Occult Hepatitis C Virus Infection and Its Relevance in Clinical Practice

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Hepatitis C virus (HCV) can persist in the liver, lymphoid (immune) cells, and serum of individuals long after an apparently complete therapy-induced or a spontaneous resolution of hepatitis C. This essential asymptomatic infection, called secondary occult HCV infection (OCI), usually occurs in anti-HCV antibody reactive individuals with normal liver function tests. This infection has been identified when the nucleic acid amplification assays of enhanced sensitivity were applied for the detection of HCV genome and its replication. In addition to the secondary OCI, a form of low-level HCV-RNA-positive infection of unknown etiology coinciding with moderately elevated serum liver enzymes and progressing in the absence of anti-HCV detectable by standard clinical assays has been reported. Because of its undefined origin, it can be termed cryptogenic OCI. In this review, the general characteristics of OCI, the ways of its detection and associated controversies, and the potential clinical implications of its existence will be concisely outlined. (J Clin Exp Hepatol 2011;1:185–189)

Hepatitis C virus (HCV) is a human blood-borne pathogen responsible for over 170 million chronic infections worldwide. At least 35% of the infected individuals with a symptomatic acute infection spontaneously resolve hepatitis, while the rest develop chronic hepatitis C (CHC) and persistently carry virus at levels normally detectable by clinical laboratory tests. Chronic HCV infection can lead to liver fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC), and end-stage liver disease caused by HCV is the leading cause of liver transplantation in many parts of the world.[1]

The HCV is a highly heterogeneous, single-stranded ribonucleic acid (RNA) virus with at least six major genotypes (designated as 1, 2, 3, etc.), many subtypes (designated as a, b, c, etc.), and uncountable variants. The virus propagates by making a complementary RNA negative strand. Although traditionally thought to infect primarily hepatocytes, HCV has also been shown in different studies to invade and replicate in other cell types, such as those of the immune system.[2] In the in vivo setting, T and B lymphocytes, monocytes, and dendritic cells from patients with hepatitis C have been reported to carry HCV-RNA positive and, in many instances, HCV-RNA negative (replicative) strand.[3–6] Further, the culture supernatant from ex vivo stimulated lymphoid cells from some of these patients has been shown to contain infectious HCV capable of inducing de novo infection of primary T cells.[7,8] Similarly, in the context of in vitro cell culture models, primary T cells and monocytes/macrophages, as well as various T and B cell lines, have been shown to be able to support productive HCV replication, although to a highly variable degree and usually at very low levels.[7,9,10] The latter is evidenced by the detection of HCV-RNA negative strand, synthesis of viral proteins, emergence of immune cell-associated viral variants, release of infectious HCV-like virus particles, susceptibility of virus in infected cells to anti-viral treatment, and altering host's immune responses.[7,9]

In clinical practice, HCV genotype has been used to determine the appropriate duration of the standard antiviral therapy, which is a combination of pegylated alpha-interferon (IFN) and ribavirin (RBV). For example, patients infected with genotypes 1, 4, and 6 are usually treated for 48 weeks, whereas those carrying the other genotypes typically receive a 24-week IFN–RBV treatment regimen. In this regard, it has been widely accepted among the clinical community that patients who are tested negative for HCV-RNA in serum or plasma by standard clinical assays for at least 6 months upon completion of therapy are considered to have had a sustained virological response (SVR) and deemed cured of HCV. Meanwhile, contrasting evidence has been brought forward by different investigators demonstrating the persistence at low levels of HCV-RNA

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Abbreviations: CHC: chronic hepatitis C; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IFN: interferon; IU: international unit; NAH: nucleic acid hybridization; OCI: occult HCV infection; PBMC: peripheral blood mononuclear cells; PCR: polymerase chain reaction; RBV: ribavirin; RNA: ribonucleic acid; SVR: sustained virological response
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in the circulation and/or hepatic tissue and peripheral blood lymphoid cells for many years after achievement of SVR.[11–14] This is referred to as occult HCV infection (OCI), owing to the seemingly asymptomatic presentation, normal levels of liver function tests, and detection of small amounts of viral genomes. Another form of OCI has also been documented in individuals who have persistently moderately elevated liver function tests despite the lack of a known history of exposure to HCV.[15] As in the case of OCI continuing after SVR (termed as secondary OCI), low levels of HCV-RNA can be found in both the circulation and cellular compartments in the latter form, termed as 'cryptogenic OCI'. Nevertheless, just as there have been studies challenging the occurrence of secondary OCI protracting after clinical resolution of hepatitis C,[16–18] the existence of cryptogenic OCI has also been disputed.[19] The current review aims at providing an overview of the data delineating general characteristics of OCI, a discussion of potential issues behind the apparent discrepancies in the detection of this form of HCV persistence, and highlighting conceivable practical ramifications surrounding this new entity of HCV infection.

Occult Hepatitis C Virus Infection Continuing After Resolution of Hepatitis C

The HCV persistence after apparent complete resolution of hepatitis C was first reported in 2004 in a group of 16 individuals with up to 5 years follow-up following confirmation of spontaneous resolution (5 individuals) or after achieving the SVR through anti-viral IFN or IFN–RBV therapy (11 individuals).[13] In this study, despite the apparently repeated HCV-RNA negativity in serum by standard clinical assays and normal liver function tests, trace amounts of HCV-RNA were detected by a superiorly sensitive reverse transcription (RT)-polymerase chain reaction (PCR) research assays in serum and/or peripheral blood mononuclear cells (PBMC) of all patients investigated. The HCV-RNA replicative strand was identified in the majority (75%) of PBMC tested. The finding was unexpected given the well-accepted notion at the time that clinical resolution of hepatitis C had reflected complete eradication of HCV infection. It was evident that the virus detection assay used in the study likely played a major role in the identification of viral genomes that would have otherwise been unrecognized by the standard clinical tests utilized at the time. In general terms, this RT-PCR-based research assay successively involved (1) two rounds of PCR amplification (direct and nested) of cDNA transcribed from target samples, i.e., RNA extracted from serum, PBMC, or liver tissues, using virus gene-specific primer sequences and (2) a nucleic acid hybridization (NAH) step of amplified products using a radiolabeled HCV-specific fragment as a probe. The second step was aimed at augmenting the level of detection while simultaneously confirming the specificity of amplified species, to give an overall sensitivity of ≤10 virus genome copies or virus genome equivalents (vge)/mL (≤3 IU/mL) or ≤5 vge/μg total RNA (≤1.5 IU/μg) to the assay.

Subsequent to these initial findings, other studies also documented, using similar detection approaches, the presence of small amounts of HCV-RNA in plasma or serum, PBMC and/or hepatic tissue for up to 10 years after clinical resolution of hepatitis C.[11,12,14,20,21] The prevalence of OCI in these individuals varied among the different studies and, depending on how the definition of OCI was being used, the overall rate ranged between 10% and 100%.[22] Interestingly, although liver histology was generally and significantly improved after achievement of the SVR, in many individuals the detectable HCV-RNA in liver biopsy specimens coincided with histological evidence of minimal to mildly active disease, including lymphocytic infiltrations, necrosis of small groups or singular hepatocytes, and various degrees of fibrosis.[12,14,23]

The HCV-RNA levels in OCI have generally been low, in the range of typically not >200 vge/mL plasma or serum and between 10 and 100 vge/μg total RNA for circulating immune cells or liver tissues. Nevertheless, it is important to recognize that HCV genomes detected in the latter two compartments coincide in many instances with the simultaneous presence of the viral RNA negative strand, implying the ongoing HCV replication.[11–13] Further, ex vivo stimulation of peripheral immune cells, such as T cells, B cells, and monocytes, with cell-activating agents like mitogens enhanced virus expression, significantly improving virus detection.[5,13,24] An area of importance yet inadequately recognized is that in many patients with OCI, or even with CHC, viral genomes may be found predominantly in particular immune cell subsets, e.g., T cells and/or B cells and/or monocytes, and that HCV occurring in these subtypes can be genetically different than the virus present in the circulation.[5,25,26] This observation and the finding of the concurrent detection of HCV replicative strand and viral proteins in the cells collectively further the case for HCV lymphotropism.

Cryptogenic Occult Hepatitis C Virus Infection

Cryptogenic OCI was first described in 2004 by Castillo and colleagues in individuals with long-standing elevation in liver function tests of undefined causes.[15] Unlike patients with secondary OCI continuing after resolution of clinically evident hepatitis C, persons with cryptogenic HCV infection are negative for antibodies against HCV (anti-HCV). In this report, the presence of HCV-RNA was found in liver biopsy specimens of nearly 60 individuals and in PBMC of 40 individuals from 100 patients investigated. Importantly, in the vast majority of these cases (>80%), the presence of the HCV-RNA genomic (positive) strand was accompanied by that of the viral RNA replicative (negative) strand, again indicating the existence of

active HCV replication. As in the case with secondary OCI, the documentation of cryptogenic HCV infection was made possible through the use of a highly sensitive RT-PCR-based research assay capable of detecting minute amounts of viral genome (sensitivity, 10 IU/mL).[15] Among the most recent studies on this subject, HCV-RNA was reportedly detected in about 10% of singular PBMC samples obtained from 69 anti-HCV antibody-negative patients with an etiologically undefined chronic liver disease.[21]

Causes Underlying Inconsistent Detection of Occult Hepatitis C Virus Infection

In parallel with studies documenting the existence of OCI, there have been other investigations arguing against the existence of low-level HCV infection. In the reports by Maylin et al,[17] and George et al,[16] the authors argued that HCV-RNA did not persist in serum[16,17] or unfractionated PBMC[16] from the vast majority (>99%) of individuals who achieved SVR. Similarly, the findings of cryptogenic OCI have also been disputed. Among these studies, those reported by Halfon et al,[19] Nicot et al,[18] and Coppola et al,[27] submitted that HCV-RNA was not present in plasma or serum, PBMC or liver tissues in anti-HCV negative patients with elevated liver enzymes. Considering the fact that OCI may have a significant impact on how patients' convalescent from hepatitis C or those with etiologically undefined chronic liver disease should be monitored and that the findings supporting the existence of OCI and those against it both seemed compelling, it would be prudent to tease out possible reasons behind such discrepancy.

It is accepted that the levels of HCV-RNA in OCI, if detectable, are low, i.e., below 200 vge/mL plasma or serum. Therefore, subtle differences in how patients' material is processed, preserved, and analyzed, as well as variations in the sensitivity of assays used in different laboratories, may cumulatively influence the ability to detect virus. Among the factors, the quality and the amount of recovered RNA from the sample analyzed are of principal significance since inappropriate handling of blood, cell, and liver tissue material, delays in RNA isolation, and suboptimal extraction procedures, even when commercial kits are employed, may substantially diminish or extinguish viral genomes, particularly if it occurs at very low levels.[22] Further, since HCV loads tend to fluctuate over the course of patients' follow-up, testing serial samples collected at a few weeks or month intervals and using nonstandard amounts of plasma or serum for RNA extraction (up to 4–5 mL) or greater amounts of RNA from PBMC or liver biopsy may be necessary to properly identify or credibly exclude virus presence.[22,23,28] Further, it has been shown that ex vivo mitogen stimulation of PBMC meaningfully augments HCV replication and, in consequence, increases HCV-RNA detection in otherwise seemingly HCV nonreactive cells.[24] This means that testing of untreated PBMC may result in underestimation of the OCI occurrence in this compartment. In fact, while HCV-RNA on average is identifiable in about 30% of persons with secondary OCI when a singular PBMC sample is tested by RT-PCR/NAH, up to 75% of the same individuals can be found HCV-RNA positive in PBMC if the cells were stimulated ex vivo before analysis.[5] In addition, since HCV may occur in different immune cell subsets in different patients,[5,25,26] testing HCV-RNA in the unfractionated PBMC population alone may lead to false negative results. Further, the accumulated evidence also indicates that the analysis of plasma or serum, PBMC, and liver tissue samples obtained at the same time point of the patient's follow-up improves detection of low-level HCV infection.

Taken together, the elements briefly outlined above together with variable sensitivities of commercial assays currently utilized in clinical laboratories and the uncertainty in the way this sensitivity is defined (i.e., 1 international unit (IU) could range from 2 vge to 7 vge depending on the assay)[29] illustrate how the task of identifying low levels of HCV—a paramount characteristic of OCI—is complex and far from uniform. Finally, low-level occult HCV persistence is not just about mere detection of HCV-RNA. Persistent expression of HCV-RNA replicative strand in liver tissue and PBMC, the display of HCV proteins in circulating immune cells, virus genome polymorphism in PBMC compared with plasma and liver, distinct cytokine expression profiles in immune cells, and the presence of infectious HCV virion-like particles have also been documented in patients with secondary OCI.[22]

Potential Clinical Relevance of Occult Hepatitis C Virus Infection

It has been well recognized that successful anti-viral therapy significantly improves clinical outcomes of CHC.[30] In the majority of patients who achieved SVR, post-treatment liver biopsies revealed an improvement in inflammation and fibrosis scores. Nevertheless, the observation is not universal as at least a subset of individuals does exhibit histological evidence of persistent hepatic alterations, including lymphocytic infiltrations, limited hepatocyte necrosis, and variable degree of fibrosis.[12,14,23] In the context of OCI, the studies showed that histological activity of the protracted liver disease was more pronounced in patients with detectable hepatic HCV-RNA, although the levels of HCV-RNA did not correlate with the extent of fibrosis.[12,14] Interestingly, similar findings have also been reported for patients with cryptogenic OCI.[15,31] In fact, histological examinations of liver biopsies from patients with an etiologically undefined liver disease revealed a higher frequency (~30%) of individuals with necroinflammatory lesions, or chronic hypertransaminasemia, having concurrent low-level HCV or hepatitis B virus (HBV) infection.[31] In similar cohorts, patients with detectable HCV-RNA

in the liver were more likely to demonstrate inflammatory lesions than those without (35% vs 14%, respectively).[15] The continuing presence of hepatic minimal to moderate inflammation accompanied by lymphocytic infiltrations in patients with OCI likely indicates an involvement of the cellular anti-viral immune responses. Indeed, in an elegant study by Hoare and colleagues,[32] individuals who were serum HCV-RNA negative by a standard laboratory test had normal levels of serum alanine aminotransferase (ALT; < 40 IU/L), and were anti-HCV reactive, were found to have hepatic fibrosis with inflammatory infiltrates enriched with CD4+ and CD8+ T cells, similar to what was observed in their viremic counterparts. Both groups were followed up for a minimum of 5 years (range, 5–12 years). Considering the apparent link between OCI and persistent subclinical hepatic alterations, an implication from this study is that HCV may have persisted and could likely be detected if a more thorough analysis using more sensitive assays was applied.

The possibility of OCI contributing to disease reactivation in situations where the immune system is compromised due to a coexisting disease or suppressive treatment needs further research. On the one hand, there have been data linking viral relapse after SVR (or spontaneous resolution) to immune suppression, suggesting that clinical resolution may not lead to a full restoration of anti-viral immunity and that a complete elimination of virus would seem unlikely.[33–37] On the other hand, recent findings from a study on patients under immunosuppressive therapy for oncohematological diseases reported no evidence of HCV-RNA in serum or in PBMC.[27]

There are clinical and molecular data suggesting that HCC may develop in patients following therapy-induced resolution of CHC. The studies of large groups of patients, who achieved SVR and became HCV-RNA nonreactive by standard clinical assays, reported development of HCC in 2–3.5% of the patients.[38–41] This percentage is comparable with that estimated for patients with CHC who developed cirrhosis.[42] While it is likely that chronic liver injury induced by HCV infection can initiate the oncogenic process, the role of low-level HCV replication and potential liver injury progressing during OCI in the carcinogenic transformation was not investigated. Despite the sparse data accumulated so far, it would be prudent to consider monitoring for potential HCC development in patients with a past history of HCV infection, even when virus elimination has been apparently complete and long-lasting.

The need of anti-viral treatment of patients with OCI is yet to be established. Nevertheless, very limited data suggest that it might be of benefit.[43,44] In the case of cryptogenic OCI accompanied by persistently elevated serum ALT, it has been reported that treatment of 10 such patients with IFN/RBV for 6 months resulted in normalization of ALT levels and clearance of HCV-RNA from PBMC in 8 of them.[44] However, only 3 patients remained HCV-RNA nonreactive in PBMC and displayed normal ALT levels after the 6-month observation period thereafter. Interestingly, liver biopsies performed after IFN/RBV therapy showed a significant decrease in the amount of intrahepatic HCV-RNA in 5 of the patients, but none of them cleared virus at this location. Liver necroinflammation and fibrosis scores had decreased in three of them comparing with those assigned prior to therapy. The issue to treat individuals with OCI or not deserves further investigations. At this stage, it appears that the treatment might be considered on an individual basis in patients with a more apparent liver disease.

The occurrence of OCI following resolution of clinically evident hepatitis C appears to be a common consequence and an unwavering element of the natural history of HCV infection. However, it cannot be excluded that it might also be a consequence of asymptomatic exposure to the virus. This infection shares many characteristics with occult HBV infection, which has been identified and extensively investigated in recent years,[45] despite the very different molecular characteristics of the viruses. Certainly, further work is required to fully recognize the nature, as well as pathogenic and epidemiological consequences of this form of HCV infection and a need for its treatment.

CONFLICTS OF INTEREST

All authors have none to declare.

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