

REVIEW

Occult hepatitis B virus infection in hepatitis C virus negative chronic liver diseases

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Abstract

Data concerning the prevalence of hepatitis B virus (HBV) occult infection (OBI) varies greatly in the different studies according to the sensitivity and specificity of the diagnostic approaches and the HBV prevalence in the different populations examined. The clinical implications of OBI are still debated. While the impact of OBI in HBV transmission as well as in HBV reactivation under immunosuppression are well established, the role of OBI in liver disease and hepatocellular carcinoma (HCC) development are still not definitively elucidated. It has been hypothesized that OBI might contribute to worsening the liver disease course when other causes of liver damage co-exist. Furthermore, much evidence suggests a role of OBI in the hepato-carcinogenesis processes through both indirect and direct oncogenic mechanisms that might favour HCC development. Data on the OBI clinical implications mainly come from studies performed in patients with hepatitis C virus (HCV) infection. However, HCV prevalence has dramatically fallen in the past years also because of the advent of specific and highly effective direct acting antivirals, with a consequent abrupt change of the worldwide scenario of chronic liver disease. Information about OBI prevalence and possible clinical impact in non-HCV-related liver disease are fragmentary, and the objective of this review is to critically summarize the available data in this field.

KEYWORDS

cirrhosis, HCV-negative chronic liver disease, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, occult hepatitis B infection

1 | INTRODUCTION

Hepatitis B virus (HBV) infection is still responsible for about 1 million deaths worldwide.^{1–3} Chronic HBV infection is a dynamic process reflecting the interaction between the virus and the host

immune response, which is also responsible for several clinical manifestations ranging from absence of liver disease to acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC).^{4–7} The natural history of chronic HBV infection can be schematically divided into five—not necessarily sequential—phases according to

Abbreviations: CLD, chronic liver disease; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OBI, occult HBV infection; PCR, polymerase chain reaction.

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virological, clinical and histologic features.⁸ Hepatitis B surface antigen (HBsAg) detection in a patient's sera is fundamental for the diagnosis of HBV infection,⁹ and the disappearance of circulating HBsAg has been considered to mark the resolution of the infection. However, despite the serum HBsAg clearance, many individuals may persistently retain residual amounts of viral DNA in the liver and much less frequently also in the blood.^{10–14} This condition is termed as “occult HBV infection” (OBI) and has been defined as the presence of replication competent HBV DNA in the liver and/or in the blood of individuals testing HBsAg negative by currently available assays.¹⁵ Moreover, according to HBV-specific antibody profiles, OBI may be categorized as seropositive-OBI (presence of antibodies to HBV core antigen [anti-HBc] and/or to HBsAg [anti-HBs]) and seronegative-OBI (anti-HBc and anti-HBs negative). Testing HBV DNA in the liver—preferably on frozen tissues—is the gold standard for the diagnosis of OBI.¹⁵ However, because of the intrinsic limitations to obtain liver specimens to be tested from a large number of individuals (i.e., few specimens available from needle liver biopsies and surgery interventions), most of the available data derives from viral DNA investigations in blood sample extracts,^{15–20} which limits the reliability of the information.

The clinical relevance of OBI is clear in terms of risks of HBV transmission (through both transfusion of blood products^{21–26} and orthotopic liver transplantation^{27–35}) as well as viral reactivation in cases under strong—and usually therapy-related—immunosuppression.^{36–48} Far more controversial is the possible role of OBI in inducing—or contributing to—chronic liver disease (CLD) and to HCC development. According to the available data, it is hypothesized that the presence of OBI is inoffensive by itself in a very large majority of cases. However, a mild necroinflammation may persist up to 30 years after the resolution of a self-limited acute hepatitis B (and—likely—of the beginning of an occult infection), despite the absence of biochemical signs of liver damage.^{49–54} This observation has contributed to the generation of the hypothesis that when other causes of liver damage co-exist, the minimal lesions produced by the CTL-specific immune response to the residual HBV proteins produced during the OBI phase might contribute to worsening the liver disease course up to cirrhosis development.^{55,56} Another relevant aspect is that a proportion of the OBI individuals clear the serum HBsAg after decades of chronic hepatitis, when cirrhosis is already established and the liver damage is no longer reversible.^{15,57,58} The possible contribution of OBI to the occurrence of cirrhosis (which is the most important liver cancer risk factor) might also be involved in the claimed role of OBI in HCC development. In this context, it is of great importance to stress that OBI appears to maintain the direct pro-oncogenic properties typical of the HBsAg positive chronic hepatitis B (i.e. capacity of viral DNA to integrate into the host's genome; persisting low-level production of X and preS/S viral proteins having transforming properties).^{57,59–63}

Most of the data concerning the possible impact of OBI on the course of liver disease and on HCC development derive from studies performed on hepatitis C virus (HCV) infected patients.^{64–74} Indeed, HCV was largely the most common cause of CLD in the richest

countries for several decades. The advent of direct-acting antiviral therapies (DAAs)—capable of curing virtually all chronic HCV infections in a few weeks—has dramatically and very quickly changed the worldwide scenario of liver illnesses. Consequently, at present, the vast majority of patients with chronic liver injury and those with HCC diagnosed in the most developed countries are patients with unidentified causes of liver injury (cryptogenic liver disease), with alcoholic or non-alcoholic fatty liver diseases or with autoimmune hepatitis. Studies concerning the prevalence and the possible impact of OBI in patients with HCV-negative liver disease are limited. In particular, few and fragmentary data derive from studies applying the gold standard for OBI diagnosis, which is the HBV DNA testing in liver DNA extracts.

The aim of this review was to summarize and comment on the available data on OBI tested by examination of liver specimens from patients with HCV-unrelated CLD or with liver cancer.

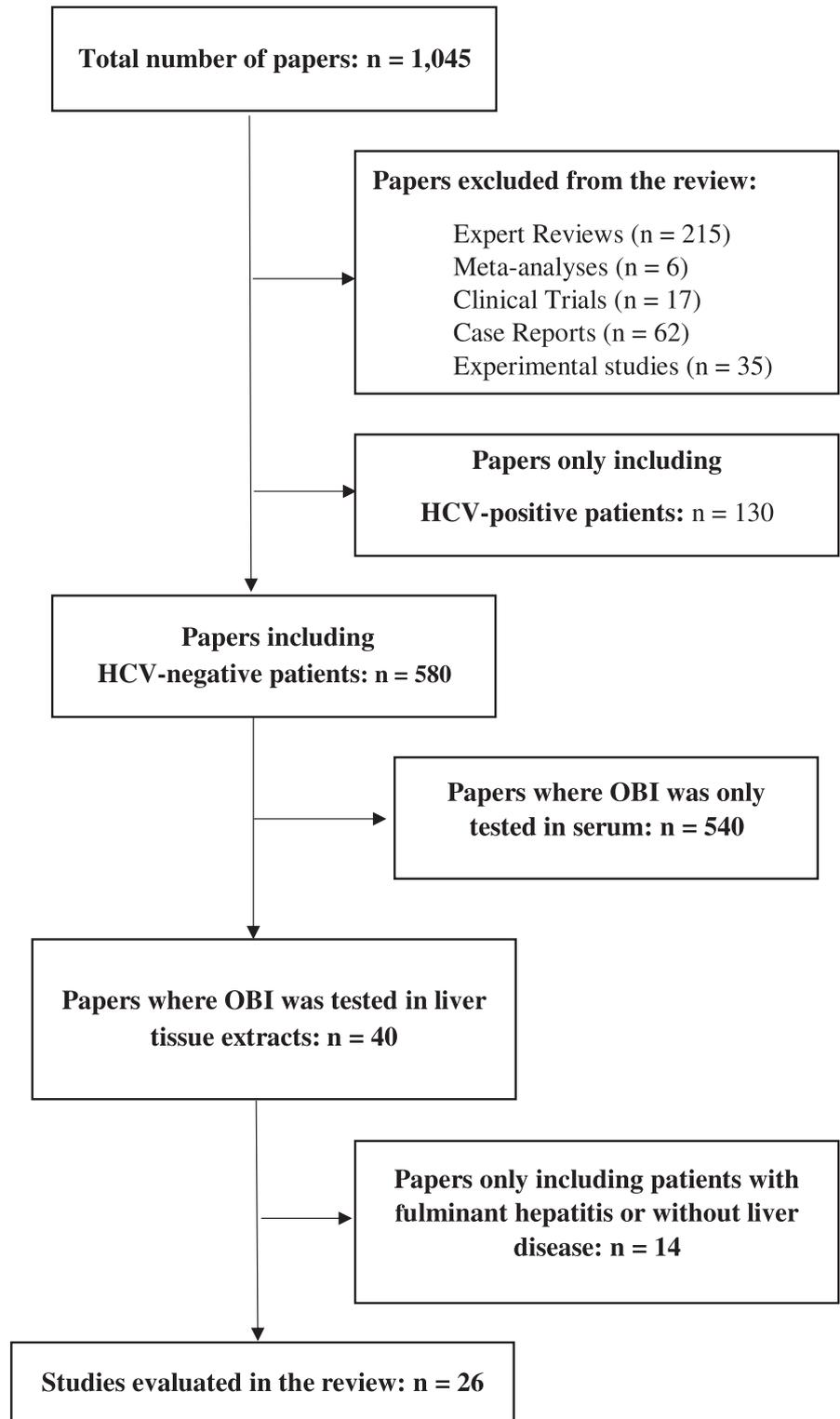
2 | METHODS

An accurate systematic literature search was conducted using PubMed MEDLINE and the following keywords: “Occult Hepatitis B,” “Occult HBV infection,” “latent HBV infection,” “silent HBV infection,” “previous HBV infection.” Initially, 1045 English-language papers were identified from January 1991 to December 2020. Subsequently, in accordance with the aim of the review, all original studies based on the diagnosis of OBI by the use of HBV DNA testing on liver tissue extracts from patients with either cryptogenic liver disease, alcoholic liver disease, non-alcoholic fatty liver disease or autoimmune hepatitis as well as from those with HCV-negative HCC or with intrahepatic cholangiocarcinoma (ICC) were analysed (Figure 1). Expert reviews, meta-analyses, clinical trials and case reports were excluded. Studies including patients with both HCV-related and unrelated CLD were considered exclusively for the data concerning the HCV-negative cases. Twenty-six papers met the inclusion criteria and are discussed in the review (Table 1; Table 2).

2.1 | OBI and cryptogenic liver diseases

Diagnosis of cryptogenic chronic liver disease (CryCLD) is—by definition—based on the exclusion of all known causes of hepatic injury, such as hepatitis virus infections, genetic or autoimmune disorders, alcohol abuse, metabolic syndrome and iatrogenic injury. In literature, 19 studies testing OBI at intrahepatic level and concerning its association with cryptogenic liver disease or HCC (9 and 10 studies respectively) are available (Table 1; Table 2). Most of them were conducted in Asia (4 in Japan, 1 in China, 1 in Korea, 1 in Taiwan, 1 in India, 1 in Iran, 1 in Israel), others in Europe (4 in Italy, 2 in France), South America (1 in Colombia, 1 in Brazil) and North Africa (1 in Morocco). Some of these studies also included HCV patients.^{16,66,75–86} Furthermore, some studies evaluated intrahepatic HBV DNA only in a few cases.^{77,79–81}

FIGURE 1 Flow chart of the studies on occult HBV infection published from January 1991 to December 2020 and evaluated in the review



2.1.1 | OBI in patients with cryptogenic CLD

Four of the 9 studies that analysed OBI in patients with CryCLD and without HCC were performed in Asia, 3 in Europe and 2 in South America. Three of these nine studies were conducted in the 1990s, 4 in the first decade of this century, and 2 in the past 10 years.

The three studies performed in the 1990s concerned patients living in Israel, China and Italy respectively. HBV DNA was tested

by single-step polymerase chain reaction (PCR) technique on formalin-fixed (FF) liver tissue specimens in two studies, whereas viral DNA was tested by *nested*-PCR in DNA extracts from frozen specimens in the Italian study. The study conducted on Israeli patients detected HBV DNA in 8/23 (34.8%) HCV-negative cases.⁷⁵ The Chinese study identified HBV DNA in 17/57 (29.8%) liver tissues, although the HCV positive/negative status of the patients was not specified.⁷⁶ In the Italian study, HBV sequences were

TABLE 1 Studies on the prevalence of occult HBV infection in HCV-negative patients according to the aetiology of the chronic liver disease (CLD)

| Study | Enrollment period | Country | OBI-positive/liver specimens tested (%) | | | |
|--------------------------|-------------------|----------|---|---------------|-----------------------------------|----------------|
| | | | Cryptogenic CLD | Alcoholic CLD | Non-alcoholic fatty liver disease | Autoimmune CLD |
| Liang et al. (1991) | NR | Israel | 8/23 (34.8%) | — | — | — |
| Zhang et al. (1993) | 1989 | China | 17/57 (29.8%) | — | — | — |
| Cacciola et al. (1999) | 1991–1997 | Italy | 6/28 (21.4%) | — | — | — |
| Chemin et al. (2001) | NR | France | 15/50 (30%) | — | — | — |
| Agarwal et al. (2003) | 1997–1999 | India | 2/14 (14.3%) | 2/14 (14.3%) | — | — |
| Pollicino et al. (2004) | 1999–2000 | Italy | 7/39 (18%) | — | — | — |
| Honarkar et al. (2005) | 2001–2002 | Iran | 4/7 (57.1%) | — | — | — |
| Georgiadou et al. (2009) | NR | Greece | — | — | — | 3/14 (21.4%) |
| Xie et al. (2013) | 2008–2012 | China | — | 18/43 (41.9%) | — | — |
| Ferrari et al. (2014) | 2009–2012 | Brazil | 1/16 (6.3%) | 1/23 (4.3%) | — | — |
| Rendon et al. (2017) | 2002–2008 | Colombia | 2/10 (20%) | — | — | — |
| Raimondo et al. (2020) | 2015–2017 | Italy | — | — | 29/226 (12.8%) | — |

Abbreviation: NR: not reported.

TABLE 2 Studies on the prevalence of occult HBV infection in HCV-negative patients according to the aetiology of the chronic liver disease (CLD) in cases with hepatocellular carcinoma (HCC)

| Study | Enrollment period | Country | OBI-positive/liver specimens tested (%) | | | |
|-------------------------|-------------------|------------------|---|---------------|-----------------------------------|----------------|
| | | | Cryptogenic CLD | Alcoholic CLD | Non-alcoholic fatty liver disease | Autoimmune CLD |
| Sheu et al. (1992) | 1988–1990 | Taiwan | 5/10 (50%) | — | — | — |
| Paterlini et al. (1993) | NR | France/ Italy | 10/19 (52.6%) | — | — | — |
| Pollicino et al. (2004) | 1999–2000 | Italy | 23/34 (67.6%) | — | — | — |
| Wong et al. (2011) | NR | Japan | 24/33 (73%) | 5/9 (56%) | — | — |
| Bai et al. (2013) | 2010–2011 | Japan | 10/24 (42%) | — | — | — |
| Kondo et al. (2014) | 1996–2006 | Japan | 15/20 (75%) | — | — | — |
| Kitab et al. (2014) | 2000–2003 | Morocco | 6/20 (30%) | — | — | — |
| Saitta et al. (2015) | 2006–2008 | Italy | 7/11 (63.6%) | 7/12 (58.3%) | 8/12 (66.6%) | — |
| Coppola et al. (2016) | 2013–2014 | Italy | — | — | 2/9 (15.4%) | — |
| Shim et al. (2017) | 2000–2009 | Korea | 41/78 (52.6%) | 14/44 (31.8%) | — | — |
| Koga et al. (2017) | 1984–2012 | Japan | — | 7/19 (36.8%) | 0/8 (0%) | — |
| Muto et al. (2018) | 2005–2012 | Japan | 5/21 (23.8%) | — | — | — |

detected in 6/28 (21.4%) frozen liver tissues of HBsAg-negative individuals classified as CryCLD.⁶⁶

The four studies performed in the first decade of this century were conducted in India, Iran, France and Italy respectively. In the two Asiatic studies, HBV DNA was tested on FF liver specimens by single-step PCR testing only HBV S gene sequences, whereas in the Italian study multiple HBV genomic regions were tested on frozen liver specimens by *nested*-PCR technique. Finally, in the French study viral DNA was tested for in either FF or frozen specimens by *nested* or *semi-nested* PCR technique testing both S and X HBV genes. In the Iranian study, seven liver specimens from patients with

well-characterized cryptogenic liver disease were tested, and HBV DNA was detected in 4 of them (57.1%).⁷⁹ HBV DNA was detected in 4/14 (28.6%) cases in the Indian study, although the HCV status of the patients was not mentioned.⁷⁸ In the Italian study, aimed at investigating OBI in HCC patients, HBV sequences were detected in 7/39 (18%) cases from individuals with HCV-negative CLD included as the control group in the study.¹⁶ HBV DNA was detected in 15/50 (30%) cases in the French study.⁷⁷

In the last decade, only two studies evaluated OBI in cryptogenic liver disease. Both of them were performed in South America (Brazil and Colombia respectively), and OBI was diagnosed by testing HBV

DNA in at least two different HBV genomic regions by *nested*-PCR on frozen liver DNA extracts from patients with cirrhosis or end-stage liver disease who underwent orthotopic liver transplantation (OLT). HBV DNA was detected in 1/16 (6.3%) cases in the Brazilian study and in 2/10 (20%) in the Colombian study.^{80,81} Very few and contrasting data concerning a possible association between OBI and liver fibrosis stage are available.⁷⁷

In summary, data concerning both prevalence and significance of OBI in patients with CryCLD is still largely incomplete and controversial. There are only nine available studies, which have been conducted across three continents and eight different countries, some of which show a high and others a low prevalence of HBV in the general population (Table 1). Different diagnostic approaches were adopted in the studies, both in terms of the applied molecular techniques and the basis of examination of either frozen or FF liver specimens. Cumulatively, 244 cases were tested, but in 57 a possible presence of HCV infection was not investigated, and the possible presence of non-alcoholic fatty liver disease (NAFLD) was mentioned in only two papers. OBI was detected in between 6.3% and 57.1% of the examined cases. The low number of cases analysed the lack of valid classification of the patients, and the largely different diagnostic approaches used in the studies clearly explain the divergence of the results.

2.1.2 | OBI in HCC patients with cryptogenic CLD

Ten studies evaluated OBI in HCC patients over the past 30 years by the analysis of liver specimens from patients with cryptogenic liver disease (Table 2). Two of them were conducted in the 1990s, one in the first decade of the 2000s, and two in the past decade. Both tumour (T) and non-tumour (NT) liver tissues were available for molecular analysis in 4/10 (40%) studies.

One of the two 1990s studies was performed in Taiwan and the other in France. In both the studies HBV DNA was tested by PCR and real-time PCR assays in frozen liver tissues by the use of pairs of primers specific for four different genes of HBV. In particular, HBV DNA was detected in 5/10 (50%) tissues from HCV-negative patients in the Taiwanese study. Furthermore, integrated HBV DNA was detected in 3/10 (30%) tumour DNA extracts and in no NT tissues by Southern blot analysis.⁸² In the French study, HBV DNA was detected in 10/19 (52.6%) T and in 1/9 (11%) NT liver tissues of 19 HBsAg-negative HCC patients.⁸⁷ Of note is that the possible presence of NAFLD in the patients included in these two studies was not reported, and even the HCV positive/negative status of the patients was not specified in the French paper.

One multicenter Italian study was performed in the first decade of the 2000s. HBV genomes were tested in DNA extracts from frozen liver tissues by *nested*-PCR technique with detection of multiple genomic regions. HBV DNA was detected in 23/34 (67.6%) cryptogenic HCC cases.¹⁶

Of the seven studies performed in the past decade, four were conducted in Japan, one in Korea, one in Morocco and one in Italy. HBV genomes were tested in DNA extracts from frozen liver tissues

by *nested*-PCR technique in two of the Japanese studies^{83,88} as well as in the Italian and the Moroccan studies,^{84,85} whereas viral DNA was tested on FF specimens by real-time PCR assay in one Japanese study and in the Korean study.^{86,89} Finally, in a further Japanese paper it was reported that real-time PCR and *nested*-PCR techniques were adopted, but it was not specified if the liver specimens had been frozen or formalin fixed.⁹⁰ In the Japanese study by Wong et al., HBV DNA was detected in 24/33 (73%) cryptogenic HCC patients, although viral DNA was more frequently found in NT than in the T portion of the livers.⁸³ In the study by Bai et al., OBI was identified in 20/24 (83%) NT and 10/24 (42%) T liver tissues from HBsAg-negative patients ($p = 0.003$).⁸⁸ Similarly, in the study by Kondo et al. HBV DNA was detected in 16/20 (80%) NT and 15/20 (75%) T liver specimens from HCV-negative HCC patients.⁸⁹ In the Moroccan study HBV DNA was detected in 6/20 (30%) tissues from patients with CryCLD, although it was not reported whether viral DNA was found in the T or NT part of the liver specimens tested.⁸⁵ Possible presence of NAFLD in the tested cases was not specified in any of the above-mentioned three papers. In the multicenter Italian study essentially focused on HBV DNA integration in HCC patients with OBI and different causes of liver disease, viral sequences were identified in 7/11 (63.6%) tissues from patients with CryCLD.⁸⁴ In the Korean study, HBV DNA was detected in 23/40 (57.5%) HCC subjects with cirrhosis and 18/38 (47.4%) HCC patients without cirrhosis, who underwent resection or OLT, although it was not reported whether viral sequences were identified in the T or NT portions of the liver in the different cases.⁸⁶ Finally, in the most recent Japanese study by Muto et al., HBV DNA was detected in 8/21 (38.1%) tissues from cryptogenic HCC patients by *nested*-PCR testing positive for at least one region of viral DNA.⁹⁰

To summarize, data on both prevalence and significance of OBI in patients with HCC and cryptogenic liver disease are quite divergent, and this is because of the same limitations highlighted above in the part of the review dedicated to the non-HCC CryCLD patients (i.e. different diagnostic approaches; patients from area with different HBV epidemics) (Table 2). Although the general prevalence of OBI in HCC cases demonstrated a wide range in the different studies (from 23.8% to 75%), it could be notable that OBI was detected in more than 50% of the HCC cases in 7/10 studies and in 42%, 30% and 23.8%—respectively—in the other three studies.

2.1.3 | OBI in patients with ICC and cryptogenic liver disease

Finally, three studies evaluated the prevalence of OBI in patients with ICC and cryptogenic liver disease over the last decade. Two studies were performed in China and one in Italy. HBV DNA was tested in FF liver tissues by real-time PCR in one Chinese study, whereas HBV genomes were detected in frozen liver specimens by performing *nested*-PCR and *seminested*-PCR amplification assays in the Italian and another Chinese study, respectively.^{91–93} Despite the differences in the diagnostic tests utilized, OBI was found to

be highly prevalent in two studies, since HBV DNA was detected in 28/44 (63.6%) tissues from ICC patients in one Chinese study, and in 26/43 (60.5%) HCV-negative, ICC patients in the Italian study.^{91,92} In the Chinese study, no PCR products specific for S and C genes were observed in either ICC tissues or non-tumour tissues of four HBV-unrelated cases.⁹³

2.2 | OBI and alcoholic liver diseases

Excessive alcohol intake is a major cause of chronic liver injury worldwide. Alcoholic liver disease (ALD) includes a wide spectrum of clinical/histological forms ranging from “simple” steatosis (without inflammation and fibrosis) to alcoholic steatohepatitis (ASH), cirrhosis and HCC. The role of OBI in patients with ALD has been subject to very little investigation.

2.2.1 | OBI in patients with alcoholic CLD

There are actually only three studies evaluating OBI by testing HBV DNA in liver extracts from patients with ALD (Table 1). One of these studies was performed in the first decade of 2000s in India. The presence of HBV DNA was evaluated by testing a single viral genomic region in FF liver tissues through a single-step PCR amplification, and viral DNA was detected in 2/14 (14.3%) cases.⁷⁸

Two studies were performed in the past decade, one in Brazil and one in China. In the Brazilian study, two different HBV genomic regions were tested in DNA extracts from frozen explanted liver specimens by *nested*-PCR technique, whereas in the Chinese study viral DNA was extracted from FF liver tissues of patients with alcoholic cirrhosis undergoing OLT. DNA extracts from explanted livers were tested by real-time PCR amplification and detection of HBV S and Core genes. OBI was diagnosed in 1/23 (4.3%) cases in the Brazilian study and in 18/43 (41.9%) in the Chinese study.^{80,94}

2.2.2 | OBI in HCC patients with ALD

Four studies investigated OBI in HCC patients with ALD by the analysis of liver specimens (Table 2). All of them were conducted in the past decade (two in Japan, one in Korea and one in Italy). None of these studies reported whether viral sequences were identified in the T or NT portions of the liver in the tested cases. HBV genomes were tested in DNA extracts from frozen liver tissues by *nested*-PCR with detection of multiple genomic regions in one Japanese study⁸³ as well as in the Italian study,⁸⁴ whereas viral DNA was tested in FF specimens by real-time PCR assay in the other Japanese study and in the Korean one.^{86,95} In the Japanese study by Wong et al., HBV DNA was detected in 5/9 (56%) liver tissues from alcoholic HCC patients.⁸³ In the multicenter Italian study, OBI was detected in 7/12 (58.3%) tissues from patients with ALD and HCC.⁸⁴ In the Korean study, HBV DNA was detected in 14/44 (31.8%) patients with ALD

and HCC who underwent resection or OLT, in particular in 5/22 (22.7%) HCC subjects with cirrhosis and 9/22 (40.4%) HCC patients without cirrhosis.⁸⁶ Finally, in the Japanese study by Koga et al., OBI was found in 7/19 (36.8%) cases of non-B/non-C HCC alcohol abusers.⁹⁵ No association between OBI and liver fibrosis stage was found in the Korean study, which was the only one that evaluated this aspect in non-cirrhotic patients.⁸⁶

In summary, there are very few available studies on OBI in HCC patients with ALD and these show the same major limitations reported above in the “OBI and CryCLD” section. Cumulatively, 84 HCC cases with ALD were tested, and prevalence of OBI detection ranged from 31.8% to 58.3%. Finally, no data are available on OBI in ICC patients with alcohol-related liver injury.

2.3 | OBI and non-alcoholic fatty liver diseases

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders, the diagnosis of which requires the exclusion of any known causes of hepatic injury.^{96,97} Liver biopsy is still the only procedure that distinguishes NAFLD patients with non-alcoholic fatty liver (NAFL) from those affected by non-alcoholic steatohepatitis (NASH), which is a more severe and potentially progressive clinical/histologic form.⁹⁸⁻¹⁰² Despite the high prevalence of NAFLD in patients with metabolic disorders (i.e. insulin-resistance, obesity and metabolic syndrome), only one Italian study evaluated the presence of OBI in liver tissues of patients with NAFLD (Table 1), and three studies (two Italian and one Japanese) evaluated the presence of OBI in liver tissues of patients with HCC and NAFLD (Table 2). All these studies were performed in the past decade.

2.3.1 | OBI in patients with NAFLD

One of the Italian studies investigated OBI in people who underwent bariatric surgery. Four different HBV genomic regions were tested by *nested*-PCR analysis in 226 frozen liver specimens. Twenty-nine (12.8%) individuals were OBI positive. Notably, 24/29 (82.8%) OBI positive cases had NASH, whereas 5/29 (17.2%) had NAFL with a clear, highly significant association between OBI and NASH ($p = 0.001$). Importantly, multivariate analysis showed a high risk of developing NASH in obese subjects with OBI. Furthermore, the contemporary presence of portal inflammation and periportal fibrosis was found more frequently in OBI-positive than in OBI-negative individuals ($p = 0.03$), thus indicating a strong association between OBI and liver fibrosis.¹⁰³

2.3.2 | OBI in HCC patients with NAFLD

Three studies evaluated OBI in patients with HCC and NAFLD. Two of them were performed in Italy and one in Japan. HBV genomes were tested in DNA extracts from frozen liver tissues in the two Italian

studies and from FF specimens in the Japanese study. HBV DNA was tested by *nested*-PCR technique of multiple genomic regions in the Italian studies, and by real-time PCR assay in the Japanese study. OBI was detected in 8/12 (66.6%) and—respectively—in 2/9 (15.4%) liver tumour specimens from patients with NASH in the two Italian studies.^{84,104} Differently, in the Japanese study, OBI was found in 10/27 (37%) subjects with type 2 diabetes mellitus. However, OBI was not detected in any of the eight cases with histologically confirmed NASH.⁹⁵ In summary it can be stated that data concerning OBI and NAFLD—with or without liver cancer—are very few and largely divergent. Finally, no data are available on OBI and ICC in patients with NAFLD.

2.4 | OBI and autoimmune liver diseases

2.4.1 | OBI in patients with chronic autoimmune liver disease

There is only one study evaluating prevalence and possible clinical significance of OBI in patients with autoimmune liver diseases. In this Greek study, HBV genomes were tested by *nested*-PCR technique in DNA extracts from FF liver tissues. HBV DNA was detected in 3/14 (21.4%) liver specimens from patients with AIH (Table 1). To note is that none of the OBI patients underwent HBV reactivation under immunosuppressive during the follow-up.¹⁰⁵

2.4.2 | OBI in HCC patients with autoimmune liver disease

No data on OBI and liver cancers (both HCC and ICC) in patients with autoimmune liver disease is available so far. Thus, the field “OBI and autoimmune liver diseases” is still an unstudied argument.

3 | CONCLUSION

Despite the dramatic and rapid decline of HCV-related liver diseases because of the advent of DAAs, chronic hepatic illnesses are not decreasing in number worldwide, and the occurrence of liver cancers (both HCC and ICC) is actually increasing globally. ALD and NAFLD are the major causes of CLD, but a non-negligible number of patients are affected by liver diseases related to autoimmune disorders as well as unknown etiopathogenetic factors (namely, cryptogenic CLD).

A relatively large body of evidence suggests that OBI may contribute to worsening HCV-related liver disease, and to favouring HCC development in these patients.

This review critically evaluated the data relating to OBI prevalence and its possible clinical implications in non-HCV related liver diseases with or without liver cancers obtained from 26 available studies and based on HBV DNA testing in liver DNA extracts, which is considered

the gold-standard approach for the diagnosis of OBI. However, these data are fragmented and widely divergent from each other because of the presence of many gaps, such as largely different diagnostic approaches, data from categories of patients at various risk of parenteral infections living in geographic areas with a different prevalence of HBV infection in the general population. Therefore, comparison of the available data is not possible, and one may affirm that the scientific information about a possible association between OBI and non-HCV-related liver disease as well as liver cancer is presently very inadequate. It is hopeful that extensive and methodologically appropriate studies aimed at identifying the real prevalence and potential pathophysiological role of OBI in all the above-mentioned clinical conditions will be performed in the near future.

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