autoimmune pathologies or nonresponders to the antiviral therapy. Likewise, their detection could identify patients with occult hepatitis C infection.

Keywords: hepatitis C virus infection; autoantibodies, rods and ring pattern

Introduction

Very often, chronic hepatitis C virus infection (HCV) associates immune dysregulation, manifested as extra-hepatic autoimmune phenomena [1,2]. In this scenario, long-term treatment with double-therapy peg-IFN-RBV may exacerbate these pre-existing conditions. Any suspicion of such a pathology should be raised on clinical aspects and subsequently confirmed by serological investigations. Appropriate immunological tests for autoimmune manifestations are mainly related to indirect immunofluorescence analysis (IIF) for detection and measuring the patients' circulating autoantibodies. Among these, a major role have antinuclear antibodies with various patterns, depending on the location of the intracellular antigen.

Cytoplasmic RR autoantibodies identified by IIF on HEp-2 cell substrate occur with a high specificity in 0.8-5% of patients undergoing long-term antiviral double peg-IFN/RBV therapy [3,4]. There are also studies that identify their presence in up to 40% of patients that undergo treatment with IFN/RBV [5,6]. Findings from recent years have not succedeed in identifying the presence of these antibodies in patients with HCV prior to IFN-RBV antiviral therapy or in patients with other medical conditions. Initially, they appear in serum around the sixth month of antiviral therapy and then persist in a plateau-like trend in the next twelfth month. Upon completion of treatment, the titers of anti-RR antibodies decrease in half of the patients, but still remain elevated in the other half.

There are two studies that have revealed a higher frequency of these anti-RR antibodies in patients with a relapse phase of the disease and non-responder to IFN/RBV therapy, especially in those who experience the reoccurrence of the circulating virus (perhaps through certain spontaneous mutations) after initially successful therapy [6,7].

From a pathogenetic perspective, the antigen recognized by these anti-RR antibodies is represented by inosine-5'-monophosphate dehydrogenase 2 (IMPDH2), which is the key enzyme in the biosynthesis of purine bases (guanosine triphosphate) involved in DNA synthesis

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[8]. Inhibition of guanosine triphosphate (GTP) and cytidine triphosphate (CTP) biosynthetic pathways induce the cell to assemble in a rod/ring structures, which consist of IMPDH2 [9]. Ribavirin is a direct inhibitor of IMPDH2, thus being able to induce the formation of RR structures *in vitro* and *in vivo*. Subsequently, in the presence of IFN/RBV therapy, these structures induce the generation of anti-RR antibodies [10-13].

Case presentation

We have studied the case of a 76-year-old female patient, retired , diagnosed in the past with HCV infection and other age-related cardio-vascular diseases (hypertension), who was admitted to the IRGH Clinical Hospital Prof. Dr. O Fodor for assessment of the therapeutic response to antiviral double therapy. Over the past eight months the patient had been treated with IFN-pegylat-ribavirin combination without any improvement in clinical and bio-humoral parameters. Prior to antiviral therapy, she had no autoimmune pathologies, all immunological tests being negative.

Clinical examination upon admisson revealed jaundice and signs of age-related heart disease, as well as osteo-articular arthrosis type disease, and recently identified signs of Hashimoto's and mixed connective tissue diseases.

Laboratory investigations revealed the full blood count within normal parameters (leucocytes = 4 $x10^3$/µl, erythrocytes=4.37$x10^6$/µl) with slightly decreased platelets number to 120$x10^3$/µl. Biohumoral: total bilirubin= 2.1 (0.1-1.2), direct bilirubin=1.05 (0-0.52), Gamma GT = 53 (7-32), ALT= 51 (5-45 U/l), AST= 64 (5-45 U/l). Abdominal ultrasound examination was suggestive for liver cirrhosis Child-Pugh class A.

Immunological test, such as Enzyme-Linked Immunosorbent assay (ELISA) showed negative results for antimitochondrial antibodies (AMA), anti-smooth muscle antibodies (SMA), anti-liver-kidney microsmal (LKM) and anti-neutrophil cytoplasmic antibody (PANCA); however, antinuclear antibodies (ANA) were positive.

Additionally, indirect immunofluorescence analysis was performed using HEp-2 cells as a substrate (INOVA Diagnostics, San Diego, CA, USA) and reading with a Zeiss Axio Imager (Carl Zeiss Inc., Germany). The assay revealed the presence of circulating ANA with a titer of 1:320 and cytoplasmic "rods and rings" pattern (Figure 1).

Other immunological tests revealed: Alpha-fetoprotein = 13.9 ng/ml (0-9.5), Crioglobulin ++ , and Hyper IgM = 276 mg/ml (40-230), Hbs antigen = negative, HBc antibody= positive and undetectable anti-delta virus antibodies.

Discussion

We report here the case of a 76-year-old female patient, known with chronic hepatitis C virus infection and age-related pathology, who associates in evolution a very rare pattern of anti-RR cytoplasmic antibodies after antiviral therapy.These anti-RR antibodies were correlated with the occurrence of autoimmune manifestations and also with unfavorable evolution of the disease even under the antiviral therapy. The data presented in this report show that these RR antibodies are strongly associated with the HCV chronic infection, and their presence is induced by combined IFN/RBV therapy, the incidence increasing in proportion to the duration of treatment.

Although the patient did not present ANA prior to initiating the antiviral therapy, circulating autoantibodies reactive against cytoplasmic antigens were detected within eight months of sustained therapy. At the same time, when presented for evaluation, the patient associated manifestations of mixed connective tissue disease. To our knowledge, this is the only published case of HCV therapy-associated RR antibodies from Romania.

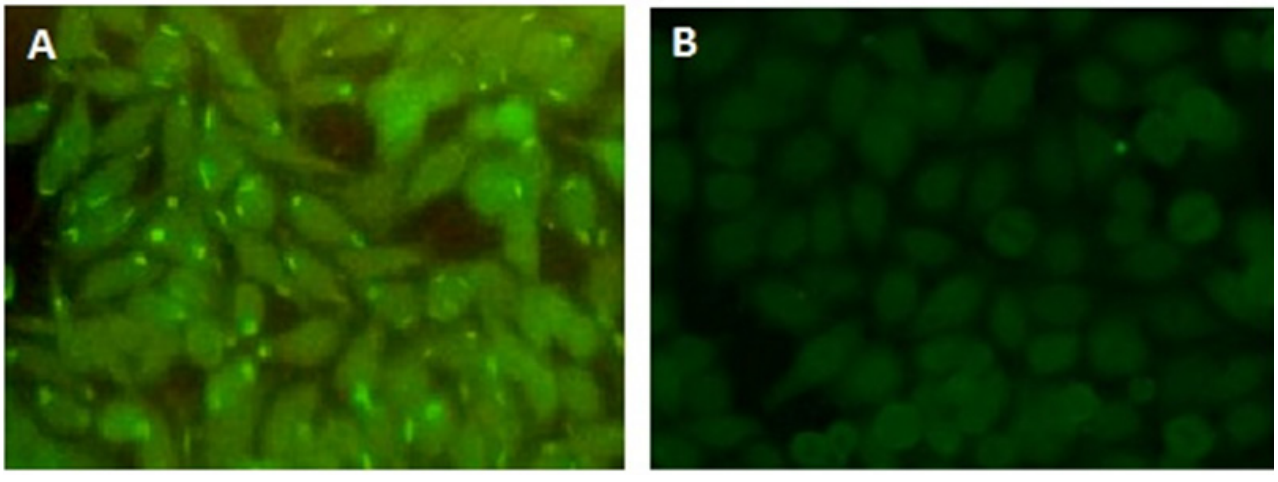

Figure 1. A. Cytoplasmatic rods and rings of HEp-2 cells in the serum from the patient with hepatitis C virus infection (x100); and B. serum from a healthy person/negative control (x100).

Rods and rings autoantibodies have become of increasing interest especially in patients with known history of HCV infection and who have undergone combined IFN/RBV therapy. The generation of these anti-RR antibodies appears to be dependent on the inhibition of IMPDH2 enzyme, which plays a key role in the synthesis of purine bases and DNA. The blocking of the enzyme function appears to be closely correlated with long-term administration of Ribavirin in the form of IFN-RBV antiviral combination therapy for HCV infection. Thus, in the presence of viral modifications and IFN therapy, the cytoplasmic rods and rings seem to be recognized as antigenic structures, thereby inducing the generation of autoantibodies [10-13].

In terms of their clinical significance, although previous reports showed no relationship between this pattern and demographic parameters, duration of diagnosis of HCV, treatment response pattern, HCV genotype or viral load, all previous studies have been found a strong association between anti RR antibodies response and HCV patients treated with with IFN/RBV [14-18]. Additionally, other studies have indicated a link between anti-RR and non-responsiveness or relapse in American and Italian HCV patient cohorts [6,7].

Our observations correlated with previous studies, lead us to the hypothesis that the occurrence of anti-RR antibodies represents a model of drug-induced immunological tolerance loss, having as main trigger the specific antiviral treatment with pegIFN and Ribavirin [19]. Thus, the early identification of these patients by IIF screening tests for the detection of anti-RR antibodies could be an indicator in early recognition of the patients with a relapse phase of HCV. Likewise, this unique exemple of autoantibody generation in human could be a very useful screening tool for identifying patients at risk of developing autoimmune conditions followed viral therapy, as well as to identify individuals with unnoticed HCV infection.

In addition, a better identification and characterization of this novel set of antibodies-associated HCV infection and therapy can help a better understanding of immuno-pathomechanism, thus potentially improving the arsenal of diagnostic test in the future.

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Ethics approval and consent to participate

A written informed consent was signed by the patient for the publication of this case report and any accompanying images.

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