DETECTION OF OCCULT HEPATITIS B VIRUS INFECTION AMONG SUBJECTS WITH ISOLATED HEPATITIS B CORE ANTIBODIES: RESULTS FROM A 3-YEAR SURVEY IN AN ITALIAN TERTIARY-CARE HOSPITAL

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Original article

DETECTION OF OCCULT HEPATITIS B VIRUS INFECTION AMONG SUBJECTS WITH ISOLATED HEPATITIS B CORE ANTIBODIES: RESULTS FROM A 3-YEAR SURVEY IN AN ITALIAN TERTIARY-CARE HOSPITAL

Running title: Occult hepatitis B virus infection detection in Northern Italy

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Highlights

- In the study population the seroprevalence is 4.6% for HBsAg and 11% for anti-HBcAg
- The isolated anti-HBcAg status shows an age-dependent increase in Italians
- Foreigners with isolated anti-HBcAg came mostly from Africa and Eastern Europe
- HBV DNA assay is scarcely prescribed for subjects with isolated anti-HBcAg status
- Among isolated anti-HBcAg subjects, 14.8% are positive for the prescribed HBV DNA

Abstract

Background: Hepatitis B virus (HBV) infection causes hepatitis, liver cirrhosis, hepatocellular carcinoma, and death. This study examines the subjects with isolated anti-HBV core antigen antibody

(anti-HBcAg), a pattern characterised by the persistent HBV carriage in the absence of HBV surface antigen (HBsAg) and anti-HBsAg antibody.

Methods: Based on medical orders, from 2017 to 2019, serological and molecular assays were performed on serum/plasma samples of 33,048 subjects (71.4% Italians, 28.6% foreigners), who referred to the Virology Unit of the University-Hospital of Parma (Northern Italy) for the laboratory diagnosis of HBV infection.

Results: The seroprevalence was 4.6% for HBsAg and 11% for anti-HBcAg. The occurrence of the isolated anti-HBcAg status was 3.1%, with higher frequency in males than in females (66.3% vs. 33.7%, $P<0.0001$), in Italians than in foreigners (54.8% vs. 45.2%, $P<0.001$), and in outpatients than in inpatients (57.4% vs. 42.6%, $P<0.0001$). Foreigners with isolated anti-HBcAg came mostly from Africa (67.9%) and Eastern Europe (26.2%). Among subjects with isolated anti-HBcAg, 14.8% had occult HBV infection, 26.3% hepatitis C virus co-infection, 2% human immunodeficiency virus co-infection, and 3.3% both of these latter co-infections.

Conclusions: The anti-HBcAg assay accurately evaluates the HBV exposure; subjects with isolated anti-HBcAg antibody should be further analysed for HBV DNA. The HBV infection prevalence in Italy is increasing, due to growing migratory flows from endemic areas.

Keywords

isolated hepatitis B core antigen antibody, hepatitis B virus DNA, epidemiology, immigrants, occult hepatitis B virus infection, laboratory diagnosis.

Abbreviations

Anti-HBcAg hepatitis B core antigen antibody

Anti-HBeAg hepatitis B e antigen antibody

Anti-HBsAg hepatitis B surface antigen antibody

HBeAg hepatitis B e antigen

HBsAg hepatitis B surface antigen

HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
OBI	occult hepatitis B infection
O.R.	odds ratio
SD	standard deviation

1. Introduction

Hepatitis B virus (HBV) infection is one of the major reasons of mortality worldwide, despite the availability of a safe and effective vaccine since 1982. The World Health Organization estimated that 325 million people are living with chronic HBV infection [1].

In Italy, the mandatory universal vaccination from 1991 for all new-born babies and 12-year-old children, the improved socio-economic conditions and the introduction of public health measures have considerably reduced the incidence of HBV infection [2,3]. Although the actual prevalence of HBV infection in Italy is about 0.6% [4], the burden of the disease remains high, in the awareness of increasing numbers of largely unvaccinated immigrants from high endemic countries [5].

The continuous implementation of measures for assessment and prevention of blood-borne virus transmission and the employ of direct-acting antivirals against hepatitis C virus (HCV) infection, which may lead to HBV reactivation in co-infected individuals [6,7], have fostered greater focus on the serological pattern called isolated HBV core antigen antibody (anti-HBcAg). The isolated anti-HBcAg status belongs to subjects positive for anti-HBcAg, but negative for both the HBV surface antigen (HBsAg) and antibody (anti-HBsAg).

The isolated anti-HBcAg serological pattern could represent the window phase between the resolution of HBsAg antigenemia and the development of anti-HBsAg, an active infection with undetectable HBsAg, a resolved infection with anti-HBsAg titers below the positive level, or a false positive reactivity [8,9]. Although the clinical implications of isolated anti-HBcAg are so far not fully understood, at least a proportion of the subjects with this profile could escape the surveillance screenings based on HBsAg and anti-HBsAg detection and could act as carrier of HBV [8]. In particular, there is an emerging evidence that occult HBV infection (OBI), defined as HBV DNA presence in the blood or liver without HBsAg antigenemia, is of clinical relevance in HBV transmission through blood transfusion, organ/bone marrow transplantation, and haemodialysis [10]. Moreover, chemotherapy and pharmacological modulation of the immune status may induce HBV reactivation, contributing to the development of cirrhosis and hepatocellular carcinoma [11–13].

The isolated anti-HBcAg pattern shows variable prevalence in different diagnostic settings [9]. In particular, it was frequently detected in individuals co-infected with HCV or human immunodeficiency virus (HIV) [14,15], as a consequence of the down-regulation of the HBsAg synthesis [16].

Since data on the isolated anti-HBcAg status in the Italian general population are scanty [17–19], the aim of this 3-year large-scale investigation in a tertiary-care hospital in Parma (Northern Italy) was both to deepen the knowledge on the prevalence of subjects presenting this serological pattern and dissect the clinical and demographic characteristics of subjects with OBI.

2. Materials and methods

2.1 Study setting

For the study, the results of the detection of the serological markers of HBV infection performed from 2017 to 2019 on 33,048 serum/plasma samples, sent for diagnostic purpose to the Virology Unit of the University-Hospital of Parma (a 1,044-bed tertiary-care center with more than 46,000 admissions per year from the city and surroundings with approximately 450,000 inhabitants [20,21]), and belonging to 33,048 subjects, were retrospectively analysed. Table 1 shows the demographic characteristics of the study population.

When the isolated anti-HBcAg pattern was observed, the results available for HBV DNA quantification, anti-HCV antibody, HIV-1 p24 antigen, and anti-HIV-1/2 antibody detection, as well as HBV and HCV genotype characterization, according to the request on the medical order, were analysed.

Patients' identity and medical information were protected and remained anonymous during the study. The selection criteria of the study population were based on the medical order, therefore there was no need to obtain an informed consent. The laboratory diagnosis results were reported in the medical records of the patients in response to a clinical suspicion.

2.2 Serological assays

Based on the medical order, all sera were submitted to the detection of at least one of the following HBV serological markers (HBsAg, quantitative HBsAg, anti-HBcAg, anti-HBsAg, hepatitis B e antigen (HBeAg) and anti-HBeAg antibody) by ARCHITECT chemiluminescent microparticle immunoassays (Abbott, Germany), according to the manufacturer’s instructions. In case of positive results for anti-HBcAg, the samples were retested in duplicate for anti-HBcAg together with HBsAg and anti-HBsAg markers. In case of first outcome of the isolated anti-HBcAg status, a second serum sample was requested to confirm sample reactivity (sample not included in the study).

The anti-HCV antibodies were determined by enzyme immunoassay (Vitros Eci HCV Ab assay, J&J Ortho Clinical Diagnostics, USA); positive or indeterminate results were confirmed by immunoblot assays (INNO-LIA HCV Score, INNOGENETICS N.V., Belgium) [22,23].

For the detection of p24 HIV-1 antigen and anti-HIV-1/2 antibodies, the VIDAS HIV DUO Quick assay (BioMérieux, France) was used; confirmatory assays were carried out by immunoblotting (HIV Blot 2.2, MP Biomedicals, Germany; New Lav blot II, Bio-Rad, Italy).

2.3 Molecular assays

HBV DNA quantification in plasma samples was carried out by COBAS AmpliPrep/Cobas TaqMan Quantitative Tests version 2.0 (Roche Diagnostics, USA). The linear range of quantification was from 2.0x10 to $1.7x10^8$ HBV DNA IU/ml.

For HBV and HCV genotyping, viral nucleic acids were extracted by NucliSens easyMAG (BioMérieux) and amplified by GeneAmp PCR System 9700 (Applied Biosystems, USA). The HBV and HCV genotypes were assessed on the AutoBlot 3000H instrument (Med Tec, Inc., USA) by INNO-LiPA HBV genotyping kit (Fujirebio, Ghent, Belgium) and Versant HCV Genotype 2.0 LiPa assays (Siemens Healthcare Diagnostics Inc., USA) [24], respectively, according to the manufacturers' instructions.

For the determination of HBV drug resistance-associated mutations, viral nucleic acids were extracted by NucliSens easyMAG (BioMérieux) and amplified by GeneAmp PCR System 9700 (Applied Biosystems). The HBV drug resistance was assessed on the AutoBlot 3000H instrument by INNO-LiPA HBV Multi-DR kit (Fujirebio, Ghent, Belgium), according to the manufacturers' instructions.

2.4 Statistical analysis

The chi-square test was used to analyse differences among age groups, gender, and country of birth. Student's *t*-test was employed to compare the mean ages. *P* values <0.05 were considered statistically significant. In addition, the odds ratio (O.R.) was calculated in order to evaluate the strength of the associations that emerged.

3. Results

In the study population, the seroprevalence was 4.6% (1,504/33,048) for HBsAg and 11% (3,644/33,048) for anti-HBcAg, with differences related to the nationality of the subjects (HBsAg: 837/1,504, 55.7%, foreigners vs. 667/1,504, 44.3%, Italians; anti-HBcAg: 1,672/3,644, 45.9%, foreigners vs. 1,972/3,644, 54.1%, Italians).

When considering specifically the subgroups of Italians and foreigners, these prevalences were significantly higher in foreigners (HBsAg: 837/9,459, 8.8%, foreigners vs. 667/23,589, 2.8%, Italians, $P<0.0001$, O.R. 3.34; anti-HBcAg: 1,672/9,459, 17.7%, foreigners vs. 1,972/23,589, 8.4%, Italians, $P<0.0001$, O.R. 2.35).

A total of 1,013 subjects (1,013/33,048, 3.1%) showed the isolated anti-HBcAg pattern (Table 2). This status was significantly more frequent in male than in female (672/1,013, 66.3% male vs. 341/1,013, 33.7%, female, $P<0.0001$; O.R. 2.69), in Italians than in foreigners (555/1,013, 54.8%, Italians vs. 458/1,013, 45.2%, foreigners, $P<0.001$; O.R. 2.11), and in outpatients than in inpatients (581/1,013, 57.4%, outpatients vs. 432/1,013, 42.6%, inpatients, $P<0.0001$; O.R. 2.14). Moreover, Italians were older than foreigners (mean age: 65.9 ± 19.5 years vs. 39 ± 19.5 years, $P<0.0001$; median age: 67 years vs. 36 years, respectively). The distribution by age of the individuals with the isolated anti-HBcAg status is shown in Fig. 1. Among Italians, the frequency of detection of the isolated anti-HBcAg status increased with age, predominating in the over-61 age group, with a prevalence significantly higher than that of the ≤60 age group (62.2%: 345 of 555 subjects vs. 37.8%: 210 of 555 subjects, $P<0.0001$). Conversely, in foreigners the isolated anti-HBcAg frequency decreased with age, predominating in the ≤40 age group (58.1%: 266 of 458 subjects), with a prevalence significantly higher than that in the older age group (41.9%: 192 of 458 subjects, $P<0.0001$).

With regard to the temporal distribution of the individuals with isolated anti-HBcAg, 372 (36.7%; 158 Italians and 214 foreigners) were detected in 2017, 338 (33.4%; 203 Italians and 135 foreigners) in 2018, and 303 (29.9%; 194 Italians and 109 foreigners) in 2019.

In this survey, out of the 458 foreigners attending the University Hospital of Parma and showing isolated anti-HBcAg, 311 (67.9 %) were from Africa (in particular 292, 93.9%, from Sub-Saharan

Africa), 120 (26.2 %) from Eastern Europe, 24 (5.2 %) from Asia, and 3 (0.7 %) from the Caribbean region.

Among the 1,013 subjects with isolated anti-HBcAg, 267 (26.3%) were co-infected with HCV, 20 (2%) with HIV (19 with HIV-1 and 1 with HIV-2), and 33 (3.3%) with both HCV and HIV-1. On the contrary, 382 (37.7%) subjects were negative for both HIV and HCV infections. For 263 subjects (26%), only partial information were available: 247 HCV-negative subjects were not tested for HIV infection and 16 HIV-negative subjects were not tested for HCV infection. For the remaining 48 subjects (4.7%), no information about HCV and HIV infections were available. Among the overall 300 HCV co-infected individuals, the HCV genotype was performed in 198 cases (66%). The predominant HCV genotypes were 1b (35.3%) and 1a (23.2%), followed by 3a (12.1%).

In particular, among the 432 inpatients with isolated anti-HBcAg, 177 (40.9%) were admitted to Infectious Disease Units, 121 (28%) to Medical Units, 101 (23.4%) to Oncology Units, and 28 (6.5%) to Nephrology Units. The remaining 5 subjects (1.2%) were from the Blood Donor Center. According to the clinical and anamnestic information available for 122 inpatients (28.2%), the prevailing diagnoses were cancer (24.8%), transplant or kidney failure (1.8%), and cirrhosis or advanced liver fibrosis (1.6%).

Among the 1,013 isolated anti-HBcAg subjects, HBV DNA quantification was performed in 122 cases (12%), according to the medical order. In 104 subjects (85.2%) HBV DNA was not detected, whereas in 18 subjects (14.8%) was present (Table 3). Of those, 14 subjects (77.8%) showed very low viral DNA loads (<20 IU/ml), while in 4 subjects (22.2%) HBV DNA ranged from 35 to 550 IU/ml.

Although no data were available for HBV genotyping in isolated anti-HBcAg subjects, the most frequent HBV genotypes circulating in 115 subjects (0.3%) of the study population were genotype D (48 cases, 41.7%), genotype E (29 cases, 25.2%), and genotype A (17 cases, 14.8%).

Among subjects with OBI, no HIV co-infections were detected, whereas two subjects (2/18, 11.1%) showed HCV co-infection.

Table 3 illustrates the clinical and demographic characteristics of the 18 subjects with OBI. Of these subjects, 13 (72.2%) were Italians and 5 (27.8%) foreigners, mostly coming from Sub-Saharan Africa. In addition, 3 (16.7%) subjects showed liver-related clinical manifestations.

Only for subjects with OBI, the follow-up analysis was extended to January 2022 (Table 3) in order to verify the occurrence of changes in the clinical status.

Importantly, the analysis showed that an African dialysed patient underwent HBV reactivation with high HBV DNA load (35,261 IU/ml), before administration of the antiviral therapy with lamivudine. In parallel, severe anemia, hypertension, and hepatic steatofibrosis of medium degree have been diagnosed in this subject in association to a significant deterioration of the renal function and consequent need to start hemodialysis. In-depth molecular assays evidenced that the HBV strain in question belongs to genotype E and does not present mutations associated to drug-resistance. Accordingly, the data reported in the follow-up analysis of Table 3 (*i.e.* HBV DNA not determined) showed the efficacy of the antiviral therapy.

4. Discussion

The majority of the screening programs for HBV infection tests only HBsAg and anti-HBsAg, missing the detection of subjects with anti-HBcAg as the only detectable marker, namely the isolated anti-HBcAg status. This pattern poses the question whether individuals are susceptible to HBV infection, have an active infection, or have resolved the infection.

The prevalence of isolated anti-HBcAg is closely related to the level of endemicity of HBV infection [25], ranging from 1% to 32% [9]. Of note, in subjects with isolated anti-HBcAg receiving chemotherapies or biologic therapies, the risk of HBV reactivation varies from 1% to 2.7% [26]. Noteworthy, the antiviral prophylaxis is scarcely administered and an insufficient percentage of serological screenings is carried out [27].

In this study, we observed a higher HBsAg positivity (4.6%) than those detected by other Authors [28–31]. In addition, the overall rate of subjects exposed to HBV infection (anti-HBcAg-positive subjects with or without HBsAg) was 11%.

The majority of HBsAg-positive subjects (55.7%) were foreigners, mostly coming from Africa. By specifically analysing the subgroup of foreigners in comparison with that of Italians, the HBsAg and anti-HBcAg prevalences were significantly higher among foreigners (8.8% vs. 2.8%, $P<0.0001$, and 17.7% vs. 8.4%, $P<0.0001$, respectively). Our issues evidenced that in Northern Italy circulates a large number of migrants with serological markers of either active or past HBV infection, in agreement with previous observations [19,32]. High HBV infection prevalence was previously assessed among African residents in Italy [33,34], whose majority was infected by the genotype E of HBV, showing virological characteristics of immune escape [34]. Accordingly, the genotype E was frequently revealed (25.2%) in the study population.

The frequency of the isolated anti-HBcAg pattern in the study population was 3.1%; in this regard, other Authors reported either lower [35] or higher [31] prevalences for the general population, while significantly higher prevalences were assessed for high-risk categories [36–38]. The proportion of the isolated anti-HBcAg status showed a significant age-dependent increase in Italians, in agreement with other Authors [39]. On the contrary, among foreigners, this status prevailed in the less than 40-year age group (58.1%). These findings clearly evidence the effects of vaccine administration made

compulsory in Italy since 1991. On the contrary, for immigrants coming from Africa the vaccination coverage remains low [40].

In addition, this pattern was associated with both country origin and sex, being significantly more frequent among Italians than foreigners (54.8% vs. 45.2%, $P<0.001$), and among males than females (66.3% vs. 33.7%, $P<0.0001$). Of interest, in this study the isolated anti-HBcAg status was assessed in subjects at high risk of transmission/reactivation of HBV infection, such as dialysis patients, cancer patients and blood donors.

Individuals co-infected with HIV have frequently anti-HBcAg as the only marker assessing HBV infection [41,42]. Moreover, the reactivation of OBI is rather frequent among immunocompromised subjects [43,44]. It has also been assessed that HCV co-infection counteracts HBV replication [45,46]. Of interest, severe hepatitis have been found in subjects with OBI co-infected with HIV and/or HCV [47,48]. In the present survey, among subjects with isolated anti-HBcAg, 26.3% were co-infected by HCV, 2% by HIV and 3.3% by both viruses; this high frequency of HCV co-infections, overall accounting for 29.6%, as well as the predominant HCV genotypes revealed in the study population, are consistent with previous data [24].

Although the precise mechanism of onset of OBI remains in part unknown, host and viral factors, viral co-infections, and epigenetic mechanisms concur to its manifestation [49]. Specifically, OBI has been reported in blood donors [50], with a high risk of HBV transmission [51]. Significantly, blood donors with isolated anti-HBcAg might be carrier of viral loads below the detection limit [52]. To date, it does not exist a shared global algorithm for OBI recognition among blood donors [53]. Although in Italy the anti-HBcAg screening is not recommended for blood donors to avoid the shortage of blood supply, two cases of transfusion-transmitted HBV infection by carriers of OBI have been documented [54]. In this view, the vaccination against HBV for subjects with isolated anti-HBcAg positivity is recommended to reduce the risk of transfusion-transmitted HBV infection [55,56]. Moreover, considering that the amount of HBV DNA in subjects with OBI is generally low, as also demonstrated by our data, and intermittently detectable [57,58], the anti-HBcAg assay has been suggested to compensate the results of nucleic acid amplification testing [59]. Specifically, the anti-HBcAg

screening was voluntarily adopted in several Italian blood centres [60]. Accordingly, Fiedler et al. [61] assessed that the anti-HBcAg assay is effective for the detection of OBI in blood donors.

Of note, a dialysed foreign patient underwent HBV reactivation with high viral DNA loads (35,261 IU/ml) and detection of hepatic steatofibrosis in 2020, requiring the administration of antiviral therapy to control it. This event is emblematic of the possibility of transmission of HBV from subjects with OBI, and highlights the need to carry out constant serological and molecular monitoring in high-risk categories of individuals. Considering that a high rate of irregular migrants infected by HBV is out of the Italian public healthcare system and no measures against HBV are carried out [62], the overall findings reinforce the need to establish accurate screening programs and allow the access to care for immigrants.

This study has the limitation that HBV DNA detection in subjects with isolated anti-HBcAg was performed in accordance with the medical order; therefore, only partial data on HBV DNA assay were available in the study period as well as in the follow-up carried out from January 2020 to January 2022. This aspect has promptly stimulated the change of our diagnostic algorithm, since for outpatients with isolated anti-HBcAg we recommend the search of HBV DNA in the medical report, while for inpatients we perform the HBV DNA assay on an aliquot of the sample submitted for HBV serology.

Conclusions

Although a large amount of information emerged in recent years, the data are still incomplete to exhaustively describe the clinical impact of HBV infection. This large-scale survey provides new information on the OBI proportion among isolated anti-HBcAg, highlighting that the anti-HBcAg assay is a useful biomarker for further diagnostic insights, and demonstrating that HBV epidemiology in Italy is changing, due to increasing migratory flows especially from high endemic areas.

Author contributions

FDC conceived the study and wrote the manuscript. FDC, MLD, MCA, MM, AC acquired the data. FDC, MB, CM, MCA, MM, and GM analysed the data. MB and GM represented graphically the data. CC and AC critically revised the manuscript. FDC, MB, MLD, CM, GM, MCA, MM, CC, and AC approved the version of the manuscript to be submitted.

Declaration of competing Interest

The Authors declare that they have no conflict of interest.

Ethical approval

This is an observational study. This article does not describe any studies with human participants or animals performed by any of the authors.

Informed consent

The samples analysed in this study were sent to the Virology Unit of the University-Hospital of Parma for routine diagnostic purposes. The laboratory diagnosis results were reported in the clinical records of the patients as answer to a clinical suspicion indicated in the medical order. Patients' identity and medical information were protected and remained anonymous during the study. Ethical approval at the University Hospital of Parma is required in cases in which the clinical samples are to be used for applications other than laboratory diagnosis.

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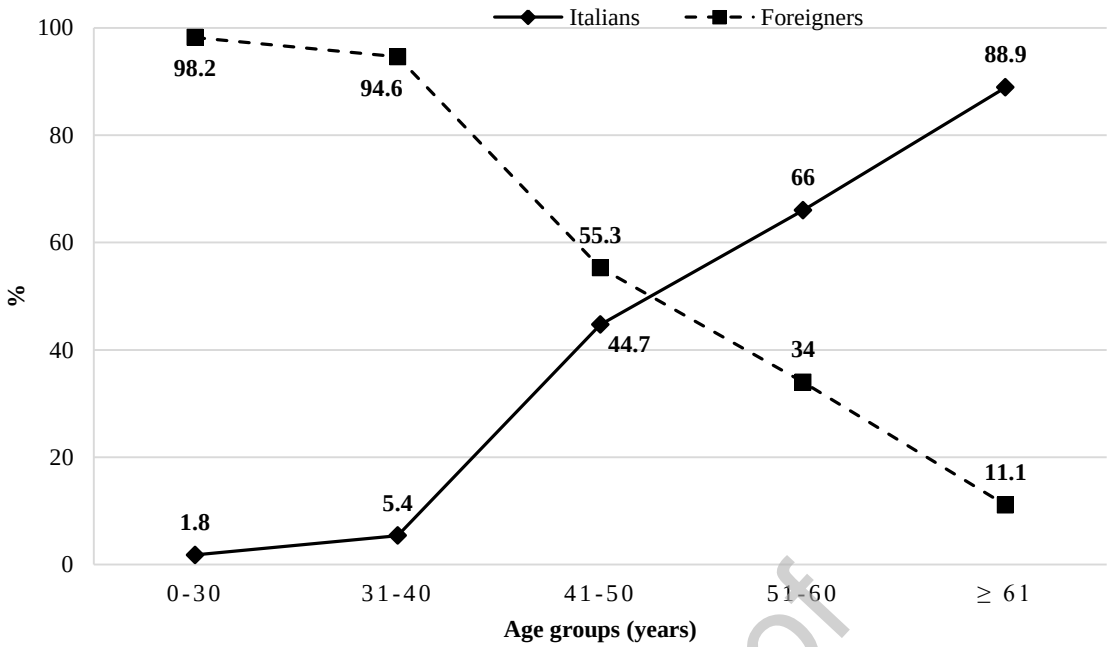

Italians	3/163 (1.8%)	6/112 (5.4%)	63/141 (44.7%)	138/209 (66%)	345/388 (88.9%)
Foreigners	160/163 (98.2%)	106/112 (94.6%)	78/141 (55.3%)	71/209 (34%)	43/388 (11.1%)

Fig. 1 Prevalence of the isolated anti-HBcAg serological status in Italians and foreigners, according to the age groups. The table shows the number of subjects for each age group and the related percentages.

Table 1 Demographic data of the 33,048 subjects whose samples were analysed for the laboratory diagnosis of HBV infection from 2017 to 2019.

Features	Number (%)
Males	14,226 (43)
Females	18,822 (57)
Mean age ± SD	45.3 ± 18.8
Median age	41
Italians	23,589 (71.4)
Foreigners	9,459 (28.6)
Inpatients	12,340 (37.3)
Outpatients	20,708 (62.7)

SD - standard deviation.

Table 2. Demographic characteristics and co-infections assessed in 1,013 subjects with the isolated anti-HBcAg serological pattern.

Features	Number (%)
Males	672 (66.3)
Females	341 (33.7)
Mean age ± SD	53.8 ± 19.5
Median age	54
Italians	555 (54.8)
Foreigners	458 (45.2)
Inpatients	432 (42.6)
Outpatients	581 (57.4)
Co-infections:	
HCV	267 (26.3)
HIV	20 (2)
HCV and HIV	33 (3.3)

SD - standard deviation.

Table 3 Clinical and demographic data of 18 subjects with OBI detected in the study population from 2017 to 2019.

Year of detection	Sex	Age (years)	Nationality	Ward	Diagnosis	HBV DNA (IU/mL)	HBeAb	HCV co-infection	HIV co-infection	Follow-up year 2020	Follow-up January 01, 2021 – January 31, 2022
2017	F	29	Morocco	outpatient	NA	< 20	NA	Absent	Absent	NA	NA
2017	M	58	Senegal	outpatient	NA	< 20	NA	Absent	Absent	NA	HBsAg Negative
2017	M	75	Italy	outpatient	Orchiepididymitis	< 20	NA	NA	NA	NA	NA
2017	M	79	Italy	outpatient	Polyneuropathy; heart disease	< 20	NA	Absent	Absent	NA	NA
2017	M	88	Italy	Oncology	Liver metastases	< 20	Positive	Absent	Absent	death (cardiac arrest)	–
2018	M	57	Philippines Islands	Medicine	Hepatocellular carcinoma; decompensated liver cirrhosis	35	NA	Absent	NA	NA	NA
2018	M	67	Italy	Nephrology	Renal failure	< 20	Positive	Absent	Absent	NA	NA
2018	F	75	Italy	outpatient	Cancer	< 20	NA	Absent	Absent	HBV DNA < 20 UI/mL	NA
2019	F	22	Camerun	Hematology	Anemia	< 20	NA	Absent	Absent	NA	NA
2019	M	44	Italy	Infectious disease	Hepatitis C	< 20	Positive	Present HCV RNA ND	Absent	NA	HBsAg Negative HBV DNA ND
2019	M	49	Ghana	Nephrology	Pre-dialysis chronic renal failure	550	Positive	Absent	Absent	HBV DNA 35,261 IU/mL HBsAg Positive Lamivudine therapy *Staphylococcus aureus* sepsis interstitial pneumonia	HBV DNA ND
2019	M	62	Italy	Nephrology	Cancer; polycystic kidney; liver cysts; chronic ischemic heart disease	93	Positive	Absent	Absent	HBV DNA < 20 IU/mL	HBsAg Negative HBV DNA < 20 IU/mL
2019	F	63	Italy	outpatient	NA	< 20	NA	NA	NA	NA	NA
2019	M	70	Italy	outpatient	Chronic lymphocytic leukaemia	< 20	NA	Absent	NA	NA	NA
2019	M	71	Italy	outpatient	NA	< 20	NA	Absent	NA	NA	HBsAg Negative HBV DNA < 20 IU/mL
2019	M	74	Italy	Neurosurgery	Non-Hodgkin lymphoma	281	NA	Absent	Absent	NA	Lamivudine therapy pancreatitis biliary obstruction death (brain haemorrhage)
2019	F	75	Italy	outpatient	NA	< 20	NA	NA	NA	NA	NA
2019	M	88	Italy	outpatient	NA	< 20	NA	Present HCV RNA 194 IU/mL 2a/2c genotype	NA	HBV DNA < 20 IU/mL HCV RNA 95 IU/mL	HBV DNA 43 IU/mL HCV RNA 146 IU/mL

M – Male; F – Female; NA – Not available; ND – Not detected.