

New perspectives in occult hepatitis C virus infection

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Abstract

Occult hepatitis C virus (HCV) infection, defined as the presence of HCV RNA in liver and in peripheral blood mononuclear cells (PBMCs) in the absence of detectable viral RNA in serum by standard assays, can be found in anti-HCV positive patients with normal serum levels of liver enzymes and in anti-HCV negative patients with persistently elevated liver enzymes of unknown etiology. Occult HCV infection is distributed worldwide and all HCV genotypes seem to be involved in this infection. Occult hepatitis C has been found not only in anti-HCV positive subjects with normal values of liver enzymes or in chronic hepatitis of unknown origin but also in several groups at risk for HCV infection such as hemodialysis patients or family members of patients with occult HCV. This occult infection has been reported also in healthy populations without evidence of liver disease. Occult HCV infection seems to be less aggressive than chronic hepatitis C although patients affected by occult HCV may develop liver cirrhosis and even hepatocellular carcinoma. Thus, anti-HCV negative patients with occult HCV may benefit from antiviral therapy with pegylated-interferon plus ribavirin. The persistence of very low levels of HCV RNA in serum and in PBMCs, along with the maintenance of specific T-cell responses against

HCV-antigens observed during a long-term follow-up of patients with occult hepatitis C, indicate that occult HCV is a persistent infection that is not spontaneously eradicated. This is an updated report on diagnosis, epidemiology and clinical implications of occult HCV with special emphasis on anti-HCV negative cases.

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Key words: Occult hepatitis C virus; Hepatitis C virus RNA; Liver; Peripheral blood mononuclear cells; T-cell response

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INTRODUCTION

The hepatitis C virus (HCV), an enveloped single-stranded RNA virus, was identified in 1989 and it was classified within the Flaviviridae family as a separate genus (Hepacivirus)^[1]. The virus replicates by the synthesis of the complementary RNA strand (the so-called negative or antigenomic strand)^[2]. So far, six major genotypes (HCV-1 to HCV-6) have been described, each containing multiple subtypes^[3], with significant differences in their global distribution and prevalence^[4]. It is estimated that about 170 million people, 3% of the world's population, are infected with HCV^[5] and it is a leading cause of chronic liver disease worldwide including cirrhosis and hepatocellular carcinoma^[6,7]. The diagnosis of HCV infection is made by the detection of antibodies against HCV (anti-HCV) and/or by detecting the presence of the HCV RNA in serum^[7,8].

However, a new entity of HCV infection was first described in 2004 in patients with persistently elevated liver function tests and who were anti-HCV and serum HCV RNA negative^[9]. Despite the absence of conventional HCV markers, 57% of these patients had HCV RNA in the liver and so this clinical situation was termed “occult HCV infection”. Moreover, it was proven that the antigenomic HCV RNA strand could be detected also in the hepatocytes of a high proportion of those patients with occult HCV infection, this indicating an active viral replication. Occult HCV infection has also been described in two other different clinical settings. One of these is in anti-HCV positive, serum HCV-RNA negative subjects with persistent normal values of liver enzymes (asymptomatic HCV carriers), of whom nearly 90% have detectable viral RNA in liver and in peripheral blood mononuclear cells (PBMCs)^[10-12]. The second one is in anti-HCV positive individuals who resolved HCV infection either spontaneously or after antiviral treatment^[13-16]. In these patients, HCV RNA is detected in liver and in PBMCs years after apparent recovery from the disease (normalization of liver enzyme values and loss of serum HCV RNA). This occult HCV infection is related to the persistence of necroinflammation activity in the liver of the sustained responders. Thus, there are two types of occult HCV infection: one can be found among anti-HCV seropositive individuals with normal values of liver enzymes and the other is found among anti-HCV seronegative patients with abnormal levels of liver enzymes. The present review focuses on the latest studies of occult HCV infection performed in anti-HCV negative and serum HCV RNA negative patients.

IDENTIFICATION OF OCCULT HCV INFECTION

Occult HCV infection was first identified in liver of anti-HCV and serum HCV RNA negative patients with abnormal liver function tests and it was also found that viral RNA could be present in the PBMCs of nearly 70% of these patients^[9]. Furthermore, it was demonstrated that occult HCV replicates in these cells^[17]. By detecting HCV RNA in liver biopsies or in PBMCs, other groups in Japan, Italy, Egypt, Colombia, Pakistan and Iran^[18-23] have confirmed the existence of occult HCV infection in patients with elevated liver enzymes and without conventional HCV markers (Table 1). Occult HCV infection has also been found in hemodialysis patients who were persistently anti-HCV and serum HCV RNA negative but with abnormal values of liver enzymes^[24], in the family setting of patients with occult hepatitis C^[25] and even in healthy subjects with normal alanine aminotransferase (ALT) levels and no clinical evidence of liver disease^[26].

Since HCV was replicating in the liver and PBMCs of patients with occult HCV infection, it was speculated that it should exist as circulating viral particles but at such low levels that the virions could not be detected even using the most sensitive reverse-transcription polymerase chain

reaction (RT-PCR) techniques. This hypothesis was tested by concentrating HCV virions by ultracentrifugation of 2 mL of serum from patients with occult HCV prior to HCV RNA detection by RT-PCR^[27]. In this way, serum viral RNA was found in nearly 60% of the patients. In addition, it was found that the density of the viral particles isolated from patients with occult HCV infection was similar to the highly-infectious lipoviral particles present in the serum of patients with classical chronic hepatitis C^[27,28], suggesting that serum from patients with occult HCV is potentially infectious.

HCV-SPECIFIC T-CELL RESPONSES AND THE OCCULT INFECTION

Functional virus-specific memory CD4⁺ and CD8⁺ T-cells have been documented in the circulation of patients with HCV RNA persistence in the liver and so assaying cellular immunity has been proposed as a surrogate marker of occult HCV infection^[29,30]. To test HCV-specific T-cell responses, PBMCs isolated from fresh heparinized venous blood by gradient centrifugation are washed twice in phosphate-buffered saline and resuspended in RPMI-1640 medium, supplemented with 10% heat-inactivated fetal bovine serum, 20 mmol/L HEPES, 2 mmol/L glutamine and antibiotics. PBMCs are cultured in triplicate (1.0×10^5 viable cells/100 μ L) in flat-bottomed 96-well culture plates at 37 °C, 5% CO₂ and humidity in the presence or absence of 1 μ g/mL HCV proteins core, NS3 and NS4; *Staphylococcus aureus* enterotoxin B (10 μ g/mL) is used as positive control. On day 6, cultures are pulsed with 1 μ Ci/well of 3H-thymidine for 16 h and then harvested and transferred to filters and the incorporated radioactivity measured^[31].

T-cell responses found in occult HCV infection are similar to those described in anti-HCV-positive patients following spontaneous or treatment-induced recovery^[32-34]. HCV-specific T-cell responses have been detected often among occult HCV-infected hemodialysis patients^[35], family members of patients with occult or overt HCV infection^[36] and among HCV-seronegative sexual partners of patients with chronic hepatitis C (Aguilar-Reina J, personal communication), supporting exposure to trace amounts of HCV RNA. The maintenance of such immune responses may require only a low level productive infection. In fact, sporadic reappearance of minute amounts of HCV RNA stimulates cellular immunity^[37]. However, persistence of occult HCV in face of adaptive cellular responses indicates that the latter do not ultimately result in sterilising immunity. The actual impact on the natural history of the occult infection is still a matter of debate. Indeed, HCV-specific T-cell responses have been described in apparently healthy persons, as well as in those who likely have resolved the infection or who have been exposed to, but who apparently did not become infected by, HCV^[30]. To summarize, T-cell responses are more frequent and stronger compared with chronic hepatitis C patients^[29], contributing to control the

Table 1 Prevalence and hepatitis C virus genotype distribution of occult hepatitis C virus infection

Type	Country	Prevalence ¹	Genotype	Cohort/setting	Ref.
Anti-HCV negative	Spain	57/100 (57%)	1b	Cryptogenic chronic hepatitis	[9]
	Japan	Not applicable	Not reported	Cryptogenic cirrhosis with HCC	[18]
	Italy	2/5 (40%)	Not reported	Cryptogenic cirrhosis with HCC	[19]
	Egypt	4/40 (10%)	Not reported	Cryptogenic chronic hepatitis	[20]
	Colombia	Not applicable	1a	Liver retransplantation	[21]
	Pakistan	23/31 (74%)	1a, 3a, 3b	Cryptogenic chronic hepatitis	[22]
	Iran	7/69 (10%)	1a, 1b, 3a	Cryptogenic chronic hepatitis	[23]
	Italy	9/276 (3.3%)	1a, 1b, 2a	General population without liver disease	[26]
	Anti-HCV positive	United States/Poland	11/11 (100%) ²	1a, 1b	Asymptomatic anti-HCV carrier
Cuba/Mexico		17/18 (94%)	Not reported	Asymptomatic HCV carrier and therapy response	[11]
Spain		10/12 (83%)	1b	Asymptomatic anti-HCV carrier	[12]
Canada		16/16 (100%) ²	1a, 1b, 2a	Spontaneous recovery and therapy response	[13]
United States/Poland		15/17 (88%) ²	1a, 1b, 2a, 3a	Therapy response	[14]
Spain		19/20 (95%)	1b, 2, 3	Therapy response	[15]
Canada		24/24 (100%) ²	1, 1a, 3a	Therapy response	[16]
Egypt		7/62 (11%)	Not reported	Therapy response	[20]

¹Hepatitis C virus (HCV) RNA detection in liver and/or peripheral blood mononuclear cells; ²Including HCV RNA detection in serum with nested reverse-transcription polymerase chain reaction-nucleic acid hybridization assay. HCC: Hepatocellular carcinoma.

extent of the infection and thus prevent HCV RNA detection in serum.

HUMORAL IMMUNITY TO HCV DURING THE OCCULT INFECTION

It is unknown how HCV persists in persons who remain anti-HCV non-reactive by currently available antibody screening tests^[38]. Antibodies to HCV proteins usually develop within 4–12 wk following exposure to the virus, those directed to the core and non-structural-3 region being the earliest and more frequently detected. Anti-HCV continues to be detectable throughout the duration of the infection although antibody reactivity declines over time after apparent clinical recovery of chronic hepatitis C^[39–41]. In immunocompetent individuals, the etiology of the primary occult HCV infection is most likely explained by the sporadic exposure to low infective virus doses resulting in a latent seronegative infection. Thus, anti-HCV reactivity remains undetectable due to prolonged very low antibody titres^[38], excluding persons with immunodeficiencies, immunosuppressed or suffering from a concomitant chronic infection.

Isolated reactivity to single proteins or peptides has been reported on supplemental anti-HCV assays in blood donors in samples which are either HCV RNA-positive or -negative^[42,43]. Such pattern of anti-HCV indeterminate results resembles the profile of antibody reactivity documented in some international seroconversion panels when tested on supplemental anti-HCV assays. These panels are composed of sequential samples from a single-source donor obtained throughout the antibody development which frequently show single-antigen reactivity at the initial stages of anti-HCV seroconversion. In addition, reactivity recorded as “faint band(s)” has been shown among at risk persons but

seronegative by screening anti-HCV tests^[44]. At this point the criteria recommended by the supplier of the supplemental assay to validate the testing as reactive or anti-HCV-positive *vs* indeterminate result should be discussed. But this issue would require a thorough comparison of the HCV antigens employed by the licensed tests and the interpretation of their reactivity in particular populations, which is out of the scope of this review and deserves future investigation.

The majority of persons exposed to HCV who become infected and seroconvert to anti-HCV remain asymptomatic. Up to 80% of seropositive infections are not diagnosed because persons belong to low, or supposedly null, risk groups. Screening programs in the general population would promote awareness and prevention of HCV spread because seropositive persons may be identified and offered appropriate counselling. However, this strategy still will not identify the seronegative infections using the current screening anti-HCV tests, as evidenced by the existence of the primary occult HCV infection.

In an attempt to overcome this, an anti-HCV assay based on a well-conserved core-derived epitope has been reported recently^[45]. Briefly, wells of a microtitre plate are coated overnight with HCV-core 5-19-peptide. Wells are washed and non-specific sites are blocked with phosphate buffer saline containing Tween-20 plus heat-inactivated fetal bovine serum. Diluted serum samples are added to the HCV-core coated wells and after incubation for 1 h, wells are washed five times and incubated with horseradish peroxidase-conjugated rabbit polyclonal anti-human IgG for 1 h. After five washings wells are reacted in the dark with 2, 20-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]-diammonium salt. Absorbance is measured at 405 nm with a reference at 620 nm. In contrast to the NS3 sequence which shows considerable inter-genotypic heterogeneity^[46], the core sequence is largely conserved

among genotypes 1 through 6^[47]. Antibody to HCV core was tested in a cohort of 145 anti-HCV screening-negative patients with occult HCV infection of whom 40% were found to be anti-HCV core-positive, including 10% of individuals who were antibody non-reactive at the time of the first sample testing. Also, the anti-HCV core was detected in 99% of chronic hepatitis C patients but in none of the patients with HCV-unrelated liver disease. Thus, anti-HCV core testing allowed serological identification of up to 40% of the anti-HCV screening-negative infections on repeated testing^[45]. The finding that a number of patients with occult infection who were initially nonreactive for anti-HCV core antibodies became positive upon subsequent testing underscores the necessity of screening serial samples as proposed by other authors^[32,48].

In addition, the anti-HCV core assay has been able to track HCV exposure among relatives of patients with occult HCV. Intrafamilial spread of occult HCV infection seems to occur as often as that of chronic hepatitis C^[25]. So, anti-HCV core was detected in 23% anti-HCV-screening-negative relatives of patients with occult HCV infection. Thus, antibody testing to HCV core detected frequent exposure to and possible transmission of HCV among family members of HCV-infected patients compared with screening anti-HCV tests^[56]. On the other hand, because patients undergoing hemodialysis are at risk of occult HCV infection^[24,35], testing for anti-HCV core has been evaluated in repeatedly anti-HCV screening-negative and serum HCV RNA-negative hemodialysis patients with abnormal liver enzymes. Anti-HCV core antibodies were detectable in 34% hemodialysis patients who have been exposed to HCV and who might have developed occult HCV infection (unpublished results).

Therefore, anti-HCV fails to be detected by screening tests available in some populations of at-risk patients^[49], including those individuals multi-exposed such as intravenous drug users or prison inmates^[44,50-52]. The ultimate utility of the anti-HCV core-based antibody assay in those cases and other settings such as blood donors warrants further investigation.

MAY OCCULT HCV INFECTION BE DIAGNOSED WITHOUT A LIVER BIOPSY?

Detection of HCV RNA in the liver biopsy is the gold-standard method for the diagnosis of an occult HCV infection. However, as commented before, viral RNA is detectable in the PBMCs and in ultracentrifuged serum of patients with occult HCV^[9,27] and anti-core HCV tested by a non-commercial enzyme-linked immunosorbent assay (ELISA) is also found in a substantial proportion of these patients^[45]. Therefore, in a recent report it was determined whether all cases of occult HCV infection could be diagnosed without performing a liver biopsy by combining these methods^[53]. A total of 122 patients, who were diagnosed of an occult HCV infection by the presence of viral RNA in a liver biopsy and with avail-

able serum samples and PBMCs were included in the study. Anti-core HCV (tested with the non-commercial ELISA) was found positive in 44/122 (36%) of the patients. After ultracentrifugation of serum samples, HCV RNA was found in 70/122 (57%) of the patients, while 74/122 (61%) had viral RNA in PBMCs. When combining the detection of anti-core HCV and the detection of HCV RNA in ultracentrifuged serum and in PBMCs, 91% of the patients (111/122) were positive for at least one of these markers. So, in the light of these results, occult HCV infection may be properly diagnosed in up to 91% of the patients without the need to perform a liver biopsy by testing for anti-core HCV and for HCV RNA in ultracentrifuged serum and in PBMCs.

In summary, when occult HCV infection is suspected and a liver biopsy is not available for HCV RNA detection, the diagnosis can be made by testing, with a highly sensitive real-time PCR technique, for the presence of viral RNA in PBMCs (that identifies between 60%-70% of the cases)^[9,53] or in ultracentrifuged serum (that allows identification of occult HCV in around 60% of the patients)^[27,53]. The combination of these two approaches along with the detection of anti-core HCV improves the diagnosis of occult HCV infection in more than 90% of the cases. Nevertheless, in order to increase the percentage of patients diagnosed of occult HCV infection with non-invasive methods, more studies should be done in the future to improve the sensitivity of the above mentioned techniques.

CHARACTERISTICS OF OCCULT HCV INFECTION AND RESPONSE TO ANTIVIRAL TREATMENT

Clinical characteristics of patients with occult HCV infection have been compared to those of patients with chronic hepatitis C matched with respect to age, gender and known duration of the disease^[54]. In the study it was found that patients with occult HCV presented significantly lower values of iron, alanine aminotransferase, γ -glutamyl transpeptidase and α -fetoprotein whereas triglycerides and cholesterol levels were significantly higher than those of patients with chronic hepatitis C. In the liver biopsies, patients with chronic hepatitis C frequently had more necroinflammation activity (96%) and fibrosis (75%) than patients with occult HCV infection (31% and 15%, respectively), but liver cirrhosis was diagnosed with a similar frequency in both groups (4.4% in occult HCV vs 7.2% in chronic hepatitis C). Although cholesterol and triglyceride levels were significantly higher in patients with occult HCV infection than in patients with chronic hepatitis C, the percentage of cases with liver steatosis did not differ significantly between these two groups, suggesting that dyslipidemic disorders did not play a predominant role in the development of steatosis in patients with occult HCV. So, occult HCV infection seems to be a milder form of the disease caused by HCV with less liver damage. However, it is important to point out that

occult HCV may lead to liver cirrhosis and therefore to the development of hepatocellular carcinoma. Regarding this issue, the presence of viral RNA in the tumour and non-tumour tissue of anti-HCV and serum HCV RNA negative patients with liver cancer has been reported^[18,19]. Nevertheless, the number of patients analyzed in these reports was low and further studies are needed to ascertain the role of occult HCV in causing hepatocellular carcinoma.

Taking into account that patients with occult HCV present with abnormal liver function tests and may have histological damage, a study was conducted to determine whether these patients could respond to antiviral treatment with pegylated-interferon (PEG-IFN) plus ribavirin^[55]. A total of 10 patients with occult HCV genotype 1b infection (anti-HCV and serum HCV-RNA negative but HCV RNA positive in liver) who were HCV RNA positive in PBMCs, had abnormal values of alanine aminotransferase for at least 12 mo and had necroinflammatory activity in a liver biopsy performed within one year before the study entry, were treated with standard doses of PEG-IFN plus ribavirin for 24 wk. The patients received a 24-wk treatment course instead of the recommended 48-wk course for HCV genotype 1 because they were serum HCV RNA negative^[56-58]. After treatment, patients were followed for 24 wk. At the end of therapy, 80% of the patients had normalized ALT values and were HCV RNA negative in PBMCs, but at the end of the post-treatment follow-up, only 3 cases remained with normal ALT values and without HCV RNA in PBMCs (complete responders). Five of the patients (2 of them with a complete response) underwent a second liver biopsy at the end of the follow-up period. Necroinflammatory activity and fibrosis scores had decreased in the post-treatment liver biopsy of 3 patients, while scores in the other 2 cases remained unchanged. Viral RNA persisted in the liver of the 5 patients but HCV RNA load was significantly lower in the post-treatment biopsy than in the basal one. Thus, treatment with PEG-IFN plus ribavirin may be beneficial in patients with occult HCV because intrahepatic HCV RNA load decreases and histological liver damage may improve but, as it has been described for chronic hepatitis C^[13-16], occult HCV infection is not eradicated.

In conclusion, although occult HCV infection appears to be milder than “classical” chronic hepatitis C, liver fibrosis is present in up to 5% of the patients^[4,42]. In addition, necroinflammatory activity is detected in the liver of nearly 35% of the cases^[9,54]. This suggests that occult HCV infection may progress to a more serious chronic liver injury. Supporting this notion is the fact that occult HCV infection has been identified in patients with liver cirrhosis and even in hepatocellular carcinoma^[18,19]. Thus, the treatment of patients with occult HCV infection with PEG-IFN plus ribavirin is a reasonable option, as it is proven for chronic hepatitis C^[59] because the histological liver damage may improve with treatment. However, the infection is not completely eradicated, as described for patients with chronic hepatitis C who respond to antiviral

treatment^[13-16].

IS OCCULT HCV A TRANSIENT OR A PERSISTENT INFECTION?

In order to determine whether occult HCV infection remains over time, we have performed a study including 37 patients who were anti-HCV and serum HCV RNA negative but with viral RNA in the liver biopsy^[60]. Patients were followed for a mean time of 55 mo and serum and PBMCs samples were collected periodically for HCV RNA testing. Evidence of viral persistence in patients with occult HCV infection was found by the detection (over the observational period) of intermittent or persistent HCV RNA positivity in the ultracentrifuged serum or in PBMCs in all but one patient. These results suggest that anti-HCV negative occult HCV is a permanent infection as it has been reported in anti-HCV positive patients who resolved HCV infection^[16,61]. Nevertheless, in order to extend the knowledge in the natural history and the pathogenesis of occult HCV infection, a more prolonged follow-up is needed.

GENOTYPES OF OCCULT HCV INFECTION

In the initial studies of occult HCV infection the only HCV genotype detected was 1b^[4,19,22]. This result was predictable because HCV genotype 1b is the most prevalent genotype in Spain^[62,63]. However, later studies performed worldwide (Table 1) have reported occult HCV infection belonging to HCV genotypes 1a, 2a, 3a and 3b^[21-23,26]. So, it can be assumed that occult HCV infection is a universal phenomenon and all genotypes may be involved in this infection. This hypothesis should be proven with several studies performed in countries with different prevalence of HCV genotypes.

ROLE OF OCCULT HCV INFECTION IN LIVER TRANSPLANTATION

In anti-HCV positive patients with occult HCV, the reactivation of HCV infection (with reappearance of serum HCV-RNA) is well documented in special clinical situations, such as immunocompromised patients, patients on long term chemotherapy for cancer or patients receiving immunosuppressive therapy (including those who have undergone liver, kidney or bone marrow transplant)^[64-69]. By contrast, there are no studies of the serologically silent (anti-HCV negative) occult HCV infection in these settings, except for a reported case of occult HCV infection as the cause of a liver transplantation and retransplantation^[21]. The patient was a 29-year-old man who was transplanted due to liver cirrhosis of unknown etiology. Ten months after the first liver transplant, the patient was retransplanted because of liver failure secondary to severe chronic cholestasis of unknown origin. Liver

samples of both explants were available for study. The patient has remained anti-HCV and HCV RNA negative in serum and plasma since the initial diagnosis, but viral RNA was detected in the liver tissue samples from the two explants. Furthermore, a phylogenetic analysis demonstrated that the HCV RNA isolated from the two liver samples belonged to genotype 1a. Although HCV RNA was undetectable in the PBMCs of the patient, he had an occult HCV infection and probably, the liver graft was infected by PBMCs.

This case suggests that occult HCV infection may play a role as an etiological agent of liver failure in transplanted patients. Also, this report strongly supports the need for performing studies not only in liver transplant, but also in other immunocompromised patients with unexplained elevation of liver enzymes to determine the real magnitude, clinical significance and long-term consequences of occult HCV infection.

OCCULT HCV INFECTION IN HEALTHY POPULATION WITH NORMAL LIVER ENZYMES

Although occult HCV infection was identified in patients with abnormal values of liver function tests, a recent work by De Marco *et al.*^[26] describes the existence of occult HCV infection among healthy people with normal alanine aminotransferase and normal aminotransferase values. These healthy subjects were enrolled in the frame of three different epidemiological studies: the Italy cohort series of the European Prospective Investigation into Cancer and Nutrition, the Turin Case-Control Bladder Cancer Study and the Italian project in Cervical Cancer Screening. Subjects were tested for anti-HCV and for HCV RNA in plasma and in PBMCs. All of them were anti-HCV and serum HCV RNA negative, but viral RNA was detected in the PBMCs of 9/276 (3.3%) healthy controls with normal liver enzymes. Interestingly, in the studied population, blood donors were over-sampled but the authors do not indicate if any of these blood donors had an occult HCV infection.

So, this work potentially has important implications. Thus, the frequency of occult HCV infection may be underestimated since until now this infection has been exclusively studied among patients with abnormal values of liver enzymes. In this sense, in the study of De Marco *et al.*^[26] the frequency of occult HCV infection in their health population was 3.3% *vs* the 2.7% prevalence of anti-HCV positivity detected in the general Italian population. Although the number of participants in the former study (276 subjects) should be increased, there is a potential risk for HCV spread from an occult HCV healthy population. Thus in blood donations, despite approaches to reduce the risk of leukocyte-related disease, such as leukodepletion, the efficacy in reducing the risk of transmitting viruses is still under debate^[70]. Furthermore, as HCV RNA may be detected after ultracentrifugation of serum samples in more than 50% of patients with occult HCV

infection^[27] and this test was not performed in the Italian study, it may be possible that healthy blood donors have HCV RNA in serum undetectable by conventional PCR assays. If this is the case, blood donors with occult HCV infection may potentially transmit this occult infection as it is undetectable by the current applied screening tests for HCV in blood banks. However, more studies should be performed in healthy subjects with normal liver enzymes and especially among blood donors to definitively establish the possible magnitude of this infection.

CONCLUSION

Occult HCV infection has been found in two different settings: in anti-HCV positive, serum HCV RNA negative patients with normal levels of liver enzymes and in anti-HCV negative, serum HCV RNA negative patients with abnormal liver function tests of unknown etiology. Occult HCV is distributed worldwide and all viral genotypes may be involved. Although seronegative occult HCV infection seems to be less aggressive than classical chronic hepatitis C, it has been detected in patients with liver cirrhosis and even in hepatocellular carcinoma. Occult HCV infection has been described also in a healthy population with no evidence of liver disease, indicating that this infection may be present in a wide spectrum of clinical situations. Further studies on the natural history and the clinical significance of occult HCV infection are needed to determine its global prevalence, infectivity, implication in causing extrahepatic diseases and its long-term complications in special circumstances such as immunocompromised patients.

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