

# Contributions to the study of the pathogenic mechanisms of HCV and their implications for a better understanding of the disease

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## ABSTRACT

Electronic transmission and confocal microscopy were used to study hepatic biopsies and peripheral blood mononuclear cells (PBMC) from 150 Cuban chronic hepatitis C patients. The study yielded novel data about the detection of the capsid of HCV in extra-parenchymal liver cells. Apoptotic cells were detected in liver tissue and PBMC using the TUNEL technique and the activation of apoptotic effectors. For the first time it was possible to observe the presence of positive- and negative-polarity RNA in hepatocytes and PBMC for 80% of the samples taken from serum RNA-negative patients with histological and biochemical remission. Another new contribution was the demonstration that extra-parenchymal liver cells and PBMC constitute reservoirs for HCV replication. The presence of HCCAg and core particles of HCV, observed for the first time in stellate cells, proved that HCV can directly infect this cell type. Since stellate cells can trans-differentiate into myofibroblast-like cells and thus modulate the process of hepatic fibrogenesis, this finding underscores the relevance of these cells, together with fibroblasts, for the development of hepatic cirrhosis. The application of these findings has important repercussions for the diagnosis, follow-up and prognosis of hepatitis C.

**Keywords:** Hepatitis C Virus, capsid protein, Peripheral Blood Mononuclear Cells, Transmission Electron Microscopy

## Introduction

Currently, the cellular and molecular processes associated to the pathogenic mechanisms taking place in the liver of hepatitis C virus (HCV)-infected patients are not well elucidated.

In Cuba, transmission electron microscopy and confocal microscopy were employed to study hepatic biopsies and peripheral blood mononuclear cells (PBMC) from 150 chronic hepatitis C patients.

## Methodology and results

The study of the samples from Cuban patients revealed the presence of the HCV capsid protein (HCCAg) in hepatocytes and 'non-parenchymal' cells. It was also possible to detect the presence of both, positive- and negative-polarity RNAs in hepatocytes and PBMC from more than 80% of the samples from RNA-negative patients' sera.

The TUNEL technique and the activation of apoptotic effectors (caspases 3 and 7) were used to visualize the presence of apoptosis in hepatic cells and PBMC. It was detected that the extra-parenchy-

mal cells of the liver (Pit, endothelial, Kupffer and stellate cells) and PBMC constitute reservoirs for the replication of HCV.

Transmission electron immunomicroscopy was applied to detect the relationship between the apoptosis mediated by the Fas and TNF systems and the development of hepatic lesions, hepatic steatosis, the evolution to chronicity during HCV infection, and hepatocarcinoma. The presence of HCCAg and capsid particles from HCV was reported for the first time in stellate cells, which proves that HCV can directly infect these cells, induce their differentiation into myofibroblasts, and therefore modulate the process of hepatic fibrogenesis.

Additionally, the study of the patients revealed that the short-term induction of fibrosis and cirrhosis was correlated with an increase in the number of stellate cells and fibroblasts. This constitutes a potential diagnostic parameter for the prognosis of these diseases. This method also evidenced the process of apoptosis in hepatocytes from patients with chronic HCV infection.

Signs of apoptosis (cellular constriction, chromatin condensation, formation of apoptotic bodies, phagocytosis of neighboring cells) were detected by transmission electron microscopy (TEM), as well as fragmentation of nuclear DNA using the TUNEL technique.

Immunocytochemical assays showed the activation of apoptotic effectors (caspases 3 and 7) and the association of HCCAg with the rough endoplasmic reticulum, mitochondria and nucleus of apoptotic hepatocytes. Furthermore, E1 antigen was immunolocalized to the cytoplasm and mitochondria of some hepatocytes, together with crystalloid bodies co-localized with this protein. Data suggest that the intracellular localization of HCCAg and E1 is directly associated with apoptosis and the pathogenesis of the disease [1].

Positive- and negative-polarity RNAs from HCV were detected in 80% of the samples from RNA-negative patients' serum, both in hepatocyte and PBMC samples, as shown in Figure 1. The negative strand RNA was expressed at lower levels than the positive strand in the liver of patients. Additionally, core E1 and E2 antigens were detected inside the hepatocytes. Hybridization and immunofluorescence signals were located inside the cytoplasm, indicating that this compartment is the site for active replication of HCV. However, there were no statistically significant differences between the levels of cellular expression of both RNA strands in the case of PBMC. These results provide evidences on the possible persistence of replicative intermediates of HCV at the liver and the PBMC of chronic patients with apparent clinical remission from HCV infection [2].

On the other hand, electron immunomicroscopy was employed to detect the presence of HCCAg in hepatic «non-parenchymal» cells of HCV patients; specifically at the cytoplasm and nucleus of lymphocytes, Kupffer, Pit, endothelial and stellate cells, as well as in fibroblasts. HCCAg was also found at bile canaliculi, suggesting the implication of the biliary system on this disease.

Data suggest that the 'non-parenchymal' hepatic cells can constitute a reservoir for HCV replication. Additionally, this virus may contribute to the modulation of the immune response, fibrosis and steatosis in the liver [3].

The study also evidenced the presence of HCV nucleocapsid-like particles in the nucleus of hepatocytes from chronic HCV patients. These particles were similar in size and shape to recombinant core particles from HCCAg obtained in *Pichia pastoris*. Besides, the HCV core antigen was detected in the cytoplasm, nucleus and nucleolus of hepatocytes using electron immunomicroscopy. This finding constitutes the first report on the nuclear localization of the HCV core antigen and nucleocapsid-like particles in hepatocytes during an infection by HCV *in vivo* [4].

### Relevance of the study

The relevance of this study is based on the fact that it was possible to detect, for the first time, the presence of positive- and negative-polarity HCV RNAs in hepatocytes and PBMC from more than 80% of the samples from sera of RNA-negative Cuban chronic hepatitis C patients. This showed that the extra-parenchymal cells from liver and PBMC constitute reservoirs for HCV replication. The evidences shed light on some long-standing questions about the short-term reinfection of liver transplant patients.

The presence of HCCAg and core particles of HCV was also detected for the first time in stellate cells, proving that HCV can directly infect these cells, inducing their differentiation into myofibroblast-like cells and therefore modulating the process of hepatic fibrogenesis.

It can be stated that the study of the PBMC by TEM constitutes a quick method for the confirmation of HCV infections. Such a method is an important tool for establishing evolution/remission criteria for

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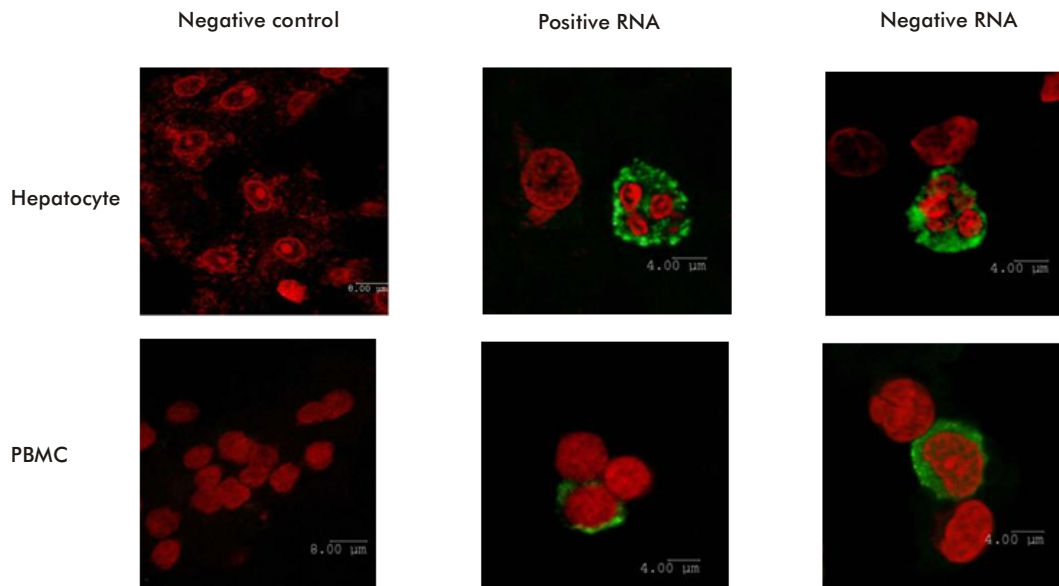


Figure 1. The figure shows the presence of positive- and negative-polarity RNAs in hepatocytes and PBMC (green label) from more than 80% of the samples from serum RNA-negative patients.

this disease, with a wide potential for practical application.

The present study was performed for the first time in Cuban patients which were serum RNA-negative, presented histological and clinical remission and had negative-polarity (viral replication) RNA in PBMC and hepatocytes.

## Conclusions

This study provides a number of new contributions to the knowledge on HCV pathogenic mechanisms. The practical application of these findings about the chronic infection with HCV constitutes a high-prior-

ity goal for the management of this public health problem. From a scientific point of view, results provide relevant information about the cellular processes implied in HCV pathogenesis.

The diagnostic criterion generated here, backed by a number of scientific publications, constitutes a significant contribution to the detection by TEM of viral HCV replication in PBMC and hepatocytes from RNA-negative patients' sera. It allows a better comprehension of the proper practices for the diagnosis, follow-up and prognosis of chronic HCV patients. This knowledge can be incorporated to the design of future clinical trials in the treatment of this infection.