

Occult Hepatitis B Virus Infection: An Old Entity With Novel Clinical Involvements

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Background. Occult hepatitis B infection (OBI) is recognized as a risk factor for cirrhosis and hepato-cellular carcinoma. However, OBI brings together a large spectrum of patients who might harbor different characteristics and prognosis.

Methods. We analyzed the databases of a university hospital in Paris to identify OBI among patients (n = 3966) concomitantly tested for hepatitis B virus (HBV) DNA and serology during a 7-year period. OBI patients were gathered into clinical entities according to their clinical records.

Results. Forty-seven OBIs were identified (1.2%). All patients had detectable anti-HBc, isolated (n = 26) or associated with anti-HBs (n = 21). The proportion of OBIs was 3.4% for patients with isolated anti-HBc and 4.2% for patients with both anti-HBc and anti-HBs. Four clinical categories of OBI patients were identified: patients with a passed HBV infection with HBs Ag clearance (group A, 23.4%); HBV-exposed patients receiving immunosuppressive therapy (group B, 29.8%); HIV/HBV-coinfected patients with therapy discontinuation (group C, 17%); HBV-exposed patients with severe liver conditions (group D, 29.8%). Significant follow-up was available for 32 patients, showing a more deleterious prognosis in group D patients, associated more with their underlying condition than the OBI status.

Conclusions. OBI is a heterogeneous condition with various clinical implications.

Keywords. hepatitis B virus; immunosuppression; occult B infection.

Chronic hepatitis B infection (CHB) is the most common cause of liver disease worldwide, leading to liver cirrhosis or hepato-cellular carcinoma (HCC). CHB is defined by the persistence of HBs antigen (Ag) in the sera at least 6 months after acute infection. In untreated patients, HBs Ag positivity is associated with various levels of viral replication, according to the natural history of chronic infection and the clinical stages of the disease. However, sensitive polymerase chain reaction (PCR) assays have defined another subgroup of the CHB condition, occult hepatitis B infection (OBI), characterized by the presence of detectable viral hepatitis B virus (HBV) DNA in plasma, without any detection of HBs antigen [1, 2]

The OBI condition, which always implies the persistence of HBV in the liver, appears to be a heterogeneous entity according to the presence of key parameters such as (1) a past known HBs Ag-positive CHB; (2) detectable anti-HBc (+/- anti-HBs)

antibodies; (3) mutations in specific regions of the HBs Ag coding gene; (4) other conditions such as liver disease, HIV, or hepatitis C virus (HCV) coinfection or immunosuppressive therapies [2]. This heterogeneity reflects various mechanisms that account for this atypical profile and partially explains the variation of OBI prevalence across studies. The respective sensitivity of virological assays for HBs Ag and HBV DNA detection is also of critical importance for detecting OBI. However, OBI is clearly recognized as a risk factor for cirrhosis and HCC, as well as for HBV reactivation under immunosuppressive therapy [3–5]. To better understand the clinical and virological characteristics and consequences of OBI, we estimated its prevalence and risk factors in a large retrospective observational study conducted in a mixed university teaching hospital and community-based setting.

METHODS

Study Design

The study was conducted in the Saint-Antoine University Hospital in Paris. To be included in the present analysis, participants had to have HBV screening from January 2010 to December 2016. The laboratory databases were used to retrieve results of HBV virological markers and to collect demographic and other laboratory parameters such as liver enzymes and HIV or HCV status. Through a unique identifying number allocated after anonymization, biological databases were linked to clinical databases to collect data focused on the HBV history of each patient, liver disease related or not to HBV, and ongoing

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immunosuppressive therapy known to reactivate HBV [6]. When available, the outcome of patients diagnosed with OBI was also analyzed.

OBI was defined by the concomitant detection of HBV DNA without detection of HBs Ag. When the positive detection of viral DNA was below 20 UI/mL (the lower quantification limit [LLQ] of the PCR method used), the plasma sample was systematically controlled on a second extraction. If the result was confirmed, the patient was classified as OBI.

As the study was purely observational and retrospective from medical databases, informed consent did not have to be individually obtained according to French ethical law. However, the databases were declared to the Conseil National Informatique et Libertés (www.cnil.fr).

Virological Methods

The Abbott Architect HBsAg Qualitative II kit was used for detection of HBs Ag, with a sensitivity of 0.02 IU/mL. The Architect immunoassay (Architect, Abbott Diagnostics, Rungis, France) was also used for the detection of anti-HBc Abs, as well as the quantification of anti-HBs Abs.

Plasma HBV-DNA viral load (VL) was quantified using a commercial PCR assay (COBAS AmpliPrep/COBAS TaqMan v2.0). The lower detection limit (LLD) of the technique is 9 IU/mL, and the LLQ is 20 IU/mL.

DNA Sequencing and Analysis

The Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS) consensus method for sequencing the overlapping Pol/S genes was used to detect potential escape mutants for HBs Ag detection [7]. This procedure was applied for all OBI patients with an HBV viral load above 500 IU/mL.

Briefly, a 630 base pair fragment covering the entire S gene starting from amino acid 33 was generated by a nested PCR and sequenced on both stands using an ABI 3500 XL Dx (Applied Biosystems, Forster City, CA). Sequence alignment and analysis used SeqScape software, v2.7.

The S and Pol open reading frames were analyzed using the Internet tools HIV grade HBV Drug Resistance Interpretation and Geno2Pheno (HBV) [8]. Every amino acid substitution in the S gene was verified, taking into account potential polymorphisms linked to HBV-infecting genotype.

Statistical Analysis

Data were analyzed using STATA (v12.1, College Station, TX), and significance was determined using a *P* value <.05. Data were expressed as median (interquartile range [IQR]) for continuous variables and as absolute numbers and percentages for discrete variables. The prevalence of OBI was estimated by the number of positive results within the total number of screened individuals and expressed with its confidence interval (95% CI) using the exact binomial method. The chi-square test of independence or Fisher exact test based on a 2 × 2 contingency table was used for discrete data, whereas clinical and virological

characteristics were compared between OBI patients with various HBV serologic profiles using the Kruskal-Wallis test for continuous variables.

RESULTS

Global HBV Profiles

During the study period, 33 996 patients were screened for HBs antigen, anti-HBc, and anti-HBs antibodies. The serological HBV profiles, determined from the first available serum sample of each included individual, were as follows: 16 083 (47.3%) were negative for all HBV serological markers (nonimmune status); 11 186 (32.9%) had a postvaccination profile (anti-HBs positive alone), whereas 3235 (9.5%) exhibited a profile of “resolutive past infection” (negative for HBs Ag and positive for anti-HBc and anti-HBs antibodies). Additionally, 1892 (5.5%) patients had the typical profile for current HBV infection (associating the positivity of HBs Ag and anti-HBc Ab with no anti-HBs Ab). Apart from these classical profiles, we observed 25 (0.07%) patients who were positive for all 3 markers, whereas 1575 (4.6%) patients presented an isolated positivity of anti-HBc Ab.

Among these 33 996 individuals with available serology, 3966 had a concomitant quantitation of HBV VL. [Figure 1](#) summarizes the general workflow of the study, whereas [Table 1](#) describes the results of HBV VL according to the HBV serological profiles of the study population. None of the vaccinated or nonimmune patients displayed a positive PCR, which means that no OBI was detected among HBV-seronegative patients. On the contrary, 70% of the HBs Ag-positive patients had a detectable HBV VL at the first sample evaluated in this study. Interestingly, all the 14 patients who were positive for the 3 HBV serological markers had a positive PCR, confirming that this atypical profile indeed corresponds to an HBV infection, despite of the presence of detectable anti-HBs Abs (median [IQR], 50.5 [22–197] mIU/mL). The median HBV VL was furthermore statistically higher for this last population (median [IQR], 101 566 [171–855 000 UI/mL) compared with the HBV-infected population showing a classical profile without anti-HBs Abs (median [IQR], 923 [112–9211] UI/mL; *P* = .001). These data likely result from a delay in anti-HBV therapy initiation. Indeed, only 1 out of the 14 patients was receiving anti-HBV treatment at the time of sample testing.

Virological Characterization of OBI Profile

Among the 2283 individuals negative for HBs Ag who were tested for HBV VL, 51 were initially positive. A significant viremia above 20 UI/mL was found in 37 individuals, whereas 14 displayed a very low viremia, under the 20 UI/mL LLQ. After retest, 4 samples out of 14 had an undetectable HBV VL; therefore, 47 individuals were considered to have a confirmed OBI, leading to an estimated prevalence of OBI of 1.2% (95% CI, 0%–5.2%) in our population concomitantly evaluated for HBV serology and PCR.

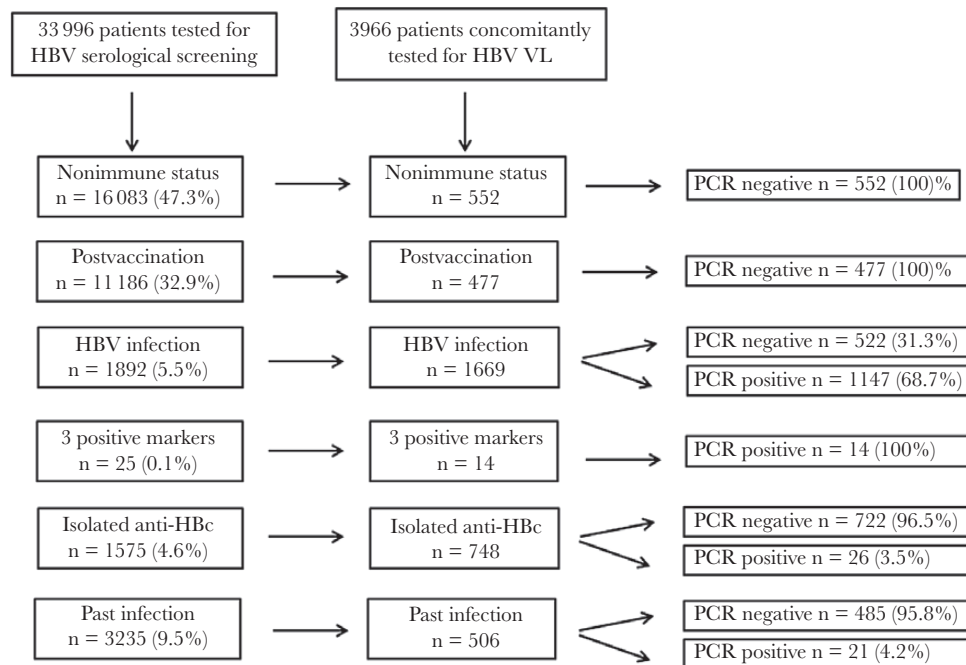


Figure 1. Global flowchart of the study. HBV infection-related tests were conducted from January 2010 to December 2016. Abbreviations: HBV, hepatitis B virus; PCR, polymerase chain reaction; VL, viral load.

These 47 subjects belong to 2 different serological groups: (1) individuals with isolated anti-HBc Abs (n = 26, 3.5%) and (2) individuals with past resolved HBV infection (n = 21, 4.2%). The HBV VL was lower in the OBI group (median [IQR], 61 [19–139] UI/mL) than in the HBs Ag-positive populations (median [IQR], 923 [112–9211] UI/mL; $P = .0001$).

We sequenced the overlapping HBV Pol and S gene in all OBI patients with an HBV VL above 500 UI/mL (n = 8), except 1, for whom no stored plasma was available (Table 2).

Six out of 7 exhibited potentially relevant amino acid substitutions located in the major hydrophilic region (MHR) of the S gene, 4 being more precisely located in the cysteine-rich domain “a” determinant, the major target of neutralizing antibodies and HBs Ag detection tests.

Clinical Characterization of OBI Individuals

The main clinical and biological characteristics of OBI patients are presented in Table 3. The population was predominantly male, with a median age around 50 years. The HBV VL was low (median [IQR], 61 [19–139] UI/mL) with no difference between groups. HIV coinfection was very frequent (40.9%, 18/47), with a tendency to a higher frequency in the “isolated anti-HBc” group (53.8%, 14/26) compared with the “anti-HBc/anti-HBs” group (22.2%, 4/21; $P = .06$). Importantly, HIV RNA viral loads were high in these coinfecting patients (median, 56 833 copies/mL), reflecting the frequency of OBI among HIV-infected patients who stopped antiretroviral (ARV) therapies (n = 8) or were discovered at a late stage of HIV infection (n = 5). HCV coinfection was observed in 6.4% of OBI individuals, with no difference between the 2 serological groups.

Table 1. HBV–Viral Load in Patients Concomitantly Tested With HBV-DNA PCR (n = 3966), According to HBV Serology

HBV Profile	Total Patients, No. (%)	HBV Viral Load Undetectable, No. (%)	HBV Viral Load Detectable, No. (%)	HBV Viral Load, Median (IQR), UI/L ^a
Nonimmune status	552 (13.81)	552	0	NA
Postvaccination profile	477 (11.93)	477	0	NA
Resolutive past infection	506 (12.66)	485 (95.8)	21 (4.2)	45 (23–89)
HBV infection	1669 (41.7)	522 (31.3)	1147 (68.7)	923 (112–9211)
Simultaneous positivity for HBs Ag and anti-HBs Ab	14 (0.25)	0	14	101 566 (171–855 000)
Isolated anti-HBc Ab	748 (18.7)	722 (96.6)	26 (3.5)	69 (19–207)

Significance between HBV viral loads in HBs Ag-positive groups vs HBs Ag-negative groups was performed by Kruskal-Wallis test (not shown in table; $P = .001$).

Abbreviations: HBV, hepatitis B virus; NA, not applicable; PCR, polymerase chain reaction.

^aOnly applicable for HBV PCR-positive patients.

Table 2. Description of S Mutations in OBI Patients With HBV VL Above 500 UI/mL

Patient	HBV serological Profile	HBV VL	HBV-Genotype	Relevant S Mutations	Region	Relevant Pol Mutations
Patient 1	Isolated anti-HBc	1022	E	T118R	MHR	None
Patient 2	Isolated anti-HBc	959	A	P120T	MHR	None
Patient 3	Isolated anti-HBc	5787	E	G145R	"a" determinant	None
Patient 4	Isolated anti-HBc	513	E	M133I	"a" determinant	None
Patient 5	Resolutive past infection	5045	B	G145S	"a" determinant	None
Patient 6	Resolutive past infection	16516	E	F134V	"a" determinant	None
Patient 7	Resolutive past infection	1374	E	None	NA	None

Abbreviations: HBV, hepatitis B virus; NA, not applicable; OBI, occult hepatitis B infection; VL, viral load.

Most of the patients (44/47) did not receive any therapy active against HBV at the time of OBI diagnosis. Regarding liver assessment, 26 out of 47 were totally asymptomatic with normal liver parameters, whereas 21 OBI cases were associated with liver abnormalities and/or disease, such as acute hepatitis (n = 13), liver failure (n = 3), cirrhosis (n = 2), HCC (n = 2, including 1 without cirrhosis), and liver metastasis (n = 1). Liver assessment showed a more severe presentation in the "anti-HBc/anti-HBs" profile compared with the "isolated anti-HBc group" ($P = .0003$), as reflected by higher liver enzyme measurements in this group ($P < .01$).

By analyzing medical records, the 47 OBI cases could be associated with 4 major clinical categories (Table 4):

1. group A: a past HBV infection, with a clearance of HBs Ag, either spontaneous or under specific antiviral therapy;
2. group B: the administration of immunosuppressive therapy known to induce HBV reactivation in a patient previously exposed to HBV;
3. group C: the interruption of ARV therapy in an HIV/HBV-coinfected patient previously controlled for HBV viral load;
4. group D: severe liver disease in a patient previously exposed to HBV.

Among the 11 group A patients (23.4%), HBs Ag clearance was spontaneously observed in all but 1 untreated individual with HBe Ag–negative chronic infection (formerly called "inactive carriers"). They globally had a milder level of transaminases

Table 3. Characteristics of OBI Individuals According to HBV Serological Profiles

	Total (n = 47)	HBV Serological Profiles		<i>P</i> ^a
		HBcAb+/HBsAb- (n = 26)	HBcAb+/HbsAb+ (n = 21)	
Sex ratio, male/female (% males)	35/12 (74.5)	20/6 (76.2)	15/6 (71.4)	.7
Age, y ^b	51 (42–65)	49 (41–62)	57 (42–70)	.2
Viral load HBV, UI/L ^b	61 (19–139)	69 (19–207)	45 (23–89)	.9
AST ^b	34.5 (24–76)	31 (23–46)	79 (29–208)	.006
ALT ^b	25.2 (17–69)	21 (15–31)	49 (19–266)	.01
Prothrombin time ^b	84.5 (71–91)	86 (74.5–91)	79.5 (65–90)	.3
HIV infection ^{c,d}	18 (40.9)	14 (53.8)	4 (22.2)	.06
HIV-RNA viral load, copies/mL ^b	56 833 (11 506–110 996)	61 951 (15 412–208 050)	38 734 (9695–73 807)	.8
HCV infection ^{d,e}	3 (6.4)	1 (3.7)	2 (10)	.3
Significant mutations in the S gene ^f	5	4	1	NS
Asymptomatic OBI with no liver disorder	26	21	5	.0003
Immunosuppressive therapy ^d	14 (29.8)	7	7	.3
First-class immunosuppressive therapy	11	5	6	.3
Second-class immunosuppressive therapy	1	1	0	.5
Third-class immunosuppressive therapy	2	1	1	.5

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; OBI, occult hepatitis B infection; NS, not significant.

^aSignificance between groups was determined using the Kruskal-Wallis test for continuous variables and the Pearson χ^2 test or Fisher exact test for categorical variables.

^bMedian (interquartile range).

^cData available for 44 patients.

^dNumber (%).

^eData available for 35 patients.

^fData available for 5 patients.

Table 4. Clinical Classification^a of OBI Cases

	Group A (n = 11)	Group B (n = 14)	Group C (n = 8)	Group D (n = 14)	PValue
Total OBI Cases (n = 47)					
Sex ratio, male/female ^b 35/12 (75.5)	9/2 (81.8)	11/3 (78.6)	5/3 (62.5)	10/4 (71.4)	.7
Age, y ^c 51 (42–65)	42 (36–51)	66 (63–72)	41.5 (38.5–49)	45 (42–65)	.0001
Viral load HBV, UI/L ^c 61 (16–139)	68 (24–159)	76.5 (19–130)	83 (19–318)	65 (34–176)	.8
AST ^c 34.5 (24–76)	30.5 (23–41.5)	40.5 (19–70)	32.5 (27–43.5)	203 (46–310)	.02
ALT ^c 25.2 (17–69)	25.5 (19.5–33.5)	32 (19–77)	18 (13–23.5)	86 (22–356)	.04
Prothrombin time ^c 84.5 (71–91)	85.5 (18–96)	79 (68–88)	89.5 (88–95)	65 (39–86)	.03
HIV infection ^b 18 (40.9)	6 (66.7)	0	7 (100)	2 (18.2)	<.0001
“Isolated anti-HBc”/“resolutive past infection” profile 26/21	9/2	7/7	8/0	2/12	.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; OBI, occult hepatitis B infection.

^aDefinition of clinical categories is specified in the text.

^bNumber (%).

^cMedian (interquartile range).

than other categories, and two-thirds were HIV-infected. They mostly exhibited an isolated HBc Ab profile. The only subject who cleared HBs Ag under treatment was an HIV/HCV/HBV-tri-infected patient receiving tenofovir-containing antiretroviral therapy for several years.

Among the 14 group B patients (32%), 11 received drugs from the highest-risk class of immunosuppression for HBV reactivation [9, 10]. They were older than the other groups, and half of them had a resolutive past infection profile.

All 8 group C patients (16.3%) stopped ARV therapy containing dually active drugs against HIV and HBV for adherence issues. They all had an isolated anti-HBc profile.

Finally, most of the 14 group D patients (29.8%) suffered from an acute liver condition unrelated to HBV, such as drug-induced liver injury (n = 5), severe sepsis (n = 2), primary hepatitis A infection (n = 1), severe flare of auto-immune hepatitis (n = 1), or acute gallstone (n = 1). We also observed 1 case of NASH-associated cirrhosis in that category, 1 case of HCC without cirrhosis, and 1 case of liver metastasis after colon cancer. This group presented a higher level of transaminases and a lower prothrombin time level, and most had a “resolutive past infection” profile.

Outcome of Individuals With OBI

Significant follow-up (median [IQR], 468 [155–1148] days) was available for 32/47 OBI individuals (Table 5). Of these, only 2 received anti-HBV therapy at the time of OBI diagnosis, whereas 20 were secondarily treated for HBV. A significant proportion of anti-HBV therapy initiation in this context was part of ARV multitherapy, either initiated in naïve patients with recent HIV diagnosis (n = 6) or re-introduced in nonadherent patients (n = 6).

Most OBI outcomes were favorable, especially in patients from group A or group C. However, among 10 patients belonging to group B, 1 died at day 28 from an underlying disease and 2 experienced, several months after OBI diagnosis, a severe hepatic flare with HBs Ag repositivation. This occurred while patients were not receiving therapy against HBV, and both responded favorably to entecavir.

In group D, the clinical outcome was less favorable, with 3 deaths occurring among 7 patients. However, this negative progression had more to do with the underlying liver condition than the OBI diagnosis itself.

DISCUSSION

The clinical significance of OBI remains largely unknown, whereas its frequency varies across series. Using sensitive assays for HBs Ag and HBV DNA detection, we identified 47 cases of confirmed OBI, among almost 4000 patients routinely followed during a 7-year period in a university teaching hospital with outpatient clinics in Paris. This is, to our knowledge, one of the largest series of OBIs ever published, which delivers some important information.

In this series, all OBI cases were observed in patients with a positive detection of anti-HBc Abs, which is reassuring regarding the accuracy of this marker to identify previous contact with HBV. More surprisingly, almost half of the cases occurred in patients who also showed significant positivity for anti-HBs antibodies. The anti-HBs Abs titers observed in this subgroup of OBI patients were furthermore rather high (median [IQR], 155 [40–396] UI/mL), suggesting a true defect in their ability to neutralize the infection, at least for replicative capacity. These data confirm that apparent “recovery” from HBV infection

Table 5. OBI Outcome in OBI Patients With a Significant Follow-up

Categories*	Follow-up, d	HBV Serology at OBI dg	HBV VL at OBI dg	Anti-HBV at OBI dg	Anti-HBV After OBI dg	Background at OBI dg	Liver Condition	OBI Outcome
A	2636	Isolated anti-HBc	104	TDF	TVD	HBV/HIV coinfectd	Asymptomatic OBI	Anti-HBs apparition (669 UJ/L) and OBI resolution under TVD
A	1816	Isolated anti-HBc	75	0	TVD	Inactive HBV + HIV diagnosis	Asymptomatic OBI	OBI resolution under TVD
A	1499	Isolated anti-HBc	373	0	TVD	Inactive HBV + HIV diagnosis	Asymptomatic OBI	OBI resolution under TVD
A	1084	Isolated anti-HBc	66	0	0	Inactive HBV carrier	Asymptomatic OBI	Spontaneous OBI resolution
A	387	Isolated anti-HBc	61	0	K/VX	Inactive HBV + HIV	Asymptomatic OBI	OBI resolution under KVX
A	644	Isolated anti-HBc	486	0	TVD	Inactive HBV + HIV	Asymptomatic OBI	OBI resolution under TVD
A	17	Isolated anti-HBc	19	0	0	Inactive HBV carrier	Asymptomatic OBI	Spontaneous OBI resolution
A	938	Isolated anti-HBc	214	0	0	Inactive HBV carrier	Asymptomatic OBI	Spontaneous OBI resolution
A	1834	Resolutive infection	29	0	TVD	Inactive HBV + HIV untreated	Asymptomatic OBI	OBI resolution under TVD
B	1179	Isolated anti-HBc	26	0	0	Myeloma under bortezomib/steroids	Asymptomatic OBI	OBI resolution after stopping IS
B	1881	Isolated anti-HBc	513	0	0	Prostate cancer under chemotherapy	Asymptomatic OBI	OBI resolution after stopping IS
B	415	Isolated anti-HBc	72	0	LMV	Myeloma under bortezomib/steroids	Asymptomatic OBI	OBI resolution under LMV
B	1324	Isolated anti-HBc	207	ETV	ETV + TVD	Liver recipient under ciclosporin	Asymptomatic OBI	OBI resolution after TVD addition
B	28	Isolated anti-HBc	81	0	TVD	Myeloma under carfilzomib/pomalidomide/steroids	Liver failure	Death at D28 for DRESS syndrome and refractory myeloma
B	71	Isolated anti-HBc	130	0	ETV	NHL under CHOP	Asymptomatic OBI	OBI resolution under TVD
B	1117	Resolutive infection	5045	0	TVD	Refractory idiopathic AI thrombopenia under rituximab	Acute hepatitis	Liver enzyme normalization and OBI resolution under TVD
B	2117	Resolutive infection	89	0	ETV	Severe RA under tocilizumab/MTX/steroids	Asymptomatic OBI	Hepatitis flare and HBs Ag positivation at D793 after stopping ETV, resolutive after ETV reintroduction
B	465	Resolutive infection	19	0	0	NHL under R-CHOP	Asymptomatic OBI	Hepatitis flare with HBs Ag positivation at D.245, resolutive under ETV
B	100	Resolutive infection	19	0	ETV	Pancreatic cancer under Folfirinox cure	Acute hepatitis	Anti-HBs persistence (430 UJ/L) and OBI resolution under ETV
C	709	Isolated anti-HBc	43	0	TVD	HIV with ARV interruption	Asymptomatic OBI	Anti-HBs apparition (300 UJ/L) and OBI resolution under TVD
C	232	Isolated anti-HBc	400	0	TVD	HIV with ARV interruption	Asymptomatic OBI	Persistence of asymptomatic OBI with adherence issue
C	136	Isolated anti-HBc	19	0	TVD	HIV with ARV interruption	OH/HBV cirrhosis	Ag HBs repositivation and cirrhosis progression with adherence issue
C	68	Isolated anti-HBc	257	0	TVD	HIV with ARV interruption	Asymptomatic OBI	OBI resolution under TVD
C	874	Isolated anti-HBc	237	0	TVD	HIV with ARV interruption	Acute hepatitis	Liver enzyme normalization and OBI resolution under TVD
C	191	Isolated anti-HBc	19	0	TVD	HIV with ARV interruption	Asymptomatic OBI	OBI resolution under TVD
D	174	Isolated anti-HBc	1374	0	0	AIH-associated flare	Acute hepatitis	Spontaneous hepatitis and OBI resolution
D	301	Resolutive infection	176	0	ETV	NASH cirrhosis in HIV	Ascitis decompensation	OBI resolution under TVD but death at D301 for cirrhosis
D	99	Resolutive infection	65	0	LMV + anti-HBs	Drug-induced fulminant hepatitis	Liver failure	Liver transplantation
D	430	Resolutive infection	34	0	0	HCC without cirrhosis post-HBV/Delta	HCC	Spontaneous OBI resolution but cancer progression and death at d430
D	505	Resolutive infection	84	0	STBD	Drug-induced hepatitis + HIV diagnosis	Acute hepatitis	Hepatitis and OBI resolution after stopping augmentin and STBD initiation
D	14	Resolutive infection	1374	0	0	Acute lysis syndrome during CLL therapy	Liver failure	Death at D14 for multi-organ failure
D	360	Resolutive infection	19	0	0	Severe sepsis	Acute hepatitis	OBI resolution under antibiotic therapy

Thirty-two patients were evaluated, with a median follow-up of 448 days.

Abbreviations: AIH, autoimmune hepatitis; CLL, chronic lymphocytic leukemia; CHOP, Cyclophosphamide/Hydroxydramycin/Oncovin/Prednisone; Dg, diagnosis; DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms; ETV, entecavir; IS, immunosuppressive therapy; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; KVX, Kivexa (abacavir/lamivudine combination); LMV, lamivudine; MTX, methotrexate; NASH, nonalcoholic steatohepatitis; NHL, non-Hodgkin lymphoma; OBI, occult hepatitis B infection; OH, alcohol; R-CHOP, rituximab + CHOP; RA, rheumatoid arthritis; STBD, strabid (elvitegravir/cobicistat/emtricitabine/tenofovir); TDF, tenofovir; TVD, tenofovir (emtricitabine/tenofovir combination); VL, viral load.

*Definition of clinical categories is specified in the text.

implies neither immune destruction of all infected cells nor disappearance of the persistent covalently closed circular viral DNA (cccDNA) from some infected cells, thus explaining the long-life risk of HBV reactivation after HBV contact [6, 11].

Many mechanisms have been proposed to explain OBI profiles. The first is a loss of antigenicity of circulating HBs Ag caused by specific mutations in the S gene, impairing its recognition by anti-HBs antibodies. These “escape mutants” can be associated with HBV VL levels comparable to overt infections and are considered by some authors to be “false” OBI [1]. However, other mechanisms rely on a true decrease in liver expression of the HBs protein, leading to undetectable levels of HBs Ag in the bloodstream, associated with low levels of HBV replication. As for antigenicity loss, the reduced expression of HBs Ag may result from complex mutational patterns located in various regions of the HBV genome, including the pre-S, the pre-C, and the Core regions [12], but it can also be linked to a viral interference phenomenon (notably with HCV) [13] or to physiological steps of the natural history of HBV chronic infection, involving both viral and host factors [4].

Regarding HBV viral load, it was, as expected, very low in the 47 OBI cases we identified, excepted for 8 patients, in whom it was >500 UI/mL. The S gene was sequenced for 7 of these patients, showing in 6 cases potentially relevant mutations located in the MHR domain of HBs Ag. Among these mutations, 4 (T118R/P120T/M133I and G145R) are known vaccine or anti-HBs Abs escape variants [14–18] previously reported in OBI patients [19], whereas G145S and F134V are unreported substitutions located in the key amino acids 134 and 145 of the “a” determinant. Given the importance of this determinant for the tertiary antigenic conformation of the S protein [20], we cannot rule out a significant impact of these mutations on HBs Ag antigenicity. However, as important progress has been made in the detection of HBs Ag mutants by commercial assays, including the Abbott Architect assay used in this study [21], we decided to include these 6 patients in our cohort.

After comprehensive analysis of the medical record of the 47 OBI patients, we were able to classify them into 4 major clinical categories. The first category (n = 11) includes previously known HBs Ag-positive CHB patients who showed a clearance of HBs Ag, associated with persistent low viremia. These patients are mainly nontreated inactive carriers, for whom HBs Ag loss may occur after years of evolution. As has been shown after recovery of acute infection, both in animal models [22] and in humans [23], a low level of HBV viremia may persist after HBs Ag loss in chronically infected patients in plasma and/or peripheral mononuclear cells [24], especially before the appearance of anti-HBs Abs. This observation likely accounts for the highest representation of this category in the “isolated anti-HBc” OBI population, compared with the “anti-HBc/anti-HBs” population.” We assume that this OBI phase is a transient step before the complete stop of viral replication in this population [4], as

suggested by the positive outcome of all patients of this group (Table 5). However, in a recent cohort of CHB patients with HBs Ag sero-clearance [25], the persistence of detectable viral DNA was associated with a higher risk of HBs sero-reversion.

The second category (n = 14) concerns previously exposed patients receiving immunosuppressive therapies, such as rituximab or high-dose steroids. The OBI profile observed in such patients may precede the appearance of HBs Ag, as it can be observed in the ramping phase of an acute infection. From these 14 patients, 2 displayed acute hepatitis at the moment of OBI diagnosis, whereas 2 others experienced, several weeks after OBI detection, a severe hepatitis flare associated with reappearance of HBs Ag. These data exemplify the importance of screening any patients receiving such therapies for HBV markers to prevent severe reactivation by appropriate prophylaxis [6, 9–11].

Apart from these 2 well-known categories, 2 others accounted for the rest of the OBI cases of this series. The third category (n = 8) is represented by a homogeneous HBV/HIV-coinfected population, where the low level of HBV viremia was observed during an interruption of the anti-HBV-containing ARV regimen, most of the time for adherence issues. This category is exclusively composed of “isolated anti-HBc” carrier patients, probably because of the delayed appearance of anti-HBs Abs following HBs Ag loss in coinfecting patients under tenofovir therapy [26].

The last category represents the most surprising result of our study. Indeed, almost 30% of the OBI cases (n = 14) were observed during a severe liver disorder occurring in patients exhibiting an “anti-HBc/anti-HBs” profile. These liver conditions were most of the time acute, nonrelated to HBV, and associated with a clear increase of liver enzymes. We may assume that the low HBV viremia responsible for the OBI profile in this population results from a passive release of viral DNA from infected liver cells into the peripheral blood, even if we cannot exclude a role of immune complexes in the lack of HBs Ag reactivity. Whatever the mechanism involved, we don’t know the potential consequences of this low viremia in the progression of HBV disease in these situations. Chronic liver conditions were also observed in this category, albeit less frequently. We notably observed cases of cryptogenic cirrhosis and HCC without cirrhosis where the putative implication of HBV could be suspected, either through immune-mediated damage of hepatic parenchyma [27], ancient chromosomal integration events [28], or virus-induced modifications of cell-signaling pathways [29].

We are aware of some limitations of this work. First, OBI diagnosis was made on a single point in the HBV story of the patients, defined by the first plasma sample tested during the study period. However, the analysis of the medical records of OBI patients allowed us to expand our study beyond this single point for 32 patients in order to evaluate the long-term prognosis of OBI cases according to the clinical category they belong

to. Second, we only had access to results obtained from plasma samples, without having any knowledge on what was going on in the liver of the OBI patients. This limitation obviously results from the scarcity of liver biopsies, especially in HBs Ag–negative individuals. The last limit is the inherent bias of studies based on retrospective database analysis. HBV DNA testing performed in HBs Ag–negative patients may indeed result from specific comorbidities and/or abnormal liver function tests, which prevents us from extending the OBI prevalence found in our study population to the general population.

Despite these limitations, this comprehensive description of the OBI condition delivers important messages in the management of HBV-exposed individuals. This study indeed confirms the inherent long-life persistence of HBV after infection, even in patients exhibiting significant anti-HBs antibodies, detected in almost half of our OBI cases. This implies a need to detect and/or prevent any resumption of viral replication in every HBV-exposed individual receiving immune-suppressive therapy, and also to maintain, at least in HBV/HIV-coinfected patients, effective anti-HBV therapy, even after HBs Ag loss. The clinical implications of OBI profiles belonging to the “severe liver disease” category remain to be determined, especially when the concomitant liver condition appears to be acute and independent of previous HBV exposure.

This large cohort of OBI patients shows that occult HBV infection is a heterogeneous condition that can be associated with 4 major clinical entities, with different clinical implications for each one. A better acknowledgement of viral and host factors involved in HBV pathogenesis and persistence, as well as the use of more accurate biomarkers for staging HBV disease [30], will help us to better manage this puzzling and complex OBI status.

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