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Concise Review

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

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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 coinfecting 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

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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 pre-core 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-IFN α 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 coinfecting 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 coinfecting 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.

References

- Pontisso P, Ruvoletto MG, Fattovich G, Chemello L, Gallorini A, Ruol A, Alberti A. Clinical and virological profiles in patients with multiple hepatitis infections. *Gastroenterology* 1993; 105: 1529-33.
- Sato S, Fujiyama S, Tanaka M, Yamasaki K, Kuramoto I, Kawano S, Sato T, et al. Coinfection of hepatitis C virus in patients with chronic hepatitis B infection. *J Hepatol* 1994; 21: 159-166.
- Crespo J, Lozano JL, de la Cruz F, Rodrigo L, Rodriguez M, San Miguel G, Artinano E, et al. Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. *Am J Gastroenterology* 1994; 89: 1147-51.
- Alberti A, Pontisso P, Chemello L, Alberti A, Pontisso P, Chemello L, Fattovich G, et al. The interaction between hepatitis B virus and hepatitis C virus in acute and chronic liver disease. *J Hepatol* 1995; 22(Suppl.1): 38-41.
- Zarski JP, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, Tran van Nhieu J, et al. Characteristic of patients with dual infection by hepatitis B and C viruses. *J Hepatol* 1998; 28: 27-33.
- Liang TJ, Baruch Y, Ben-Porath E, Enat R, Bassan L, Brown NV, Rimon N, et al. Hepatitis B virus infection in patients with idiopathic liver disease. *Hepatology* 1991; 13: 1044-51.
- Zhang YY, Hansson BG, Kuo LS, Widell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. *Hepatology* 1993; 17: 538-544.
- Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med* 1999; 341: 22-26.
- Raimondo G, Balsano C, Craxi A, Farinati F, Levrero M, Mondelli M, Pollicino T, et al. Occult hepatitis B virus infection. *Dig Liver Dis* 2000; 32: 822-6.
- Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; 34: 194-203.
- Raimondo G. Occult HBV infection and liver disease: fact or fiction? *J Hepatol* 2001; 34: 471-473.
- Torbenson M & Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; 2: 479-486.
- Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 2002; 9: 243-257.
- Mimms LT, Mosley JW, Hollinger FB, Aach RD, Stevens CE, Cunningham M, Vallari DV, et al. Effect of concurrent acute infection with hepatitis C virus on acute hepatitis B virus infection. *Br Med J* 1993; 307: 1095-1097.
- Sagnelli E, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, et al. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; 36: 1285-1291.
- Ganem D, Prince AM. Hepatitis B virus infection - Natural history and clinical consequences. *N Engl J Med* 2004; 350: 1118-29.
- Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Craxi A, Farinati F, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. *Gastroenterology* 2004; 126: 102-10.
- Wieland SF, Spangenberg HC, Thimme R, Purcell RH, Chisari FV. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proc Natl Acad Sci USA* 2004; 100: 2129-34.
- Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during Adefovir-Dipivoxil therapy. *Gastroenterology* 2004; 126: 1750-58.
- Brechot C. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004; 127(5 Suppl 1): S56-61.
- Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. *Gastroenterology* 2004; 127(5 Suppl 1): S72-8.
- Hillman MR. Overview of the pathogenesis, prophylaxis and therapeutic of viral hepatitis B, with focus on reduction to practical applications. *Vaccine* 2001; 19: 1837-1848.
- Stuver SO. Towards global control of liver cancer? *Semin Cancer Biol* 1998; 8: 299-306.
- Arauz-Ruiz P, Norder H, Robertson BH, Magnus LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; Aug83(Pt 8): 2059-73.
- Arauz-Ruiz P, Norder H, Visona KA, Magnus LO. Genotype F prevails in HBV infected patients of hispanic origin in Central America and may carry the precore stop mutant. *J Med Virol* 1997; 51: 305-12.
- Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. *Hepatology* 2002; 35: 1274-6.
- Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; Sep 9; 2: 588-91.
- Brunetto MR, Stamler M, Schodel F, Will H, Ottobreilli A, Rizzetto M, Verme G, et al. Identification of HBV variants which cannot produce precore derived HBeAg and may be responsible for severe hepatitis. *Ital J Gastroenterol* 1989; 21: 151-154.
- Raimondo G, Costantino L, Caccamo G, Pollicino T, Squadrito G, Cacciola I, Brancatelli S. Non-sequencing molecular approaches to identify preS2-defective hepatitis B virus variants proved to be associated with severe liver diseases. *J Hepatol* 2004; 40: 515-519.
- Santantonio T, Jung MC, Pastore G, Angarano G, Gunther S, Will H. Familial clustering of HBV pre-C and pre-S mutants. *J Hepatol* 1997; 26: 221-7.
- Pollicino T, Zanetti AR, Cacciola I, Petit MA, Smedile A, Campo S, Saggiocca L, et al. Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. *Hepatology* 1997; 26: 495-9.
- Le Seyec J, Chouteau P, Annie I, Guguen-Guillouzo C, Gripon P. Role of the pre-S2 domain of the large envelope protein in hepatitis B virus assembly and infectivity. *J Virol* 1998; 72: 5573-8.
- Fan YF, Lu CC, Chen WC, Yao WJ, Wang HC, Chang TT, Lei HY, et al. Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. *Hepatology* 2001; 33: 277-86.
- Blum H, Galun E, Liang TJ, von Weizsacker F, Wands JR. Naturally occurring missense mutation in the polymerase gene terminating hepatitis B virus replication. *J Virol* 1991; 65: 1836-42.
- Yamamoto K, Horikita M, Tsuda F, Itoh K, Akahane Y, Yotsumoto S, Okamoto H, et al. Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; 68: 2671-76.
- Carman WF, Van Deursen FJ, Mimms LT, Hardie D, Coppola R, Decker R, Sanders R. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa and Sardinia. *Hepatology* 1997; 26: 1658-66.
- Figus A, Blum HE, Vyas GN, De Virgili S, Cao A, Lippi M, Lai E, et al. Hepatitis B viral nucleotide sequences in non-A, non-B or hepatitis B virus related chronic liver disease. *Hepatology* 1984; 4: 364-368.
- Thiers V, Nakajima E, Kremsdorf D, Mack D, Schellekens H, Driss F, Goudeau A, Wands J, Sninsky J, Tiollais P, et al. A transmission of hepatitis B from hepatitis B seronegative subjects. *Lancet* 1988; 2: 1273-76.
- Kaneko S, Miller RH, Feinstone SM, Unoura M, Kobayashi K, Hattori N, Purcell RH. Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. *Proc Natl Acad Sci* 1989; 86: 312-316.

40. Wang JT, Wang TH, Sheu JC, Shih LN, Lin JT, Chen DS. Detection of hepatitis B virus DNA by polymerase chain reaction in plasma of volunteer blood donors negative for hepatitis B surface antigen. *J Infect Dis* 1991; 163: 397-399.
41. Liang TJ, Blum HE, Wands JR. Characterization and biological properties of a hepatitis B virus isolated from a patient without hepatitis B virus serologic markers. *Hepatology* 1990; 12: 204-212.
42. Lorient MA, Marcellin P, Bismuth E, Martinot-Peignoux M, Boyer N, Degott C, Erlinger S, et al. Demonstration of hepatitis B virus DNA by polymerase chain reaction in the serum and the liver after spontaneous or therapeutically induced HBeAg to anti-HBe or HBsAg to anti-HBs seroconversion in patients with chronic hepatitis B. *Hepatology* 1992; 15: 32-36.
43. Zhang Y-Y, Hansson BG, Kuo LS, Widell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. *Hepatology* 1993; 17: 538-544.
44. He C, Nomura F, Itoga S, Isobe K, Nakai T. Prevalence of vaccine-induced escape mutants of hepatitis B virus in the adult population in China: a prospective study in 176 restaurant employees. *J Gastroenterol Hepatol* 2001; 16: 1373-7.
45. Lu M, Lorentz T. De novo infection in a renal transplant recipient caused by novel mutants of hepatitis B virus despite the presence of protective anti-hepatitis B surface antibody. *J Infect Dis* 2003; 187: 1323-6.
46. Basuni AA, Butterworth L, Cooksley G, Locamini S, Carman WF. Prevalence of HBsAg mutants and impact of hepatitis B infant immunisation in four Pacific Island countries. *Vaccine* 2004; 29: 2791-9.
47. Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, Brown N, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. *Hepatology* 1998; 27: 1670-7.
48. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, et al. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. *Hepatology* 2000; 32: 1078-88.
49. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; 125: 1714-22.
50. Dienstag JL, Cianciara J, Karayalcin S, Kowdley KV, Willems B, Plisek S, Woessner M, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. *Hepatology* 2003; 37: 748-55.
51. Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, Brosgart C, et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* 2003; 125: 292-7.
52. Villeneuve JP, Durantel D, Durantel S, Westland C, Xiong S, Brosgart CL, Gibbs CS, et al. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. *J Hepatol* 2003; 39: 1085-9.
53. Werle B, Cinquin K, Marcellin P, Pol S, Maynard M, Trepo C, Zoulim F. Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. *J Viral Hepat* 2004; 11: 74-83.
54. Robertson B, Myers G, Howard C, Brettin T, Bukh J, Gaschen B, Gojobori T, et al. Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. *Arch Virol* 1998; 143: 2493-503.
55. Penin F. Structural biology of hepatitis C virus. *Clin Liver Dis* 2003; 7: 1-22.
56. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; 345: 41-52.
57. Di Bisceglie AM, McHutchison J, Rice CM. New therapeutic strategies for hepatitis C. *Hepatology* 2002; 35: 224-231.
58. Thomson M, Liang TJ. *Molecular biology of hepatitis C virus*. In: Hepatitis C. Edited by TJ Liang and J Hoofnagle. Boston: Academic Press 2000: 1-23.
59. Pawlotsky JM. Hepatitis C virus genetic variability: pathogenic and clinical implications. *Clin Liver Dis* 2003; 7: 45-66.
60. Orland JR, Wright TL, Cooper S. Acute hepatitis C. *Hepatology* 2001; 33: 321-327.
61. Troisi CL, Hollinger FB, Hoots WK, et al. A multicenter study of viral hepatitis in a United States hemophilic population. *Blood* 1993; 81: 412-418.
62. Afdhal NH. The natural history of hepatitis C. *Semin Liver Dis* 2004; 24 Suppl 2: 3-8.
63. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; 362: 2095-100.
64. Alberti A, Chemello L, Benvegno L. Natural history of hepatitis C. *J Hepatol* 1999; 31 (Suppl 1): 17-24.
65. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. *Hepatology* 1997; 26 (Suppl 1): 34S-38S.
66. Colombo M. Natural history and pathogenesis of hepatitis C virus related hepatocellular carcinoma. *J Hepatol* 1999; 31 (Suppl 1): 25-30.
67. Agnello V, De Rosa FG. Extrahepatic disease manifestations of HCV infection: some current issues. *J Hepatol* 2004; 40: 341-52.
68. Zignego AL, Brechot C. Extrahepatic manifestations of HCV infection: facts and controversies. *J Hepatol* 1999; 31: 369-76.
69. Hadziyannis SJ. The spectrum of extrahepatic manifestations in hepatitis C virus infection. *J Viral Hepat* 1997; 4: 9-28.
70. Di Marco V, Lojaco O, Cammà C, Vaccaio A, Giunta M, Martorana G, Fuschi P, et al. The long-term course of chronic hepatitis B. *Hepatology* 1999; 30: 257-264.
71. Guptan RC, Thakur V, Raina V, Sarin SK. Alpha-Interferon therapy in chronic hepatitis due to active dual infection with hepatitis B and C viruses. *J Gastroenterol Hepatol* 1999; 14: 893-898.
72. Squadrito G, Orlando ME, Pollicino T, Raffa G, Restuccia T, Cacciola I, Di Marco V, et al. Virological profiles in patients with chronic hepatitis C and overt or occult HBV infection. *Am J Gastroenterol* 2002; 97: 1518-1523.
73. Gaeta GB, Stornaiuolo G, Precone DF, Lobello S, Chiamonte M, Stroffolini T, Colucci G, et al. Epidemiological and clinical burden of chronic hepatitis B virus/hepatitis C virus infection. A multicenter Italian study. *J Hepatol* 2003; 39: 1036-41.
74. Fong TL, Di Bisceglie AM, Waggoner JG, Banks SM, Hoofnagle JH. The significance of antibody hepatitis C virus in patients with chronic hepatitis B. *Hepatology* 1991; 14: 64-67.
75. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. *Hepatology* 1995; 22: 1101-1108.
76. Sagnelli E, Coppola N, Scolastico C, Filippini P, Santantonio T, Stroffolini T, Piccinino F. Virological and clinical aspects of reciprocal inhibitory effect of hepatitis B, C, and Delta viruses in patients with chronic hepatitis. *Hepatology* 2000; 32: 1106-1110.
77. Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; 36: S35-S46.
78. Simonetti RG, Cammà C, Fiorello F, Cottone M, Rapicetta M, Marino L. Hepatitis C virus infection and a risk factor for hepatocellular carcinoma in patients with cirrhosis. *Ann Intern Med* 1992; 116: 97-102.
79. Benvegno L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, Alberti A. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. *Cancer* 1994; 74: 2442-2448.
80. Chiamonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U, Lobello S, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. *Cancer* 1999; 85: 2132-2137.
81. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* 2002; 36: S74-S83.
82. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer* 1998; 75: 347-354.
83. Tanaka H, Tsukuma H, Yamano H, Oshima A, Shibata H. Prospective study on the risk of hepatocellular carcinoma among hepatitis C virus-positive blood donors focusing on demographic factors, alanine aminotransferase level at donation and interaction with hepatitis B virus. *Int J Cancer* 2004; 112: 1075-1080.
84. Strader DB. Understudied populations with hepatitis C. *Hepatology* 2002; 36: S226-S236.
85. Weltman MD, Brotodihardjo A, Crewe EB, Farrell GC, Bilous M, Grierson JM, Liddle C. Coinfection with hepatitis B and C or B, C

- and delta viruses results in severe chronic liver disease and responds poorly to interferon-alpha treatment. *J Viral Hepat* 1995; 2: 39-45.
86. Mazzella G, Saracco G, Festi D, Rosina F, Marchetto S, Jaboli F, Sostegni R, et al . Long-term results with interferon therapy in chronic type B hepatitis: a prospective randomized trial. *Am J Gastroenterol* 1999; 94: 2246-2250.
 87. Villa E, Grottola A, Buttafoco P, Colantoni A, Bagni A, Ferretti I, Cremonini C, et al . High doses of α -Interferon are required in chronic hepatitis due to coinfection with hepatitis B virus and hepatitis C virus: long-term results of a prospective randomized trial. *Am J Gastroenterol* 2001; 96: 2973-2977.
 88. Villa E, Grottola A, Trande P, Seium Y, Rebecchi AM, Dugani A, Manenti F. Reactivation of hepatitis B virus infection induced by Interferon (IFN) in HBsAg-positive, antiHCV-positive patients. *Lancet* 1993; 341: 1413.
 89. Liu CJ, Lai MY, Kao JH, Jeng YM, Chen DS. Ribavirin and Interferon is effective for hepatitis C virus clearance in hepatitis B and C dually infected patients. *Hepatology* 2003; 37: 568-576.
 90. Fattovich G, Tagger A, Brollo L, Giustina G, Pontisso P, Realdi G, Alberti A, et al . Hepatitis virus infection in chronic hepatitis B virus carriers. *J Infect Dis* 1991; 163: 400-402.
 91. Sheen IS, Liaw YF, Chu CM, Pao CC. Role of hepatitis C virus infection in spontaneous hepatitis B surface antigen clearance during chronic hepatitis B virus infection. *J Infect Dis* 1992; 165: 831-834.
 92. Chu CM, Yeh CT, Liaw YF. Low-level viremia and intracellular expression of hepatitis B surface antigen (HBsAg) in HBsAg carriers with concurrent hepatitis C virus infection. *J Clin Microbiol* 1998; 36: 2084-2086.
 93. Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Galimany R, Esteban R, et al. Rule of hepatitis B, C and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. *Hepatology* 2001; 34: 404-410.
 94. Shih CM, Lo SJ, Miyamura T, Chen SY, Lee YH. Suppression of hepatitis B virus expression and replication by hepatitis C virus core protein in Hu-H7 cells. *J Virol* 1993; 67: 5823-5832.
 95. Shih CM, Chen CM, Chen SY, Lee YH. Modulation of the trans-suppression activity of hepatitis C virus core protein by phosphorylation. *J Virol* 1995; 69: 1160-1171.
 96. Schuttler CG, Fiedler N, Schmidt K, Repp R, Gerlich WH, Schaefer S. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. *J Hepatol* 2002; 37: 855-862.
 97. Chen SY, Kao CF, Chen CM, Shih CM, Hsu MJ, Chao CH, Wang SH, et al . Mechanisms for inhibition of hepatitis B virus gene expression and replication by hepatitis C virus core protein. *J Biol Chem* 2003; 278: 591-607.
 98. Ohkawa K, Hayashi N, Yuki N, Hagiwara H, Kato M, Yamamoto K, Eguchi H, et al . Hepatitis C virus antibody and hepatitis C virus replication in chronic hepatitis B patients. *J Hepatol* 1994; 21: 509-514.
 99. Ohkawa K, Hayashi N, Yuki N, Masuzawa M, Kato M, Yamamoto K, Hosotsubo H, et al . Long-term follow up of hepatitis B virus and hepatitis C virus replicative levels in chronic hepatitis patients coinfecting with both viruses. *J Med Virol* 1995; 46: 258-264.
 100. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. *Gastroenterology* 1984; 86: 230-235.
 101. Davis GL, Hoofnagle JH. Reactivation of chronic type B hepatitis presenting as acute viral hepatitis. *Ann Int Med* 1985; 102: 762-765.
 102. Raimondo G, Rodinò G, Smedile V. Hepatitis B virus (HBV) markers and HBV-DNA in serum and liver tissue of patients with acute exacerbation of chronic type B hepatitis. *J Hepatol* 1990; 10: 271-273.
 103. Raimondo G, Schneider R, Stelmier M, Smedile V, Rodino G, Will H. A new hepatitis B virus variant in a chronic carrier with multiple episodes of viral reactivation and acute hepatitis. *Virology* 1990; 179: 64-68.
 104. Arase Y, Ikeda K, Chayama K, Murashima N, Tsubota A, Suzuki Y, Saitoh S, et al . Fluctuation patterns of HCV-RNA serum level in patients with chronic hepatitis C. *J Gastroenterol* 2000; 35: 221-5.
 105. Halfon P, Bourliere M, Halimi G, Khiri H, Bertezene P, Portal I, Botta-Fridlund D, et al . Assessment of spontaneous fluctuations of viral load in untreated patients with chronic hepatitis C by two standardized quantitation methods: branched DNA and Amplicor Monitor. *J Clin Microbiol* 1998; 36: 2073-5.
 106. Pontisso P, Bellati G, Brunetto M, Chemello L, Colloredo G, Di Stefano R, Nicoletti M, et al . Hepatitis C virus RNA profiles in chronically infected individuals: do they relate to disease activity? *Hepatology* 1999; 29: 585-9.
 107. Raimondo G, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C, Squadrito G, et al . Wide spectrum of virological profiles in HBsAg/anti-HCV positive patients. An Italian multicentre study. *J Hepatol* 2005; 42 (Suppl. 2): 20 (Abstract).
 108. Villari D, Pernice M, Spinella S, Squadrito G, Rodino G, Brancatelli S, Longo G, et al . Chronic hepatitis in patients with active hepatitis B virus and hepatitis C virus combined infections: a histological study. *Am J Gastroenterol* 1995; 90: 955-958.
 109. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 1994; 94: 907.
 110. Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, Chisari FV, et al . Long-lasting memory T cell responses following self-limited acute hepatitis B. *J Clin Invest* 1996; 98: 1185-1194.
 111. Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shintani Y, Fujie H, Tsutsumi T, et al . Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 1998; 27: 1377-1382.
 112. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, Ting LT, et al . Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. *Hepatology* 1998; 28: 231-236.
 113. Blackberg J, Kidd-Ljunggren K. Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. *J Hepatol* 2000; 33: 992-997.
 114. Michalak TI, Pardoe IU, Coffin CS, Churchill ND, Freake DS, Smith P, Trelogan CL. Occult lifelong persistence of infectious hepadnavirus and residual liver inflammation in woodchucks convalescent from acute viral hepatitis. *Hepatology* 1999; 29: 928-938.
 115. Bergsland EK, Venook AP. Hepatocellular carcinoma. *Curr Opin Oncol* 2000; 12: 357-361.
 116. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; 340: 745-750.
 117. Colombo M. Hepatitis C virus and hepatocellular carcinoma. *Semin Liver Dis* 1999; 19: 263-269.
 118. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* 2002; 36: S74-S83.
 119. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. *N Engl J Med* 1981; 305: 1067-73.
 120. Paterlini P, Gerken G, Nakajima E, Terre S, D'Errico A, Grigioni W, Nalpas B, et al . Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. *N Engl J Med* 1990; 323: 80-85.
 121. Sheu JC, Huang GT, Shih LN, Lee WC, Chou HC, Wang JT, Lee PH, et al . Hepatitis C and B viruses in hepatitis B surface antigen-negative hepatocellular carcinoma. *Gastroenterology* 1992; 103: 1322-27.
 122. Korba BE, Wells FV, Baldwin B, Cote PJ, Tennant BC, Popper H, Gerin JL. Hepatocellular carcinoma in woodchuck hepatitis virus infected woodchucks: presence of viral DNA in tumor tissue from chronic carriers and animals serologically recovered from acute infections. *Hepatology* 1989; 9: 461-470.
 123. Marion PI. Ground squirrel hepatitis virus. In: McLachlan A, ed. *Molecular biology of hepatitis B virus*. Boca Raton: CRC, 1991: 39-51.
 124. Zignego AL, Fontana R, Puliti S, Barbagli S, Monti M, Careccia G, Giannelli F, et al. Relevance of inapparent co-infection by hepatitis B virus in alpha interferon-treated patients with hepatitis C virus chronic hepatitis. *J Med Virol* 1997; 51: 313-18.
 125. De Maria N, Colantoni A, Idilman R, Friedlander L, Harig J, Van Thiel DH. The impact of previous HBV infection on the course of chronic hepatitis C. *Am J Gastroenterol* 2000; 95: 3529-36.
 126. Fukuda R, Ishimura N, Hanomoto S, Moritani M, Uchida Y, Ishihara S, Akagi S, et al . Co-infection by serologically-silent hepatitis B virus may contribute to poor interferon response in patients with chronic hepatitis C by down regulation of type 1 interferon receptor gene expression in the liver. *J Med Virol* 2001; 63: 220-227.
 127. Raimondo G, Pollicino T, Squadrito G. What is the clinical impact of occult hepatitis B virus infection? *Lancet* 2005; 365: 638-640.