Case report

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

Mariano Felisberto a,⁎, Alex Sandro Jorge b, Rafael Andrade Menolli b, Laisa Vieira Gnuzmann a, Vanessa Nesi a

a Laboratório de Análises Clínicas do Hospital Universitário do Oeste do Paraná, Florianópolis, SC, Brazil
b Universidade Estadual do Oeste do Paraná (Unioeste), Florianópolis, SC, Brazil

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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).
Corresponding author.
E-mail: marianofelisberto@hotmail.com (M. Felisberto).
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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
Paciente do sexo feminino, queixando-se de astenia e dor em hipogastrio, 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 peguaildado 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 antígenico 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 antígena 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 ocorre 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.1,2 HCV was originally isolated in a serum sample of an individual with non-A, non-B hepatitis in 1989 by Choo et al.3 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.1

Interferon is a cytokine which composes the innate response of the human host. The addition of one polyethylenglycol 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).2

Case report
Female patient complaining of astenia 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
the diagnosis of autoimmune disease, a search for autoan-
tibodies against cellular antigens (PAAC-HEp-2), traditionally
known as antinuclear antibodies (ANA), was requested, show-
ing 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 under-
stand 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, scat-
tered 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 anti-
genic 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 isofoms 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 IMDH2 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 autoim-
mune 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 immunofluores-
cence (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 nei-
ther 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 forma-
tion 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 asso-
ciated 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 demo-
graphic parameters, duration of diagnosis of HCV, treatment
response pattern, HCV genotype or viral load.9

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Silva AL, Vitorino RR, Esperidião-Antonio V, Santos ET, Santana