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# Role of occult hepatitis B virus infection in chronic hepatitis C

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## Abstract

The development of sensitive assays to detect small amounts of hepatitis B virus (HBV) DNA has favored the identification of occult hepatitis B infection (OBI), a virological condition characterized by a low level of HBV replication with detectable levels of HBV DNA in liver tissue but an absence of detectable surface antigen of HBV (HBsAg) in serum. The gold standard to diagnose OBI is the detection of HBV DNA in the hepatocytes by highly sensitive and specific techniques, a diagnostic procedure requiring liver tissue to be tested and the use of non-standardized non-commercially available techniques. Consequently, in everyday clinical practice, the detection of anti-hepatitis B core antibody (anti-HBc) in serum of HBsAg-negative subjects is used as a surrogate marker to identify patients with OBI. In patients with chronic hepatitis C (CHC), OBI has been identified in nearly one-third of these cases. Considerable data suggest that OBI favors the increase of liver damage and the development of hepatocellular carcinoma (HCC) in patients with CHC. The data from other studies, however, indicate no influence of OBI on the natural history of CHC, particularly regarding the risk of developing HCC.

**Key words:** Occult hepatitis B virus infection; Silent hepatitis B virus infection; anti-hepatitis B core antibody; Hepatitis B virus infection; Cirrhosis; Hepatocellular carcinoma

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**Core tip:** Occult hepatitis B infection (OBI) is a virological condition characterized by a low level of

hepatitis B virus (HBV) replication with HBV DNA detectable in liver tissue in the absence of detectable surface antigen of HBV in serum. Some studies indicate that OBI may favor the increase of liver fibrosis and the development of hepatocellular carcinoma in patients with chronic hepatitis C, whereas other investigations refute this. Here, we review all the available data on this topic and discuss the possible influence of OBI on the natural course of chronic hepatitis C.

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## INTRODUCTION

Approximately 170 million individuals are chronically infected with hepatitis C virus (HCV) worldwide<sup>[1-4]</sup>. HCV is a small, enveloped, positive-sense, single-stranded RNA virus of the genus *Hepacivirus* of the *Flaviviridae* family. Phylogenetic analysis of HCV isolates has generated the viral classification into six major genotypes (from 1 to 6) and more than 100 subtypes<sup>[5,6]</sup>. HCV is transmitted by percutaneous exposure to infected blood through intravenous drug injection and invasive medical procedures, and by permucosal exposure through unprotected intercourse with multiple partners<sup>[7,8]</sup>, particularly in human immunodeficiency virus (HIV)-positive men who have sex with men<sup>[9-12]</sup>.

HCV causes acute hepatitis that is frequently asymptomatic, and in its symptomatic form, it is characterized by nausea, malaise, and jaundice. The acute HCV infection resolves spontaneously in about one-third of the cases<sup>[13,14]</sup>, whereas the remaining two-thirds remain infected, circulate anti-HCV and HCV RNA, and usually show an indolent course or a slow progression to liver cirrhosis and hepatocellular carcinoma (HCC)<sup>[15]</sup>. In some cases, however, spontaneous acute exacerbations may develop, characterized by one or more peaks of the aminotransferase serum levels above the previous values<sup>[16-22]</sup>, which can frequently induce a deterioration of the liver disease. In some cases the progression to liver cirrhosis and HCC is rapid<sup>[15]</sup>, particularly when co-morbidities, an unfavorable genetic background, and unsafe lifestyle factors are present. Indeed, the outcome of chronic hepatitis C (CHC) is influenced by associated host factors (sex, age at infection, routes of transmission, immune response, genetic background), viral factors (HCV genotype and viral quasiespecies), co-morbidities (viral co-infection, insulin-resistance, liver steatosis, immunosuppressive clinical condition) and lifestyle factors (alcohol intake)<sup>[23-30]</sup>.

The development of sensitive assays to detect small amounts of hepatitis B virus (HBV) DNA has favored the identification of occult HBV infection (OBI), a virological condition characterized by a low level of HBV replication with HBV DNA detectable in the liver cells in the absence of detectable surface antigen of HBV (HBsAg) in serum. In patients with CHC, OBI has been identified in about one-third of HBsAg-negative/anti-HCV-positive subjects in the Mediterranean Basin and in more than 50% in East Asian countries<sup>[31-36]</sup>. Considerable data suggest that in patients with CHC, OBI may contribute to chronic liver damage and to the development of HCC<sup>[24,31,37-40]</sup>. Other studies, however, indicate that OBI does not influence the natural history of HCV infection, particularly as regards the risk of HCC development<sup>[41-43]</sup>. In this review article, which takes into account all the available literature data, the possible role of OBI in modifying the clinical course of CHC is evaluated and discussed.

## DEFINITION OF OBI

OBI has been defined as the presence of viral DNA in the liver tissue (regardless of HBV DNA detectability in serum) of individuals testing negative for serum HBsAg<sup>[36]</sup>. The gold standard to diagnose OBI is the detection of HBV DNA in the hepatocytes by highly sensitive and specific techniques [real-time polymerase chain reaction (PCR), nested PCR, and the use of oligonucleotide primers specific for different HBV genomic regions], a diagnostic procedure requiring liver tissue to be tested and the use of non-standardized non-commercially available techniques. Consequently, in everyday clinical practice, the detection of anti-hepatitis B core antibody (anti-HBc) in serum of HBsAg-negative subjects, a sign of previous acute hepatitis B (AHB), is used as a surrogate serum marker to identify subjects with OBI<sup>[39,43-49]</sup>. This option is supported by the observation that in patients experiencing immunosuppression, OBI, as defined by the presence of HBV DNA in liver tissue, mostly occurs in HBsAg-negative/anti-HBc-positive patients<sup>[44,50,51]</sup>. The data from a previous investigation on 89 HBsAg-negative patients with CHC showed the presence of HBV DNA in plasma, peripheral blood mononuclear cells, and/or liver tissue in 60% of the anti-HBs/anti-HBc-positive patients, in 80% of the anti-HBs-negative/anti-HBc-positive patients, and in 10% of those lacking both antibodies<sup>[44]</sup>.

## MECHANISMS OF LIVER DAMAGE BY OBI

In some cases, an underhand activity of the HBV genome is the persistence of mild hepatocellular necrosis for years after the resolution of self-limiting AHB<sup>[52,53]</sup>. The mechanism of liver damage due to OBI is still unclear, but there is some evidence that viral

factors may play a role in its development and in the related liver damage. In fact, the persistent synthesis of minute undetectable amounts of the virus or other viral transcripts produced by the HBV covalently closed circular DNA (cccDNA) seems capable of maintaining the HBV-specific memory T-cell response<sup>[33,54]</sup> and the production of cytokines, such as tumor necrosis factor- $\alpha$  and interferon- $\gamma$ <sup>[55,56]</sup>. In addition, mutations in the X region of HBV may reduce the ability of the X protein to transactivate host cellular proteins essential for viral replication, which may lead to the reduction of HBV DNA replication and the lack of HBsAg serum expression<sup>[57]</sup>.

We should remember, however, that a rare escape mutation in the S region decreases the reactivity in the HBsAg detection assays<sup>[58]</sup> and is responsible for an "overt" HBsAg-negative infection that might mimic OBI.

## OBI AND THE PROGRESSION OF LIVER FIBROSIS

### *The impact of OBI, as detected by the presence of serum anti-HBc, on the progression of liver fibrosis in patients with CHC*

As mentioned above, the detection of serum anti-HBc has been used as a surrogate marker of the presence of liver HBV DNA to detect OBI in numerous investigations exploring the correlation between this virological condition and liver fibrosis in patients with CHC (Table 1). In the year 2000, our group<sup>[24]</sup> published a cross-sectional, case-control study on 174 Caucasian HBsAg-negative CHC patients. We showed that the prevalence of cases with cirrhosis in the anti-HBc-positive subgroup was significantly higher than in the anti-HBc-negative subgroup, suggesting a role of OBI in fibrosis progression. Similar data were obtained in a cross-sectional study performed in the same period by De Maria *et al.*<sup>[37]</sup> on 285 HCV-infected patients. A few years later, a cross-sectional study<sup>[38]</sup> confirmed the relationship between the presence of anti-HBc and liver cirrhosis in 119 Italian anti-HCV-positive/HBsAg-negative patients. A study on 129 Portuguese anti-HCV-positive patients published in 2005 found an independent association between previous HBV infection and biopsy-proven liver cirrhosis<sup>[59]</sup>. Subsequent studies further confirmed the unfavorable influence of OBI, as detected by the presence of anti-HBc in HBsAg-negative patients, on the clinical course of CHC. A cross-sectional Brazilian study<sup>[60]</sup> found OBI was a predictor of significant necroinflammation and fibrosis; El-Sherif *et al.*<sup>[61]</sup> demonstrated that the prevalence of cases with advanced liver disease was higher in patients with OBI than in those without. Coppola *et al.*<sup>[62]</sup> demonstrated that OBI was an independent predictor of HCV-related cirrhosis in a cross-sectional study on 222 patients from southern Italy.

Conflicting data have been published by other authors. Verbaan *et al.*<sup>[63]</sup> did not find any association between OBI and the progression to cirrhosis in 99 CHC patients, whereas in an Egyptian study, patients with OBI were more likely than those without to show HCV-related cirrhosis<sup>[64]</sup>. No association was observed between serum anti-HBc and the degree of liver fibrosis in a study from Spain<sup>[65]</sup> or between anti-HBc and the entity of necroinflammation or fibrosis in a French study<sup>[66]</sup>.

### *The impact of OBI, as detected by the presence of HBV DNA in liver tissue, plasma, or peripheral blood mononuclear cells, on the progression of liver fibrosis in patients with CHC*

Table 2 shows the data from several studies on the relationship between the degree of liver fibrosis and the presence of OBI, as demonstrated by detecting HBV DNA in the liver tissue, plasma, or peripheral blood mononuclear cells (PBMC) of HBsAg-negative patients with CHC (Table 2). One of the first studies to suggest a clinical impact of OBI was a cross-sectional study published in 1999 by Cacciola *et al.*<sup>[31]</sup>, which showed in 200 CHC patients that 33.3% of those with detectable liver HBV DNA had cirrhosis whereas only 19.4% of HBV-DNA-negative patients did. Similar data were observed in 203 HCV-infected patients in a French study published in 2007<sup>[67]</sup>, in which patients with plasma HBV-DNA showed more advanced fibrosis ( $P < 0.001$ ) than those who were HBV-DNA-negative. In 2008, Matsuoka *et al.*<sup>[68]</sup> tested 468 Japanese HBsAg-negative patients with CHC for the presence of plasma HBV DNA and found over a mean follow-up period of 6.7 years that cirrhosis and HCC occurred more frequently in those with OBI than in those without. These data were confirmed in a prospective study from Italy, in which HBV DNA was detected in the liver tissue of 326 CHC patients<sup>[69]</sup>, and progression to cirrhosis or development of HCC were more frequent in those with OBI than in those without.

Conflicting data, however, have come from several studies, with all but one detecting HBV DNA only in plasma. In a Japanese study on 65 HCV-infected patients, liver cirrhosis was detected with a similar frequency in CHC patients with or without OBI<sup>[35]</sup>. In 2004, Toberson *et al.*<sup>[70]</sup> reported no association between OBI and the grading or staging in 180 anti-HCV-positive drug users. A cross-sectional study published in the same year on 59 Brazilian patients<sup>[71]</sup> showed a similar degree of liver fibrosis in those with or without OBI. Hui *et al.*<sup>[72,73]</sup> published in 2006 two retrospective cohort studies on 74 CHC patients and 118 subjects with a recurrent HCV infection after liver transplantation, respectively. In both studies, liver fibrosis, as detected by comparing two consecutive liver biopsies, showed a similar increase in patients with or without OBI. In addition, Sagnelli *et al.*<sup>[44]</sup> did not find any association between the degree of liver

**Table 1** The studies evaluating the role of anti-HBc in the development of cirrhosis in HBsAg-negative patients with chronic hepatitis C

First author, year	No. of patients	Country	Type of study	Cirrhosis, positive/tested, n/n (%)		P value
				HBcAb <sup>+</sup>	HBcAb <sup>-</sup>	
Verbaan 1998 <sup>[63]</sup>	99	Sweden	Cross-sectional	10/44 (22.7)	10/55 (18.2)	NS
De Maria 2000 <sup>[37]</sup>	285	United States	Cross-sectional	29/90 (32.2)	41/195 (21.0)	< 0.05
Sagnelli 2000 <sup>[24]</sup>	174	Italy	Case-control	9/76 (11.8)	6/98 (6.1)	< 0.005
Giannini 2003 <sup>[38]</sup>	119	Italy	Cross-sectional	20/48 (41.6)	15/71 (21.0)	0.020
Dinis-Ribeiro 2005 <sup>[59]</sup>	129	Portugal	Cross-sectional	14/30 (46.6)	32/99 (32.3)	HR:1.35 (1.01-2.69) <sup>1</sup>
Helmy 2006 <sup>[64]</sup>	169	Saudi Arabia	Cross-sectional	14/85 (16.5)	45/84 (53.6)	0.0001
Laguno 2008 <sup>[65]</sup>	238	Spain	Cross-sectional	78/142 (55) <sup>2</sup>	49/96 (51) <sup>2</sup>	0.720
Carvalho-Filho 2009 <sup>[60]</sup>	111	Brazil	Cross-sectional	24/31 (77.4) <sup>3</sup>	40/80 (50.0) <sup>3</sup>	0.017
El-Sherif 2009 <sup>[61]</sup>	100	Egypt	Cross-sectional	68/71 (95.8)	23/29 (79.3)	0.009
Levast 2010 <sup>[66]</sup>	140	France	Cross-sectional	5/45 (11.1)	16/95 (16.8)	NS
Coppola 2014 <sup>[62]</sup>	222	Italy	Cross-sectional	21/77 (27.3)	12/145 (8.3)	< 0.009

<sup>1</sup>Hazard ratio for progression to cirrhosis in HBsAb/HBcAb<sup>+</sup> patients; <sup>2</sup>Advanced fibrosis (Scheuer score > 2); <sup>3</sup>Advanced fibrosis (Metavir score F2-F4). NS: Not significant.

**Table 2** The studies evaluating the role of hepatitis B virus DNA in serum and/or liver tissue in the development of cirrhosis in surface antigen of hepatitis B virus-negative patients with chronic hepatitis C

First author, year	No. of patients	Country	Type of study	Liver disease	Sample for HBV-DNA detection	Cirrhosis, positive/tested, n/n (%)		P value
						OBI <sup>+</sup>	OBI <sup>-</sup>	
Cacciola 1999 <sup>[31]</sup>	200	Italy	Cross-sectional	CH/cirrhosis	Liver	22/66 (33.3)	26/134 (19.4)	0.040
Fukuda 1999 <sup>[35]</sup>	65	Japan	Cross-sectional	CH/cirrhosis	Serum	5/34 (14.7)	3/31 (9.7)	NS
Giannini 2003 <sup>[38]</sup>	119	Italy	Cross-sectional	CH/cirrhosis	Serum	2/8 (25.0)	32/111 (28.8)	NS
Silva 2004 <sup>[71]</sup>	59	Brazil	Cross-sectional	CH/cirrhosis	Serum	2/10 (20.0)	6/49 (12.0)	NS
Toberson 2004 <sup>[70]</sup>	180	United States	Cross-sectional	CH/cirrhosis	Serum	8/81 (9.9) <sup>1</sup>	11/99 (11.1) <sup>1</sup>	NS
Hui 2006 <sup>[72]</sup>	74	United States	Cohort	CH/cirrhosis	Serum	6/31 (19.4) <sup>2</sup>	8/43 (18.6) <sup>2</sup>	NS
Hui 2006 <sup>[73]</sup>	118	United States	Cohort	Liver transplantation	Serum	8/41 (19.5) <sup>2</sup>	13/77 (16.9) <sup>2</sup>	NS
Mrani 2007 <sup>[67]</sup>	203	France	Cross-sectional	CH/cirrhosis	Serum	28/47 (60.0)	52/156 (33.3)	< 0.001
Laguno 2008 <sup>[65]</sup>	90	Spain	Cross-sectional	CH/cirrhosis	Serum	8/15 (53.3) <sup>3</sup>	37/75 (49.3) <sup>3</sup>	NS
Matsuoka 2008 <sup>[68]</sup>	468	Japan	Cross-sectional	CH/cirrhosis	Serum	37/204 (18.1)	28/264 (10.6)	0.002
Sagnelli 2008 <sup>[44]</sup>	89	Italy	Cohort	CH/cirrhosis	Serum/PBMC/liver	10/37 (27.0) <sup>3</sup>	19/52 (36.5) <sup>3</sup>	NS
Emara 2010 <sup>[74]</sup>	155	Egypt	Cross-sectional	CH/cirrhosis	Serum	0/6 (0.0)	4/149 (2.7)	0.020
Squadrito 2013 <sup>[69]</sup>	326	Italy	Cohort	CH/cirrhosis	Liver	30/128 (23.4)	25/198 (12.6)	< 0.01

<sup>1</sup>Advanced fibrosis (Ishak score 3-6); <sup>2</sup>Severe fibrosis (Metavir score F3-F4); <sup>3</sup>Advanced fibrosis (Scheuer score > 2); <sup>4</sup>Severe fibrosis (Scheuer score 3-4). CH: Chronic hepatitis; PBMC: Peripheral blood mononuclear cells; NS: Not significant; HBV: Hepatitis B virus; OBI: Occult HBV infection.

fibrosis and OBI in a prospective study where OBI was assessed through the detection of HBV DNA in plasma, PBMC, and liver tissue in 89 patients with CHC. Finally, Emara *et al.*<sup>[74]</sup> studied 155 Egyptian CHC patients and found the prevalence of cases with cirrhosis in patients with circulating HBV DNA was significantly lower than in those without.

## OBI AND THE OCCURRENCE OF HCC

There is biological, epidemiological, and clinical evidence demonstrating that the oncogenic potential of HBV may induce the development of HCC both in patients with cirrhosis and in those with a milder liver disease. Chronic HBV infection accounts for approximately 50% of the total cases and for virtually all childhood HCC, and prospective cohort studies showed a 5- to 100-fold increase in the risk of developing HCC among HBsAg carriers compared with uninfected subjects<sup>[75]</sup>. In spite of this, the role of OBI

in the development of HCC in patients with chronic hepatitis due to etiological agents other than HBV, firstly HCV, is still a matter of debate in the scientific community. Using anti-HBc positivity or the presence of HBV DNA in plasma or liver tissue as a sign of OBI, several research groups have investigated the role of OBI in the development of HCC in HBsAg-negative patients with CHC.

### *The impact of OBI, as detected by the presence of serum anti-HBc, on the development of HCC in patients with CHC*

The studies that evaluated the impact of OBI, as detected by the presence of anti-HBc in serum, on the development of HCC are listed in Table 3. In 1996, Chiba *et al.*<sup>[76]</sup> published data from a cohort study on 412 Japanese patients with CHC with or without cirrhosis and showed a higher incidence of HCC in those with OBI than in those without (23.7% vs 7.5%,  $P = 0.02$ ). The same authors reported similar



**Table 3** The studies evaluating the role of anti-hepatitis B core in the development of hepatocellular carcinoma in surface antigen of hepatitis B virus-negative patients with chronic hepatitis C

First author, year	No. of patients	Country	Type of study	Liver disease	HCC, positive/tested, n/n (%)		P value
					Anti-HBc <sup>+</sup>	Anti-HBc <sup>-</sup>	
Takano 1995 <sup>[84]</sup>	61	Japan	Cohort	CH	9/36 (25.0)	2/25 (8.0)	NS
Chiba 1996 <sup>[76]</sup>	412	Japan	Cohort	CH/cirrhosis	47/198 (23.7)	16/214 (7.5)	0.020
Chiba 1996 <sup>[77]</sup>	204	Japan	Cross-sectional	cirrhosis	92/128 (71.9)	36/76 (47.4)	0.0005
Shiratori 1997 <sup>[86]</sup>	502	Japan	Case-control	CLD	111/263 (42.2)	81/239 (33.9)	NS
IIHCSG 1998 <sup>[85]</sup>	451	Italy	Cohort	CLD	34/206 (16.5)	32/245 (13.1)	NS
Dutta 1999 <sup>[78]</sup>	51	Australia	Case-control	CH/cirrhosis	10/17 (58.8)	7/34 (20.6)	0.010
Marusawa 1999 <sup>[34]</sup>	2366	Japan	Cross-sectional	CH/cirrhosis	363/1047 (34.7)	248/1319 (18.8)	< 0.01
Hiraoka 2003 <sup>[48]</sup>	202	Japan	Case-control	CLD	109/250 (43.6)	93/342 (27.2)	NS
Imazeki 2003 <sup>[79]</sup>	459	Japan	Cohort	CH/cirrhosis	37/160 (23.1)	26/299 (8.7)	< 0.05
Hasegawa 2005 <sup>[87]</sup>	140	Japan	Cohort	CH/cirrhosis	9/64 (14.0)	9/76 (11.8)	NS
Tanaka 2006 <sup>[80]</sup>	74	Japan	Cohort	CLD	13/53 (24.5)	0/21 (0.0)	0.012
Bruno 2007 <sup>[49]</sup>	160	Italy	Cohort	Cirrhosis	29/86 (33.7)	25/74 (33.8)	0.390
Ikedo 2007 <sup>[81]</sup>	846	Japan	Cohort	CH/Cirrhosis	130/392 (33.1)	107/454 (23.6)	IRR: 1.03 (0.66-1.56) <sup>1</sup> IRR: 1.58 (1.12-2.22) <sup>2</sup>
Adachi 2008 <sup>[82]</sup>	123	Japan	Cohort	Cirrhosis	57/96 (59.3)	10/27 (37.0)	0.0039
Alencar 2008 <sup>[89]</sup>	50	Brazil	Cross-sectional	Cirrhosis	5/12 (41.7)	12/38 (31.6)	NS
Miura 2008 <sup>[84]</sup>	141	Japan	Cohort	CH	22/83 (26.5)	11/58 (19.0)	0.700
Ramia 2008 <sup>[88]</sup>	3364	Lebanon	Cross-sectional	CH/cirrhosis/ healthy controls	7/408 (1.7)	2/2956 (0.07)	0.507
Stroffolini 2008 <sup>[47]</sup>	693	Italy	Cohort	Cirrhosis	44/303 (14.5)	57/390 (12.0)	0.900
Ohki 2010 <sup>[90]</sup>	1262	Japan	Cohort	CLD	160/522 (30.6)	179/740 (24.2)	0.630
Lok 2011 <sup>[43]</sup>	273	United States	Case-control	CH/Cirrhosis	38/121 (31.4)	53/152 (35.0)	0.540
Reddy 2013 <sup>[83]</sup>	459	United States	Case-control	CLD	95/229 (41.5)	27/230 (11.7)	0.010
Tsubouchi 2013 <sup>[91]</sup>	400	Japan	Cohort	CLD	24/213 (11.3)	14/187 (7.5)	0.280

<sup>1</sup>Incidence Rate Ratio for HCC in patients with chronic hepatitis; <sup>2</sup>Incidence Rate Ratio for HCC in patients with cirrhosis. CH: Chronic hepatitis; CLD: Chronic liver disease; NS: Not significant; HCC: Hepatocellular carcinoma; HBc: Hepatitis B core.

results in a cross-sectional study on 204 cirrhotic patients<sup>[77]</sup>. In 1999, a case-control study on 51 Australian patients with CHC with or without cirrhosis showed a correlation between the occurrence of HCC and male gender, lower serum albumin level, and anti-HBc positivity<sup>[78]</sup>. In the same year, Marusawa *et al.*<sup>[34]</sup> published a study on 2014 patients with CHC with or without cirrhosis and showed that patients with OBI had a significantly higher rate of HCC than those without (34.7% vs 18.8%). Similar results were reported in a cohort study<sup>[79]</sup> on 459 Japanese patients followed up for a mean period of 6.6 years, where the incidence of HCC correlated with the age of the patients, the degree of liver fibrosis, alanine aminotransferase (ALT) levels and anti-HBc positivity. Another Japanese cohort study<sup>[80]</sup> on 74 CHC patients showed a correlation between the incidence of HCC and anti-HBc positivity. Ikeda *et al.*<sup>[81]</sup> performed a prospective study on 872 Japanese CHC patients and observed in those with liver cirrhosis a significantly higher occurrence of HCC in those with OBI than in those without, a difference not observed in patients with a lower degree of liver fibrosis. Adachi *et al.*<sup>[82]</sup> followed up 123 Japanese cirrhotic patients for a mean period of 53.3 mo and identified as independent predictors of HCC development male gender, higher  $\alpha$ -fetoprotein and ALT serum values, and the presence in serum of anti-HBc but not HBV DNA. A case-control study recently conducted by Reddy *et al.*<sup>[83]</sup> in North America on 459 anti-HCV-positive patients with CHC

showed a significantly higher frequency of HCC in those with OBI than in those without.

Several studies, however, produced different results. A prospective investigation<sup>[84]</sup> on 61 CHC patients found no difference in HCC occurrence between groups of patients with or without previous exposure to HBV. The cohort study conducted in 1998 by the Italian IFN- $\alpha$  Hepatocellular Carcinoma Study Group<sup>[85]</sup> on 451 anti-HCV-positive subjects showed a similar incidence of HCC in anti-HBc-positive and -negative cases. In 1997, a Japanese study on 502 patients found a similar frequency of HCC in anti-HBc-positive and anti-HBc-negative patients<sup>[86]</sup>. Hiraoka *et al.*<sup>[48]</sup> in 2003 and Hasegawa *et al.*<sup>[87]</sup> in 2005 also published similar data. Likewise, Bruno *et al.*<sup>[49]</sup> demonstrated that anti-HBc positivity was not independently associated with HCC occurrence in 163 Italian consecutive cirrhotic patients with HCV infection followed up for a median period of 10.7 years. Similarly, Stroffolini *et al.*<sup>[47]</sup> found no association between serum anti-HBc positivity and HCC development in a multicenter retrospective cohort study of 693 Italian cirrhotic patients. This association was not found also in two cross-sectional studies, one conducted in Lebanon<sup>[88]</sup> and one in Brazil<sup>[89]</sup>. In a cohort study<sup>[90]</sup> on 1262 Japanese HCV patients, anti-HBc positivity was associated with the development of HCC in a univariate analysis but not in a multivariate analysis considering age and gender as confounding factors. Finally, Tsubouchi *et al.*<sup>[91]</sup> published in 2013 the results of a prospective study on 400 anti-HCV-positive

**Table 4** The studies evaluating the role of hepatitis B virus DNA in serum and/or liver tissue in the development of hepatocellular carcinoma in surface antigen of hepatitis B virus-negative patients with chronic hepatitis C

First author, year	No. of patients	Country	Type of study	Liver disease	Sample for HBV-DNA detection	HCC, positive/tested, n/n (%)		P value
						OBI+	OBI-	
Pollicino 2004 <sup>[39]</sup>	226	Italy	Case-control	CH/cirrhosis/ HCC	Liver	45/101 (44.5)	28/125 (22.4)	< 0.001
Tanaka 2004 <sup>[80]</sup>	93	Japan	Cross-sectional	CH/cirrhosis/ HCC	Serum	25/32 (78.1)	25/61 (41.0)	< 0.001
Hasegawa 2005 <sup>[87]</sup>	140	Japan	Cohort	CH/cirrhosis	Serum	2/11 (18.2)	16/129 (12.4)	NS
Squadrito 2006 <sup>[40]</sup>	134	Italy	Cohort	CH/cirrhosis	Liver	8/53 (15.1)	1/81 (1.2)	0.002
Branco 2007 <sup>[93]</sup>	66	Brazil	Cross-sectional	CH/HCC/health controls	Serum/liver <sup>1</sup>	7/10 (70.0)	13/56 (23.2)	0.029
Adachi 2008 <sup>[82]</sup>	123	Japan	Cohort	Cirrhosis	Serum	6/14 (42.9)	60/109 (55.0)	NS
Matsuoka 2008 <sup>[68]</sup>	468	Japan	Cohort	CH/cirrhosis	Serum/liver <sup>1</sup>	29/204 (14.2)	9/264 (3.4)	0.0001
Miura 2008 <sup>[94]</sup>	141	Japan	Cohort	CH	Serum	4/8 (50.0)	29/133 (21.8)	0.0036
Obika 2008 <sup>[41]</sup>	167	Japan	Cohort	CLD	Liver	2/25 (8.0)	10/142 (7.0)	NS
Shetty 2008 <sup>[42]</sup>	44	United States	Cross-sectional	cirrhosis	Liver	12/22 (54.5)	8/22 (36.3)	NS
Lok 2011 <sup>[43]</sup>	83	United States	Case-control	CH/Cirrhosis	Liver	3/16 (18.7)	25/67 (37.3)	NS
Squadrito 2013 <sup>[69]</sup>	94	Italy	Cohort	CH/cirrhosis	Liver	13/37 (35.1)	5/57 (8.1)	< 0.01

<sup>1</sup>OBI assessed with immunochemistry for HBsAg and/or HBeAg. OBI: Occult HBV infection; CH: Chronic hepatitis; CLD: Chronic liver disease; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

patients and showed no difference in the incidence of HCC and of cumulative liver-related mortality in patients with and without OBI.

#### **The impact of OBI, as detected by the presence of HBV DNA in serum or liver tissue, on the development of HCC in patients with CHC**

The studies listed in Table 4 investigated the correlation between HBV-DNA positivity in plasma or in liver tissue and the development of HCC in CHC patients (Table 4). Pollicino *et al.*<sup>[39]</sup> tested for HBV DNA in the tumorous tissue of 73 patients with CHC and HCC and a liver sample of 153 CHC patients used as controls and observed a significant association between OBI and HCC, irrespective of age or gender. In a cross-sectional study published in 2004, Tanaka *et al.*<sup>[92]</sup> demonstrated a significantly higher frequency of cases with HCC in CHC patients with plasma HBV-DNA positivity than in those without. Branco *et al.*<sup>[93]</sup> studied 26 Brazilian CHC patients, 20 with HCV-related HCC and 20 healthy controls, for HBV DNA in serum and for HBsAg and HBeAg immunochemistry in liver tissue and found a higher prevalence of HCC in the 10 patients with OBI than in the 56 without (70% vs 23%). Seeking HBV DNA in the liver tissue of 124 CHC patients followed up for a mean period of 82.8 mo, Squadrito *et al.*<sup>[40]</sup> found a significant association between OBI and HCC occurrence, a finding confirmed in a study they published more recently<sup>[69]</sup>. In 2008, a cohort study<sup>[94]</sup> enrolling 141 Japanese CHC patients identified OBI as an independent predictor of HCC development.

Some published studies, however, report conflicting data. A prospective study by Obika *et al.*<sup>[41]</sup> on 167 patients with CHC showed a similar incidence rate of HCC over a mean follow-up of 42.5 mo in patients with or without HBV DNA in liver tissue (8% vs 7%, respectively). In 2008, Shetty *et al.*<sup>[42]</sup> published a study on 56 patients selected for orthotopic liver transplantation (OLT), 44 of whom underwent OLT.

Serum HBV DNA was detected in 28% of the 56, and liver HBV DNA was detected in 50% of the 44. Explant-proven HCC was found in 12 of the 22 (54.5%) patients with OBI and in eight of the 22 (36.3%) without, a difference not statistically significant. Lastly, Lok *et al.*<sup>[43]</sup> tested for HBV DNA in frozen liver samples of 83 CHC patients, 28 with HCC and 55 controls, and found no association between OBI and HCC.

## **CONCLUSION**

The clinical impact of OBI on the natural history of CHC has been extensively investigated, but the available data are conflicting and do not allow for conclusions to be drawn on this topic. One of the main reasons for this inconsistency is the heterogeneity of the methods used to detect OBI. In fact, the detection of HBV DNA in liver tissue of HBsAg-negative subjects can be considered of high sensitivity and high specificity, and that of HBV DNA in plasma of high specificity and moderate sensitivity. In addition, the detection of anti-HBc in serum should be considered of moderate specificity and moderate sensitivity in this setting, although anti-HBc-negative subjects may show HBV DNA in the liver tissue. Furthermore, the variety of diagnostic molecular assays used to identify HBV DNA in plasma and liver tissue of HBsAg-negative subjects possess different sensitivities, bringing considerable heterogeneity in the results. Indeed, in the majority of studies, anti-HBc in serum or HBV DNA in plasma was used to detect OBI, since this method is cheaper, less invasive, and less time-consuming than the detection of HBV DNA in the liver tissue.

Other reasons for the substantial variability in the prevalence of OBI in published studies may be the differences in the extent of the spread of HBV infection in the various geographical areas, the variability in the viral characteristics, and the heterogeneity of the enrolment criteria regarding age, gender,

immunological and ethnic background, and social habits of the subjects examined.

In addition, OBI itself is a virological condition of different origins; most patients having a self-limiting AHB and a minority from the pool of HBsAg chronic carriers, of whom nearly 1% per year clear serum HBsAg. Subjects with OBI of different origins may be present in different proportions in the studies published, and OBI itself may have a different outcome and a different impact on the clinical course of CHC in relation to its origin.

In light of this, we should conclude that the present knowledge on the clinical impact of OBI on the progression of liver fibrosis and on the development of HCC is still insufficient.

In order to reduce the effect of different methods with different sensitivity and specificity used to detect OBI in the published studies, we performed a comprehensive analysis of the studies in which OBI was identified by the detection of HBV DNA in the liver tissue, but the results remained conflicting. In fact, regarding the progression to cirrhosis, we have only three studies, two from the same Italian group<sup>[31,69]</sup> showing a higher rate of patients with cirrhosis in CHC with OBI than in those without, and one from another Italian group<sup>[44]</sup> showing no difference. Regarding the development of HCC, six studies were analyzed, three of which were from the same Italian research group<sup>[39,40,69]</sup>, showing a higher rate of patients with HCC in the group of patients with OBI than in those without, whereas the other three studies, one from Japan<sup>[41]</sup> and two from the United States<sup>[42,43]</sup>, showed no difference. The selection criteria were certainly different from one study to another, but the methods to detect HBV DNA in the liver were similar, albeit not identical. Therefore, the question whether OBI might influence the natural course of CHC remains unanswered.

A strong contribution to defining the clinical impact of OBI could come from a prospective international study considering a large number of HBsAg-negative patients with CHC selected with pre-established criteria and using as sign of OBI the detection of HBV DNA in the liver tissue performed with a highly sensitive technique in a single, high standard laboratory.

No standardized strategy, at least to our best knowledge, is at present recommended for the management of OBI in patients with CHC. In particular, because of the uncertainty surrounding the clinical impact of OBI, it is not clear whether close monitoring is an adequate measure or whether the administration of an anti-HBV nucleot(s)ide to prevent both the progression of fibrosis and the onset of HCC is necessary. In this case, the low cost of the anti-HBV nucleoside lamivudine, which is now obsolete in other HBV treatment settings because of its low genetic barrier and the consequent high risk of inducing viral resistance, might be the drug of choice to suppress the low level of HBV replication characterizing OBI.

In conclusion, some studies indicate that OBI

unfavorably affects the progression of liver fibrosis and the development of HCC in patients with CHC, an observation not confirmed in other investigations. The data from prospective studies applying a careful selection of patients and a highly sensitive, standardized method to identify HBV DNA in the liver tissue may help clarify this important issue.

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