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Case report

Induction of cytoplasmic pattern in the form of “rods and rings” through the treatment of hepatitis C: a case report[☆]



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ARTICLE INFO

Article history:

Received 1 April 2013

Accepted 28 January 2014

Available online 27 November 2014

Keywords:

Hepatitis C

Self-antibodies

Rods and rings

Cytoplasmic pattern

ABSTRACT

Female patient, complaining of weakness and pain in hypogastric, was admitted to the emergency department of the University Hospital of the West of Paraná (HUOP). During the interview reported treatment of chronic infection with hepatitis C virus (HCV) with peginterferon and ribavirin. Among the laboratory tests ordered, the search for self-antibodies against cellular antigens, traditionally known as antinuclear factor, showed fluorescence shaped like rods and/or rings in the cytoplasm of cells. This study attempts to clarify the relationship between this pattern not yet completely understood and the clinical picture of the patient. This pattern is characterized by 3–10 μm rods or rings with 2–5 μm in diameter scattered throughout the cytoplasm of the cell. Therefore, this new standard has been designated as “rods and rings” (RR). The antigenic target of this reaction was identified as inosine-5'-monophosphate dehydrogenase type 2 (IMPDH2) which is a key enzyme in the synthesis of purine nucleotides. The IMPDH2 enzyme aggregated or modified shaped RR in those patients treated with ribavirin may become antigenic and induce an autoimmune response. It is possible that interferon alpha stimulates the occurrence of anti-RR reactivity apparently induced by ribavirin. So far it is not known why the standard RR in HEp2 cells occurs only in a fraction of patients with HCV. Previous studies presented in this paper allow affirming that these antibodies associated with the standard RR are strongly related to hepatitis C. Moreover, it can be stated that the occurrence of anti-RR reactivity is promoted by combination therapy with interferon and ribavirin.

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[☆] Study conducted at Diagnostic and Therapeutic Support Service (SADT), Hospital Universitário do Oeste do Paraná (HUOP-Unioeste).

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<http://dx.doi.org/10.1016/j.rbre.2014.01.004>

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Indução do padrão citoplasmático em forma de “bastões e anéis” através do tratamento da hepatite C: relato de caso

R E S U M O

Palavras-chave:

Hepatite C
Autoanticorpos
Bastões e anéis
Padrão citoplasmático

Paciente do sexo feminino, queixando-se de astenia e dor em hipogastro, foi admitida no pronto-socorro do Hospital Universitário do Oeste do Paraná (HUOP). Durante a anamnese relatou tratamento de infecção crônica pelo vírus da hepatite C (VHC) com interferon peguado e ribavirina. Dentre os exames laboratoriais solicitados, a pesquisa de autoanticorpos contra antígenos celulares (PAAC-HEp-2), conhecido tradicionalmente como fator antinúcleo (FAN), apresentou fluorescência em forma de bastões e/ou anéis no citoplasma das células. Esse padrão é caracterizado por bastões de 3-10 μm e anéis com 2-5 μm de diâmetro espalhados através do citoplasma da célula. Portanto, esse novo padrão tem sido designado como “bastões e anéis” (traduzido do inglês: *Rods and Rings*, RR). O alvo antigênico dessa reação foi identificado como inosina-5'-monofosfato desidrogenase tipo 2 (IMPDH2) que é uma enzima chave na síntese de nucleotídeos púricos. A enzima IMPDH2 agregada ou modificada em forma de RR nos pacientes tratados com ribavirina pode tornar-se antigênica e induzir uma resposta autoimune. É possível que o interferon alfa estimule a ocorrência de reatividade anti-RR aparentemente induzida pela ribavirina. Até o momento não se sabe por que o padrão RR em células HEp-2 ocorrem apenas em uma fração de pacientes portadores do VHC. Os dados apresentados em trabalhos anteriores possibilitam afirmar que esses anticorpos associados ao padrão RR estão fortemente relacionados com o tratamento da hepatite C. Além disso, pode-se afirmar que a ocorrência de reatividade anti-RR é promovida pela terapia combinada com interferon alfa e ribavirina.

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Introduction

Hepatitis C virus (HCV) is an RNA virus of the Flaviviridae family, genus *Hepacivirus*, with a high rate of hepatic replication. This is an enveloped virus, with a size between 30 and 40 nm.¹⁻³ HCV was originally isolated in a serum sample of an individual with non-A, non-B hepatitis in 1989 by Choo et al.⁴ Since then, hepatitis C gained special relevance among the causes of chronic liver disease worldwide. In 1992, the first test for identification of the antibody against HCV was developed, providing greater safety in blood transfusions.^{1,2}

The HCV transmission occurs through contact with infected blood due to percutaneous exposure, blood and/or blood products transfusion and transplants from infected donors. Although some patients with acute HCV infection have an immune system able of eliminating the virus, 55-58% of patients develop chronic infection, defined as the persistence of infection for no less than six months, with only 10-15% of cases reaching spontaneous healing. The hepatocellular injury, seen in chronic HCV infection, does not seem to be directly related to a viral cytopathic effect, being related to immune mediators, with natural killer cells and CD8+T lymphocytes playing a central role in the pathogenesis.^{1,2}

The treatment of HCV infection aims to control the progression of liver disease by inhibiting viral replication. Furthermore, the reduction in inflammatory activity prevents its progression to cirrhosis and hepatic carcinoma. The recommended therapy for chronic HCV infection is a combination of a formulation of interferon alpha and ribavirin.³

Interferon is a cytokine which composes the innate response of the human host. The addition of one polyethylene glycol molecule to the interferon molecule prolongs the action, increases the rate of absorption, extends the half-life and reduces the clearance of interferon. Ribavirin is a nucleoside analog antiviral agent used orally, with a wide spectrum of action against viral pathogens. Ribavirin also has the effect of modulating the immune response.^{2,3} Mori et al. recently demonstrated that ribavirin in therapeutic doses inhibits the replication of HCV RNA, and proposed that this anti-HCV activity is mediated through the inhibition of inosine-5'-monophosphate dehydrogenase (IMPDH).⁵

Case report

Female patient complaining of asthenia and pain at hypogastric area, admitted on October 4, 2011 in the emergency room of Hospital Universitário do Oeste do Paraná (HUOP) in the city of Cascavel-PR. During anamnesis, the patient reported treatment of chronic infection with hepatitis C virus with pegylated interferon alpha and ribavirin, starting on December 7, 2010 and concluded on November 1, 2011. During this period the patient developed severe pancytopenia due to the adverse effects of the medication.

On admission, routine laboratory tests were performed and the results showed significant changes in blood count parameters; and a hematocrit of 23%, associated with normal values of MCH (mean corpuscular hemoglobin), together with clinical data obtained at the time of anamnesis, raised the diagnostic hypothesis of autoimmune anemia. In an attempt to confirm

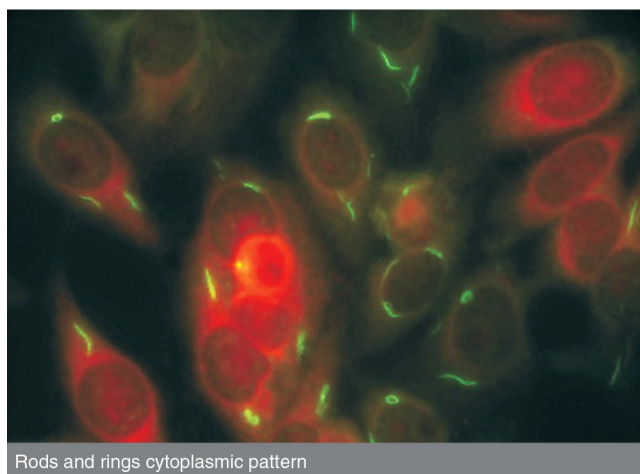


Fig. 1 – Rods measuring 3–10 μm and rings with 2–5 μm of diameter, scattered across the cell cytoplasm.

the diagnosis of autoimmune disease, a search for autoantibodies against cellular antigens (PAAC-HEp-2), traditionally known as antinuclear antibodies (ANA), was requested, showing fluorescence in the shape of rods and/or rings in the cell cytoplasm.

This description of a clinical case aims to bring current information about this fluorescence pattern found in Hep-2 cells, not yet completely understood, in an attempt to understand its relationship with the clinical picture of the patient.

Discussion

Recently, a new cytoplasmic pattern in PAAC-HEp-2 has been reported in patients with HCV. This pattern is characterized by rods with 3–10 μm and rings with 2–5 μm in diameter, scattered across the cell cytoplasm (Fig. 1). Because of that, this pattern has been designated as “rods and rings” (RR).⁶

Seelig et al., when doing a search for PAAC-HEp-2, detected the RR pattern in a serum sample of a patient, and the antigenic target of this reaction was identified through searches in databases as being IMPDH type 2, which is a key enzyme in the synthesis of puric nucleotides.⁷ In the study by Carcamo et al., cell cultures treated with ribavirin showed that this drug has the ability to induce the formation of the RR pattern; on the other hand, in *in vivo* tests 25% of HCV positive patients treated with ribavirin and interferon alpha had anti-RR antibodies, while none of the untreated subjects developed this pattern.⁸

In mammals, there are two isoforms of this enzyme, IMPDH1 and IMPDH2. While IMPDH1 is constitutively expressed in normal cells, the expression and activity of IMPDH2 are increased in malignant cells.⁸ Therefore, HEp-2 cells, by being originated from human laryngeal carcinoma, present increased expression and activity of IMPDH2 enzyme.

IMPDH2, aggregated or modified in the form of RR in these patients treated with inhibitors of this enzyme such as ribavirin, may become antigenic and induce an autoimmune response. It is possible that interferon alpha stimulates the occurrence of anti-RR reactivity apparently induced by ribavirin.^{6–8}

Keppeke et al. carried out a longitudinal study and analyzed samples from 597 patients using indirect immunofluorescence (IIF) technique in HEp-2 slides. The RR pattern was observed in 14.1% of 342 patients with HCV, and none of the 117 patients without HCV showed this pattern. Regarding treatment, anti-RR antibodies were present in 38% of 108 patients receiving interferon alpha and ribavirin, but neither of the 26 patients receiving monotherapy with interferon alpha or ribavirin nor those 166 untreated patients presented RR.

In this study we observed the presence of antibodies anti-RR just after the beginning of treatment, with its appearance starting within the first month in 6% of patients; however, at six months more than 47% of the tested samples presented the RR pattern. Another relevant fact of this study was that, among anti-RR-positive patients, 77% did not respond to treatment. On the other hand, among anti-RR-negative patients, the rate was 64%. Thus, this pattern is not related to the success of drug therapy.⁸

When it comes to RR pattern, there is discrepancy in the results obtained in PAAC-HEp-2 with IIF by using slides from different manufacturers, and this remains a not completely resolved issue, but that may stem from differences in culture conditions, sample processing, or both.^{7,8} This difficulty was also found in our laboratory, considering that, when faced with this pattern, hitherto unknown, it was necessary to confirm it with slides of various trademarks; but we observed the formation of RR pattern only in one of the commercial slides used, thus creating doubts about the relevance of the pattern. In fact, in most of the HEp-2-positive slides, serum samples positive for the RR pattern produce a non-specific spotted cytoplasmic pattern, or present no significant reaction.⁶

The identification of a new autoantibody associated with a given pathology may contribute to the understanding of its pathophysiology and can enrich the arsenal of diagnostic tests for this disease.⁶ It is therefore important that further studies are conducted, in order to understand the clinical significance of this cytoplasmic pattern.

Data presented in this study revealed that antibodies associated to RR pattern are strongly associated with the treatment of hepatitis C; that the occurrence of anti-RR reactivity is promoted by a combined therapy with interferon alpha and ribavirin; and that its frequency increases with the duration of treatment. However, the same was not observed when these drugs are used separately. Furthermore, previous studies showed no relationship between this pattern and demographic parameters, duration of diagnosis of HCV, treatment response pattern, HCV genotype or viral load.⁸

Conflicts of interest

The authors declare no conflicts of interest.

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