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Concise Review

Hepatitis B virus and hepatitis C virus co-infection: additive players in chronic liver disease?

Giovanni Raimondo;[1] Gaia Cacciamo;[1] Carlo Saitta[1]

Abstract

Hepatitis B virus (HBV) and hepatitis C virus (HCV) share modes of transmission and their combined infection is a fairly frequent occurrence particularly in areas where the two viruses are endemic and among subjects with a high risk of parenteral infections. Moreover, the number of coinfected patients is likely higher than is usually thought. In fact, many studies have shown that HBV genomes may also be present in HBsAg-negative patients, particularly in those with HCV-related chronic hepatitis. This condition is commonly called "*occult HBV infection*". Much evidence suggests that coinfection by HBV and HCV may have considerable clinical relevance. In particular, this condition is generally believed to be a factor favouring the progression of liver fibrosis toward cirrhosis and the development of liver cancer, and in case of both overt and *occult* HBV infection. In spite of its potential clinical impact, however, there is few information about the possible interplay between the two viruses. Here, we concisely reviewed the available data on the virological and clinical features of the dual HBV/HCV infection prospecting the aspects that should be highlighted in the nearest future for improving the knowledge on this important field of the hepatology.

Key words: Hepatitis B virus, Hepatitis V virus genotypes, Hepatitis C virus, Hepadnavirus, Interferon.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for most of the cases of liver disease worldwide. Both represent viral pandemics, and the World Health Organization estimates that more than 350 million and 170 million people are chronic carriers of HBV and HCV, respectively. They share modes of transmission and their combined infection is a fairly frequent occurrence particularly in areas where the two viruses are endemic and among subjects with a high risk of parenteral infections.[1-5] Moreover, the number of co-infected patients is likely higher than is usually thought. In fact, the availability of highly sensitive molecular biology techniques has allowed the identification of HBV infection also in HBV surface antigen (HBsAg) negative individuals, particularly in those with HCV-related chronic hepatitis (6-8 and reviewed in 9-13). This condition is commonly called "occult HBV infection".

Much evidence suggests that co-infection by HBV and HCV may have considerable clinical relevance, but the information concerning many aspects of such dual infection are at present largely incomplete. In particular, very few studies have been reported so far on the acute hepatitis occurring in case of combined HBV and HCV infection, although all of them agree with the occurrence of a considerable reciprocal influence between the two viruses in such cases.[14,15]

In any case, the subject of this review will be strictly focused on chronic HCV-related liver diseases with concurrent overt (HBsAg positive) or occult (HBsAg negative) HBV co-infection also excluding the pictures of hepatitis Delta virus and/or immuno-deficiency virus additional infection(s).

We will begin the review with very concise – though necessary –information on the biology of the two viruses.

HBV is a DNA virus belonging to the Hepadnavirus family. The viral DNA is a closed, circular, partially double stranded molecule of 3.2 kilobases [relaxed circular DNA (rcDNA)], and contains four partially overlapping open reading frames: the S gene, coding for the envelope proteins; the Core gene, coding for the core and "e" proteins; the P gene, coding for a protein with multiple functions, including reverse transcriptase and DNA polymerase activities; the X gene, coding for the "X" protein of yet not well defined functions, but with transcriptional transactivating properties and a likely important role in the viral replication.[16] Once it has penetrated into the hepatocyte, viral core is transported to the nucleus and the rcDNA is converted into a circular, covalently closed, fully double stranded supercoiled DNA

[1] Unit of Clinical and Molecular Hepatology. Department of Internal Medicine. University of Messina – ITALY.

Address for correspondence:
Prof Giovanni Raimondo
Unità Operativa di Epatologia Clinica e Biomolecolare
Dipartimento di Medicina Interna
Policlinico Universitario. Via Consolare Valeria, 1. 98124
Messina. ITALY

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(cccDNA). This is the template for the production of virus mRNAs including a RNA pregenome that is reverse transcribed in the cytoplasm of the hepatocytes for the synthesis of the DNA molecules.[16] The HBV cccDNA can persist throughout the natural life of the hepatic cell, providing a continuous source of infective virions that spread to new hepatocytes maintaining a long-term (life-long?) persistence of the virus in the liver of infected individuals.[17-19] Viral DNA can be directly integrated into the host DNA, and thus it becomes a predisposing factor or a trigger for the development of HCC. Moreover, HBV may exert its pro-oncogenic role also through the production of proteins, such as X and truncated preS-S proteins, which have potential transforming properties and by the chronic necroinflammation and cirrhosis that its infection may induce.[20,21] In this context, it has to be stressed that HBV is classified as a Group 1 human carcinogen, and it is considered the second most important oncogenic agent after smoking tobacco.[22,23]

Eight HBV genotypes, from A to H, have been classified on the basis of a genetic variability of 5 to 10% on the overall genome. They are equally dominant genetic variants, with a different geographic distribution: genotype D is prevalent in the Mediterranean basin, B and C are most frequent in Asia and genotypes F and H in South America.[24-26] The genetic variability of HBV also concerns the natural emergence of variant viral strains due to mutations that may occur in all portions of the viral genome and may have relevant biological and clinical impact.[16] The most common of these variants carries a stop codon in the precore genomic region that enables the synthesis of the viral "e" antigen (HBeAg).[27,28] Other frequently observed genetic variants are the preS2-defective HBV (due to a start codon mutation or a deletion at level of the preS2 genomic region) and those with mutations in the virus core-promoter that is located in the X gene.[16,29-33] Rearrangements in the HBV genome interfering with the gene expression or leading to the production of an antigenically modified HBsAg - that cannot be detected by the commercially available assays – have also been implied in cases of occult HBV infection.[34-36] However, much evidence indicates that viral genomic heterogeneity accounts for the minority of these cases since the occult HBV status is mostly a consequence of a strong suppression of the viral replication that is due to not yet defined mechanisms.[37-43]

HBV mutants may also be induced by therapeutic treatments. In fact, both HBV vaccines and high doses of anti-HBV immunoglobulins may induce the emergence of variants that are not recognized by the anti-HBs neutralizing antibodies because of mutation(s) in the "a" determinant of the HBsAg, and these variants may infect individuals despite proper HBV immunoprophylaxis.[44-46] Other very relevant HBV variants are those selected under antiviral treatment with nucleos(t)ide analogues, such as lamivudine or adefovir dipivoxil. These variants carry mutations at the polymerase gene level and once emerged induce resistance to the therapy.[47-53]

HCV is an enveloped, single-stranded, positive-sense RNA virus, with a genome of approximately 9,600 nucleotides.[54,55] It has been classified as a separate genus in the Flaviviridae family, other members of which include the viruses that cause yellow-fever and dengue.[56] Its genome consists of 5' and 3' non coding regions and a single open reading frame that encodes a single viral polyprotein of 3010-3033 amino acids.[55-57] The viral polyprotein undergoes post-translational cleavages to form functional viral proteins, both structural (core and envelope proteins) as well as non-structural (designated NS2 through NS5), which produce the enzymes required for viral growth and replication.[58] HCV has considerable sequence heterogeneity and is classified into six major genotypes with a fairly distinct geographic distribution. Because of its rapid degree of replication and the fact that the RNA-dependent RNA polymerase does not accurately reproduce the viral genome, HCV spontaneously mutates within a given infected individual, resulting in related but distinct *"quasispecies"*.[56,57] These closely related variants have genome sequences that are subtly different.[59] The generation of these mutants appears to be one of the key mechanisms by which HCV establishes and maintains persistent infection.[60] Very importantly, the high degree of variability of the envelope proteins is responsible for the non-availability of an anti-HCV vaccine.

HCV infection occurs with the highest prevalence among individuals with large or repeated percutaneous exposure to infected blood (HCV infection is founded in 55-80% of patients with thalassemia and over 90% of those with hemophilia).[61] Most of the newly infected subjects develop chronic hepatitis C (CHC), the most important clinical consequence of which is the progressive liver fibrosis leading to cirrhosis. Most studies report that cirrhosis occurs in less than 50% of patients with CHC infection over a period of 20-30 years.[62-64] Once cirrhosis is established, however, the incidence of HCC may be as high as 7% per year.[65,66] CHC appears to be responsible for the majority of cases of cirrhosis and, consequently, for HCC in the western countries.[21]

Chronic HCV infection is also associated with several extrahepatic manifestations, usually immune-mediated, that may include cryoglobulinemia, porphyria cutanea tarda, membrano-proliferative glomerulonephritis, polyarteritis nodosa vasculitis, B-cell or non-Hodgkin's lymphoma, idiopathic pulmonary fibrosis, autoimmune thyroiditis, lichen planus, sialadenitis, corneal ulcer, sicca syndrome and Raynaud's phenomenon.[67-69]

Chronic HCV and overt HBV co-infection

The classical form of HBV/HCV co-infection – identified by the contemporary positivity in the serum of the HBsAg and the anti-HCV antibodies – may occur in individuals from everywhere in the world and represents a sizable proportion of chronic hepatitis patients in many

geographic areas.[1,3,4,70-73] Almost uniformly, the literature in the field indicates the great clinical importance of this condition. In particular, this co-infection is generally considered a factor favouring the progression of the liver fibrosis and the establishment of cirrhosis (74-76, and reviewed in 77). Even more evident is the synergism of the two infections in the development of liver cancer,[78-81] and it has been shown that HBsAg/HCV positive cirrhotics have a significantly higher risk of developing HCC when compared with individuals infected either by HCV alone or HBV alone.[82,83]

In spite of its clinical importance, very little information is at present available about the therapeutic treatment of the HBV/HCV co-infected population.[84] This lack is mainly due to the fact that most studies on the natural and post-therapeutic course of chronic hepatitis C foresee, among the main exclusion criteria, the coexisting presence of HBsAg. Vice versa, anti-HCV positive subjects are excluded from the studies on HBV-related liver disease. Nevertheless, albeit numerically scarce, the few available studies on the treatment of chronic HBV/HCV hepatitis agree in considering this form of chronic viral hepatitis particularly difficult to cure.[85-87] In particular, patients with chronic hepatitis C and HBsAg positivity appear to have low possibilities of responding to standard dosage of Interferon-α (IFN-α) therapy,[85-87] whereas no data are at present available concerning treatment with PEG-IFNα and Ribavirin. Moreover, there is evidence that in some individuals an apparently suppressed HBV infection may reactivate during the phase of inhibition of the HCV replication induced by IFN therapy.[88,89] In this context, it is worth focusing attention on the several reports suggesting that HBV and HCV interact in the case of concurrent infection (reviewed in 4,75). In particular, a certain number of clinical observations indicate that most co-infected cases have detectable levels of HCV viremia and very low values of serum HBV-DNA,[2,90-93] suggesting that the interference between the two viruses is more frequently characterized by an inhibition of HBV exerted by HCV. This hypothesis would seem to be confirmed by two orders of consideration: (i) as mentioned above, the HBV is strongly suppressed in case of occult infection which is highly prevalent in HCV infected patients; (ii) *"in vitro"* studies indicate that HCV is capable of suppressing the HBV activity and this inhibitory effect is essentially mediated by the HCV core protein.[94-97] However, it has to be considered that the HCV core region is a highly conserved portion of the viral genome in all cases of HCV infection, regardless of the contemporary presence and the replicative levels of the HBV.[72] Moreover, clinical studies do not uniformly report a dominant role of the HCV in cases of combined infection, and some of them suggest a reciprocal interference or even a dominant effect of HBV.[1,3,5,98,99] Actually, patients with combined infection may show a large spectrum of virological profiles. In fact, although many cases appear to have active HCV and inactive HBV replication, some patients have high HBV viremia levels and undetectable HCV RNA; analogously, the replication activity of both viruses appears to be enhanced in some cases and suppressed in others.[4,72] In this context, one cannot exclude that in dual HBV/HCV infection one or both viruses may have alternated phases of active or suppressed replication. At present, the main limitation in this field is that all the available data come from cross-sectional studies and, consequently, we do not know whether the activity revealed for each of the two viruses in individual patients is the expression of a long-lasting state or is only a temporary effect as part of complex kinetics evolving over years of chronic infection. This point has a great relevance in terms of both correct diagnosis and therapeutic approaches in cases of HBV and HCV co-infection. In this context, one should consider that the classic anti-HBe positive chronic hepatitis B is often characterized by phases of low levels of HBV replication interspersed with episodes of viral reactivation,[100-103] and many HBsAg/anti-HCV cases are anti-HBe positive, particularly in the Mediterranean basin. In analogy, the HCV may show alternating phases of active and suppressed replication also in cases of single infection.[104-106] Consequently, one cannot exclude that at least in some HBV/HCV coinfected cases the behaviour of each virus is independent of the contemporary presence of the other. This hypothesis seems to be confirmed by a very recent Italian multicentre study that longitudinally examined the largest series of HBV/HCV coinfected patients analysed so far and showed that the virological patterns in these cases may be widely divergent and have dynamic profiles over time.[107] Thus, at least in some double infected cases each virus might exert its own pathogenetic role, causing a cumulative effect in terms of liver injury that may contribute to explain the high grade of disease severity frequently observed in case of co-infection. This hypothesis is in agreement with our previous data showing the contemporary presence of the typical histological patterns of each individual infection in cases of HBV and HCV coexistence.[108]

In any case, the most urgent need for this category of patients is to perform clinical and therapeutical trials including a numerically adequate number of patients who must be very carefully categorized on the basis of their virological profile.

Chronic HCV and occult HBV co-infection

HBV carriers of hepatitis B virus are traditionally identified by detection of the HBsAg in their blood. However, the advent of highly sensitive molecular techniques has allowed the definitive demonstration that HBV genomes may persist in the liver and, less frequently, in the serum of HBsAg-negative individuals with or without serological markers of previous infection [anti-HBs and antibody to hepatitis B core antigen (anti-HBc)] (6-11, re-

viewed in 12). This so-called occult or cryptic HBV infection represents an intense matter of debate from several biological and clinical points of view. Since this review is exclusively devoted to the HBV/HCV co-infection, we will discuss only the possible impact that such peculiar type of masked infection might exert on HCV positive patients.

Occult HBV infection occurs frequently in HCV patients, with the highest prevalence reported in Asian populations (reviewed in 12). About one third of the Italian patients with chronic hepatitis C carry such cryptic infection[8,17] with no difference in prevalence in respect to the gender, HCV genotype and HCV viremia levels.[8]

We previously showed that occult HBV infection is significantly associated with cirrhosis in HCV-infected individuals, and this observation has been confirmed by several studies from different geographic areas (reviewed in 12). These data obviously suggest that such cryptic infection may produce or contribute to liver damage through mechanisms that, however, are at present unknown. Trying to find an explanation to this critical aspect, we should consider that occult HBV may persist as episomal free genomes into liver of infected patients,[17,109-111] and we recently showed that these viruses maintain low levels of transcription and replication, as indicated by the intrahepatic persistence of HBV cccDNA and viral mRNAs.[17] Moreover, patients who have apparently resolved acute hepatitis B continue to carry in the liver episomal viral DNA even after surface antigen seroclearance, and this event is associated with mild hepatic inflammation lasting for decades.[112,113] The same results was obtained in a study on woodchucks convalescent from acute hepatitis due to the infection by the corresponding hepadnavirus (woodchucks hepatitis virus, WHV): these animals show the lifelong persistence of small amounts of replicating virus that induces a mild liver necroinflammation continuing for life.[114] These data tempt us to speculate that occult HBV infection might frequently reactivate. Such reactivations are usually suppressed by the HBV-specific memory T-cell response and may induce only very mild liver damage. However, in the event of the contemporary presence of other causes of liver injury, such as HCV infection, the minimal lesions produced by the immune response to HBV antigens might contribute in making the course of the liver disease worse over time.

The observation that occult HBV is associated with advanced chronic liver disease is a meaningful finding also considering that cirrhosis is the main risk factor for HCC development, a cancer that is currently showing a continuous increase in incidence and mortality rate also in western countries where it appears to be essentially associated with the HCV infection.[66,81,115-118] Now, considering the high prevalence of occult HBV in HCV-infected individuals and its tendency to favour the progression toward cirrhosis of the liver disease occurring in these patients, it is clear that chronic HCV and occult HBV co-infection has been suspected of being an important risk factor for HCC development (reviewed in 20). In this context, it has to be stressed that several epidemiological and molecular studies performed since the ’80s had suggested a role played by occult HBV in HCC development,[17,119-121] and that both woodchucks and ground squirrels, once infected by the corresponding hepadnaviruses (WHV and GSHV, respectively), are at high risk of developing HCC even after the apparent clearance of the virus.[122,123]

We recently examined the largest series of tumor liver tissues from HBsAg negative patients with HCC tested so far for occult HBV (107 cases, mostly HCV infected) and compared the results with those obtained by the analysis of liver biopsy specimens from 192 HBsAg negative chronic hepatitis patients. We detected viral DNA in 68/107 (63.5%) HCC cases and in 63/192 (32.8%) chronic hepatitis cases ($p<0.0001$; odds ratio = 3.6, 95% confidence interval: 2.2-5.9). Moreover, both integrated HBV DNA and viral cccDNA were revealed in patients with *occult* HBV who also showed persistence of viral transcription and replication. Thus, our findings strongly (definitively?) confirm that occult HBV is a potent risk factor for HCC development and showed that the potential mechanisms whereby overt HBV might induce tumor formation are mostly maintained in cases of occult infection.[17]

Finally, we would like to discuss a further aspect of the HCV and occult HBV co-infection that might have a fundamental importance. Several studies reported that occult HBV exerts its negative influence on chronic hepatitis C also in terms of a reduced response to IFN therapy.[8,12,123,124] Moreover, the association between cryptic HBV infection and non-response to therapy appeared to be independent of other factors, as confirmed by a multivariate analysis.[125] By which mechanisms the occult HBV may help HCV to resist IFN is at present totally unknown. We might speculate that in such cases the viral genomes present in the hepatocytes, despite their activity being inhibited, are still capable of interfering with cellular proteins having anti-viral properties and thus facilitate the HCV activity. In this context, a recent study showing decreased intrahepatic expression of IFN receptor mRNA and protein in occult hepatitis B should be mentioned.[126] Although this argument is intriguing and potentially very relevant from a practical point of view, we must note that all the cited reports concern treatment schedules using the conventional IFN therapy, whereas no prospective study has been performed so far evaluating whether occult HBV infection may interfere with the response to PEG-IFN plus Ribavirin that is at present the gold-standard therapy for the treatment of chronic hepatitis C. In this view, this particular aspect of the possible interaction between the two viruses must be completely re-evaluated.[127]

The total number of individuals with overt and occult HBV infection represents a considerable proportion of chronic HCV carriers. Considering that the available data strongly suggest that dual infection by HBV and HCV

may be associated with severe forms of liver disease poorly sensitive to interferon treatment and with a high risk of hepatocellular carcinoma development, it is clear that the attention of researchers should in the near future be much more focused on this category of patients that is at present one of the least studied among subjects affected by chronic liver disease.

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