

EDITORIAL

Occult hepatitis C virus infection: A new form of hepatitis C

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Abstract

Occult hepatitis C virus (HCV) infection is a new recently characterized entity. This occult infection can be present in two different clinical situations: in anti-HCV negative, serum HCV-RNA negative patients with abnormal liver function tests and in anti-HCV positive subjects with normal values of liver enzymes and without serum HCV-RNA. This review describes recent studies of occult HCV infection in both kinds of patients.

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INTRODUCTION

The etiology of liver disease is unknown in approximately 10% of patients with abnormal results on liver function tests. Some authors have reported that occult hepatitis B virus could be the cause of a proportion of these cryptogenic chronic hepatitis^[1,2] cases, but no conclusive results have been yielded.

In January 2004, the role of occult hepatitis C virus (HCV) infection in chronic liver disease of unknown etiology was first described by Castillo *et al*^[3]. This study included 100 patients with persistently long-standing abnormal liver function test results: alanine aminotransferase (ALT) and/or gamma-glutamyl transpeptidase (gamma-GTP). All known causes of liver diseases were excluded, and they were repeatedly anti-HCV and serum HCV-RNA negative. All these patients underwent a liver biopsy. A reverse-transcription polymerase chain reaction found that 57% of them had HCV-RNA in their liver. These results were also

confirmed by *in situ* hybridization. In addition, 48/57 (84%) of these patients with occult HCV infection also had the antigenomic HCV-RNA strand in the liver tissue, indicating an ongoing viral replication. The HCV genotype found in the liver of the patients was 1b, which was demonstrated by a commercial genotyping assay and by amplification and sequencing of the HCV-core region. Moreover, 70% of the patients with intrahepatic HCV-RNA in liver also had viral RNA in their peripheral blood mononuclear cells (PBMC). Finally, liver necroinflammatory activity and fibrosis were observed in a significantly higher proportion of patients with occult HCV infection than in those without intrahepatic HCV-RNA. In summary, this paper identified a new form of hepatitis C virus infection called "occult HCV infection". It is characterized by the presence of HCV-RNA in the liver in the absence of serological markers of infection (anti-HCV and serum HCV-RNA negative).

The existence of this kind of occult HCV infection has also been found by other authors. Thus, Stapleton and colleagues have reported several studies on "seronegative" HCV infection in patients with cryptogenic liver disease and persistently abnormal results of liver tests^[4,5]. Editorials or editors' comments have also been devoted to the role and significance of occult HCV infection, recognizing this infection as a new entity that should be taken into account for the diagnosis of patients with liver diseases of unknown cause^[6-10].

Once occult HCV infection was identified, different research fields were developed: (1) To find an alternative to the liver biopsy for the diagnosis of occult HCV infection. (2) To study if HCV replicates or not in the PBMC of patients with occult HCV infection. (3) To compare the clinical, biochemical and histological characteristics of occult with chronic HCV infection. (4) To compare virus-specific T-cell responses in patients with occult and with chronic HCV infection. (5) To assess the possible role of occult HCV infection in the development of hepatocellular carcinoma. (6) To study the prevalence of occult HCV infection in other risk populations such as hemodialysis patients. (7) To assess the efficacy of antiviral therapy for occult HCV infection. (8) To study other possible clinical situations of occult HCV infection.

ALTERNATIVES IN DIAGNOSIS OF OCCULT HCV INFECTION

Although HCV-RNA is also detected in the PBMC of a high percentage of patients with an occult HCV infection, the gold standard for diagnosis of this occult viral infection

is detection of HCV-RNA in liver cells. However, because of the invasive nature of the liver biopsy, other alternatives were studied in an attempt to increase the sensitivity of the diagnostic tests in serum. Taking into account previous data recorded in patients with chronic hepatitis C^[11], we performed a study with 21 patients diagnosed as having occult HCV infection (HCV-RNA positive in liver but negative in serum) and compared detection of viral RNA in plasma, PBMC and whole-blood^[12]. All cases had negative results for HCV-RNA in plasma. In 3 (14%) patients, viral RNA was detected in whole-blood while HCV-RNA could be detected in PBMC of 57% of the included cases. Thus, using whole-blood as the source for HCV-RNA detection does not improve the sensitivity of the diagnosis of occult HCV infection. Testing for HCV-RNA in PBMC is much more reliable in identifying patients with an occult HCV infection when a liver biopsy is not available.

OCCULT HCV REPLICATION IN PBMC

One important question regarding the transmission of occult HCV infection was whether the virus could replicate in PBMC. To study this issue, 18 patients who had been diagnosed with occult hepatitis C by testing for HCV-RNA in their liver biopsy and who also had HCV in their PBMC were selected for this study^[13]. By a strand-specific RT-PCR it was found that 61% of the patients had the antigenomic HCV-RNA strand in their PBMC, indicating that HCV was replicating in these cells. So, although the patients with occult HCV infection do not have detectable circulating virions, they could be potentially infectious.

CHRONIC VERSUS OCCULT HEPATITIS C

Once occult HCV infection is identified, one important question is if the clinical characteristics of this infection differ from those usually found in chronic hepatitis C. Trying to answer this question, the biochemical, virological and histological features of a group of 68 patients with occult HCV infection were compared with those of a group of 69 patients with histologically proven chronic hepatitis C^[14]. Groups were matched with respect to gender, age and known time duration of the disease. Triglycerides and cholesterol values were significantly higher in occult HCV infection, while alanine aminotransferase, gammaglobulin, alpha-fetoprotein and iron levels were significantly higher in patients with chronic hepatitis C. The number of patients who had necroinflammatory activity and fibrosis in the liver biopsy was significantly higher in the group with chronic hepatitis C than in the group with occult HCV infection, but no difference was found in the percentage of patients with liver steatosis between both groups. Finally, as could be expected, the percentage of HCV-infected hepatocytes (determined by *in situ* hybridization) was significantly lower in patients with occult HCV. Thus, it was concluded that occult HCV infection is a milder disease, with less liver damage than chronic hepatitis C. Nevertheless, as patients with occult hepatitis C may present dyslipidemic disorders, studies on the natural history of occult HCV infection should be performed to prove the role of occult HCV as

the cause for liver injury in these patients.

CELLULAR IMMUNE RESPONSES IN OCCULT HCV INFECTION

Why does occult HCV infection induce a less aggressive disease than chronic HCV infection? The immunological system of the patients could be involved in this situation.

Quiroga *et al*^[15] have performed a study in an attempt to determine if the cellular immune response of patients with occult HCV infection is different from that of patients with chronic hepatitis C. This work compared 50 patients with occult HCV, 141 with chronic hepatitis C and 21 patients with cryptogenic liver disease (all known causes of liver disease were discarded, including an occult HCV infection). Overall, 26/50 (52%) of patients with occult HCV infection had CD4⁺ T-cell proliferative responses. These responses were significantly more frequent in patients with occult HCV than in the group of patients with chronic hepatitis C (37/141: 26%; $P = 0.0016$) or in individuals with cryptogenic liver disease (1/21: 5%; $P < 0.001$). HCV-specific T-cells of patients with occult HCV infection proliferated more commonly in response to NS3 and NS4 proteins, and the peripheral blood mononuclear cells derived T-cell lines from these patients produced gamma interferon. Finally, patients with occult HCV infection had significantly higher amounts of HCV-specific CD8⁺ T-cells than patients with chronic hepatitis C. In summary, HCV-specific cellular immune responses are more frequent in occult HCV infection than in chronic hepatitis C. Thus, patients with an occult HCV infection had a better immune response and this could be the cause of the milder disease that these patients have in comparison to those with chronic hepatitis.

These results seem to suggest that the clinical differences observed between occult and chronic hepatitis C are a consequence of the host's immunological system.

OCCULT HCV INFECTION AND HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is one of the most common malignancies throughout the world. In a small proportion of cases, the etiology agent associated with the development of liver cancer is unknown. Two different groups, one from Japan^[16] and the other from Italy^[17], have reported the presence of occult HCV infection in non-tumoral and tumoral liver tissues of patients with HCC who were negative to serological HCV markers. Therefore, it seems that occult HCV infection may play a role in the development of HCC however, as discussed above, more studies on this issue should be performed.

OCCULT HCV INFECTION IN RISK POPULATIONS

In the presence of elevated levels of liver enzymes, hemodialysis patients are screened for hepatotropic viral infections: hepatitis B surface antigen, anti-HCV and/

or serum HCV-RNA. Nevertheless, in some patients the etiology of their elevated liver enzymes cannot be established as they are negative for all serological viral markers (including serum HCV-RNA). To investigate whether these hemodialysis patients are affected by an occult HCV infection, 6 Spanish Hemodialysis Units enrolled 42 patients with abnormal liver function tests of unknown etiology^[18]. By strand-specific RT-PCR and by *in situ* hybridization it was found that 26/42 (62%) of the patients had HCV-RNA in their PBMC. These patients with occult HCV infection had significantly higher ALT values than the negative ones. In addition, HCV was replicating in the PBMC of 15/26 (58%) of the cases of occult infection. In summary, although these patients are serum HCV-RNA negative they could be potentially infectious as HCV is replicating in their PBMC, thus preventive measures to avoid HCV spread in hemodialysis units must be considered.

ANTIVIRAL TREATMENT IN OCCULT HCV INFECTION

Regarding the possible efficacy of antiviral therapy in occult hepatitis C, Pardo *et al*^[19] treated 10 patients with occult HCV infection with pegylated-interferon plus ribavirin for 6 mo, followed by a post-treatment follow-up period of 6 mo. Although all the patients were infected with HCV genotype 1b, they were treated for 6 mo instead of 12 mo (the currently accepted duration of antiviral therapy for patients with chronic hepatitis C and genotype 1b), because they were serum HCV-RNA negative and the percentage of infected hepatocytes is lower than in chronic hepatitis C^[14]. At the end of treatment, 80% of the patients had normalized ALT levels and cleared HCV-RNA from PBMC. However, at the end of the post-treatment follow-up only 30% of the patients maintained a complete response (HCV-RNA negative in PBMC and normal ALT levels). Five patients (2 of them were complete responders) underwent a second liver biopsy after treatment and, although none of them lost HCV-RNA in liver, a significant decrease was observed in the amount of intrahepatic viral RNA in comparison to the basal levels. Moreover, in 3 patients liver necroinflammatory activity and fibrosis score had decreased with respect to the pre-treatment histological diagnosis.

It can be concluded that, as reported in chronic hepatitis C (see below), antiviral therapy in occult HCV infection does not lead to a complete eradication of HCV infection, yet it may be useful as liver damage improves. Thus, treatment with pegylated-interferon and ribavirin of patients with occult HCV infection and a stage of liver fibrosis of 2 or more seems advisable.

OTHER FORMS OF OCCULT HCV INFECTION

As commented before, occult HCV infection is characterized by the absence of anti-HCV and of serum HCV-RNA, but viral RNA is detectable in liver and PBMC. However, occult HCV infection may exist in other clinical situations such as in anti-HCV positive patients who are serum HCV-RNA negative and who present

normal liver function tests.

One of these populations is the “healthy” HCV-carriers. These patients, who are anti-HCV positive with undetectable serum viral RNA and normal ALT levels, are considered to be subjects who have cleared HCV infection after exposure to HCV. To verify if these patients could have HCV-RNA in their liver, we performed a study with 12 “healthy” anti-HCV carriers^[20]. These anti-HCV positive patients were serum HCV-RNA negative and had persistently normal ALT values for a 29 ± 19 mo follow-up period. These patients underwent a programmed interventional laparoscopy and gave their consent for obtaining a liver biopsy specimen during the laparoscopy. The genomic HCV-RNA strand was detected in the liver of 10/12 (85%) of these subjects and it was also found that HCV was replicating in the hepatocytes of the 10 patients in question. All of them were infected by HCV genotype 1b, as demonstrated by sequencing of the HCV genome amplified from the liver. Viral RNA was also found in the PBMC of 6/12 patients and 5 (83%) out of these 6 also had HCV replication in PBMC. Another research group^[21] has also demonstrated the presence of HCV infection and replication either in the liver or PBMC of nearly 90% of anti-HCV positive patients with normal ALT values.

Occult HCV infection has also been identified in a similar cohort of patients: those with chronic hepatitis C who have responded to an antiviral therapy with loss of circulating HCV-RNA and normalization of ALT levels. Several papers have reported the presence of an occult HCV infection (persistence of HCV-RNA) in the liver and in PBMC of sustained responders^[22-24]. However, other authors have not found viral RNA in these patients^[25,26]. These discrepancies could be due to different preservation methods of the liver biopsies, to differences in the sensitivity of the methods employed for HCV-RNA detection, different geographical incidence, *etc.* Thus, further studies are needed to know the real prevalence of occult HCV in complete responder patients. One of the possible consequences of occult HCV infection is the persistence of liver necroinflammation in an important number of sustained responder patients^[23,24].

The case of a patient with chronic hepatitis C who cleared serum HCV-RNA with normalization of ALT levels in whom HCV infection reactivated following prednisone therapy after 8.5 years of HCV-RNA negativity has been published. Thus, occult HCV infection should be taken into account when these anti-HCV positive patients with normal ALT levels undergo immunosuppressive therapies^[25].

CONCLUSIONS

Occult hepatitis C infection is a new entity that should be taken into account for the diagnosis of patients with a liver disease of unknown origin. Future works should deal with its possible incidence and pathologic relevance in immunosuppressed or HIV coinfecting patients, drug abusers or subjects who had received multiple blood transfusions. On the other hand, it would be convenient to perform epidemiological studies on occult HCV infection

among health care staff, patients on hemodialysis, etc to know the prevalence and spread of this infection in these populations. It is also very important to determine the possible incidence and consequences of occult HCV infection in blood donated and transplanted patients.

REFERENCES

- Diodati G**, Pontisso P, Bonetti P, Stenico D, Noventa F, Alberti A, Realdi G. Cryptogenic chronic liver disease and serum or liver hepatitis B virus markers. Their possible correlations and etiologic significance. *Digestion* 1988; **39**: 251-256
- Chemín I**, Zoulim F, Merle P, Arkhis A, Chevallier M, Kay A, Cova L, Chevallier P, Mandrand B, Trépo C. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. *J Hepatol* 2001; **34**: 447-454
- Castillo I**, Pardo M, Bartolomé J, Ortiz-Movilla N, Rodríguez-Iñigo E, de Lucas S, Salas C, Jiménez-Heffernan JA, Pérez-Mota A, Graus J, López-Alcorocho JM, Carreño V. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis* 2004; **189**: 7-14
- Schmidt WN**, Wu P, Cederna J, Mitros FA, LaBrecque DR, Stapleton JT. Surreptitious hepatitis C virus (HCV) infection detected in the majority of patients with cryptogenic chronic hepatitis and negative HCV antibody tests. *J Infect Dis* 1997; **176**: 27-33
- Stapleton JT**, Schmidt WN, Katz L. Seronegative hepatitis C virus infection, not just RNA detection. *J Infect Dis* 2004; **190**: 651-652
- Lerat H**, Hollinger FB. Hepatitis C virus (HCV) occult infection or occult HCV-RNA detection ?. *J Infect Dis* 2004; **189**: 3-6
- Coyle WJ**. Abnormal liver tests with unexplained cause in patients with hepatitis C virus infection. *Curr Gastroenterol Rep* 2004; **6**: 271-272
- Blendis L**. Occult viral hepatitis: what is the significance? *Gastroenterology* 2005; **128**: 2168-2170
- Feld JJ**, Liang TJ. HCV persistence: cure is still a four letter word. *Hepatology* 2005; **41**: 23-25
- Gordon SC**. Occult viral hepatitis and noncirrhotic hepatocellular carcinoma. *Am J Gastroenterol* 2005; **100**: 1754-1757
- Schmidt WN**, Stapleton JT, LaBrecque DR, Mitros FA, Kirby PA, Phillips MJ, Brashear DL. Hepatitis C virus (HCV) infection and cryoglobulinemia: analysis of whole blood and plasma HCV-RNA concentrations and correlation with liver histology. *Hepatology* 2000; **31**: 737-744
- Carreño V**, Castillo I, Bartolomé J, Rodríguez-Iñigo E, Ortiz-Movilla N, de Lucas S, Pardo M. Comparison of hepatitis C virus RNA detection in plasma, whole blood and peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *J Clin Virol* 2004; **31**: 312-313
- Castillo I**, Rodríguez-Iñigo E, Bartolomé J, de Lucas S, Ortiz-Movilla N, López-Alcorocho JM, Pardo M, Carreño V. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *Gut* 2005; **54**: 682-685
- Pardo M**, López-Alcorocho JM, Rodríguez-Iñigo E, Castillo I, Carreño V. Comparative study between occult hepatitis C virus infection and chronic hepatitis C. *J Viral Hepat* 2007; **14**: 36-40
- Quiroga JA**, Castillo I, Rodríguez-Iñigo E, Pardo M, Carreño V. HCV-specific cellular immune responses in patients with occult HCV infection in the liver. Abstracts of the 55th Annual Meeting of the American Association for the Study of Liver Diseases. *Hepatology* 2004; **40** Suppl 1: 278A
- Esaki T**, Suzuki N, Yokoyama K, Iwata K, Irie M, Anan A, Nakane H, Yoshikane M, Nishizawa S, Ueda S, Sohda T, Watanabe H, Sakisaka S. Hepatocellular carcinoma in a patient with liver cirrhosis associated with negative serum HCV tests but positive liver tissue HCV RNA. *Intern Med* 2004; **43**: 279-282
- Comar M**, Dal Molin G, D'Agaro P, Crocè SL, Tiribelli C, Campello C. HBV, HCV, and TTV detection by in situ polymerase chain reaction could reveal occult infection in hepatocellular carcinoma: comparison with blood markers. *J Clin Pathol* 2006; **59**: 526-529
- Castillo I**, Rodríguez-Iñigo E, Barril G, Arenas D, Espinosa M, García-Valdecasas J, González-Parra E, Sánchez C, Selgas R, Carreño V. Occult HCV infection in hemodialysis patients. Abstracts of the 56th Annual Meeting of the American Association for the Study of Liver Diseases. *Hepatology* 2005; **42** Suppl 1: 437A
- Pardo M**, López-Alcorocho JM, Castillo I, Rodríguez-Iñigo E, Pérez-Mota A, Carreño V. Effect of anti-viral therapy for occult hepatitis C virus infection. *Aliment Pharmacol Ther* 2006; **23**: 1153-1159
- Carreño V**, Pardo M, López-Alcorocho JM, Rodríguez-Iñigo E, Bartolomé J, Castillo I. Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. *J Infect Dis* 2006; **194**: 53-60
- Falcón V**, Acosta-Rivero N, Shibayama M, Luna-Munoz J, Miranda-Sánchez M, de la Rosa M-C, Menéndez I, Gra B, Dueñas-Carrera S, García W, Vilar E, Silva J, López D, González-Bravo M, Fernández-Ortega C, Casillas D, Morales J, Kouri J, Tsutsumi V. Evidences of hepatitis C virus replication in hepatocytes and peripheral blood mononuclear cells from patients negative for viral RNA in serum. *Am J Infect Dis* 2005; **1**: 34-42. Available from: [http:// www.scientificworld.net/fulltext/ajid/ajid1134-42.pdf](http://www.scientificworld.net/fulltext/ajid/ajid1134-42.pdf)
- Pham TN**, MacParland SA, Mulrooney PM, Cooksley H, Naoumov NV, Michalak TI. Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. *J Virol* 2004; **78**: 5867-5874
- Radkowski M**, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology* 2005; **41**: 106-114
- Castillo I**, Rodríguez-Iñigo E, López-Alcorocho JM, Pardo M, Bartolomé J, Carreño V. Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. *Clin Infect Dis* 2006; **43**: 1277-1283
- McHutchison JG**, Poynard T, Esteban-Mur R, Davis GL, Goodman ZD, Harvey J, Ling MH, Garaud JJ, Albrecht JK, Patel K, Dienstag JL, Morgan T. Hepatic HCV RNA before and after treatment with interferon alone or combined with ribavirin. *Hepatology* 2002; **35**: 688-693
- Tsuda N**, Yuki N, Mochizuki K, Nagaoka T, Yamashiro M, Omura M, Hikiji K, Kato M. Long-term clinical and virological outcomes of chronic hepatitis C after successful interferon therapy. *J Med Virol* 2004; **74**: 406-413

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