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The present profile of chronic hepatitis B virus infection highlights future challenges
An analysis of the Multicenter Italian MASTER-B cohort

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ABSTRACT

Background: Chronic hepatitis B virus (HBV) infection remains a primary cause of morbidity and mortality worldwide.
Aim: The study is aimed at updating the clinical and epidemiological profile of chronic HBV infection in Italy.
Methods: A cross-sectional multicenter prospective study enrolled consecutive HBsAg positive patients seen in 73 Italian centers in the period 2012–2015. Individual patient data were collected using an electronic platform and analyzed using standard statistical methods.
Results: Among 2877 HBsAg positive individuals (median age 49.8 years, 68% males), 27% were non-Italian natives (NINs); 20% had chronic infection, 58.5% chronic hepatitis and 21.5% cirrhosis. Among NINs, age was younger, male gender was less prevalent and liver disease less advanced than in Italians (all $p < 0.0001$). HBeAg positive cases were 23.6% among NINs vs 8.2% in Italians ($p < 0.0001$); HDV coinfections 11.1% vs 7.3% ($p = 0.006$) and HCV coinfections 2.3% vs 4.2% ($p = 0.017$), respectively. Anti-HDV or anti-HCV antibodies were detected more frequently in patients with cirrhosis. Fifty percent of NINs with cirrhosis were aged below 45 years.
Conclusion: The study offers an insight into the evolving burden of chronic hepatitis B virus infection in the near future and highlights new territories for public health interventions.

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Hepatitis B virus (HBV) chronic infection remains a primary cause of morbidity and mortality worldwide, although the burden of the infection tended to reduce, due to the increasing number of countries that implemented vaccination against HBV and more in general, to the improvement in socio-economic and hygienic conditions [1,2]. In addition, the availability of potent oral antiviral drugs has reduced the proportion of highly viremic patients who are the reservoir of the virus, thus contributing to the reduction in HBV circulation in the areas where these drugs have been used extensively. In contrast to this progress, some factors, such as poverty and wars, have induced a decrease in the attention to preventive measures in some countries and a parallel intense flow of migration from areas where HBV infection is highly prevalent towards developed countries [3].

Nationwide Italian surveys of patients with chronic liver disease have shown that 10–15% of chronic hepatitis or cirrhosis are HBsAg positive [4]; the same proportion was found among patients with hepatocellular carcinoma [5]. The typical Italian native patient with HBV infection was over 50, with a prevalence of HBeAg around 10%. Young patients were rare, due to the extensive vaccination campaign that involved all neonates and adolescents from 1991 [6]. Migration flow has raised exponentially in recent years; at present, non-Italian native residents are estimated to be over five million individuals, that is around 8% of the total resident population [3]. Surveys in immigrate communities, performed, using heterogeneous sample sources, showed that the prevalence of HBsAg carriers was many fold higher than that recorded in the Italian population [7,8]; few or no data on coinfection with Delta virus were available. Altogether, the data suggest that the burden of chronic HBV infection is going to change dramatically and might have a deep impact on public health resources at present and in future years.

To give an updated picture to caregivers and stakeholders, the Italian Association for the study of the liver (AISF) promoted a multicenter survey aimed at defining the current profiles of the subjects with chronic HBV infection.

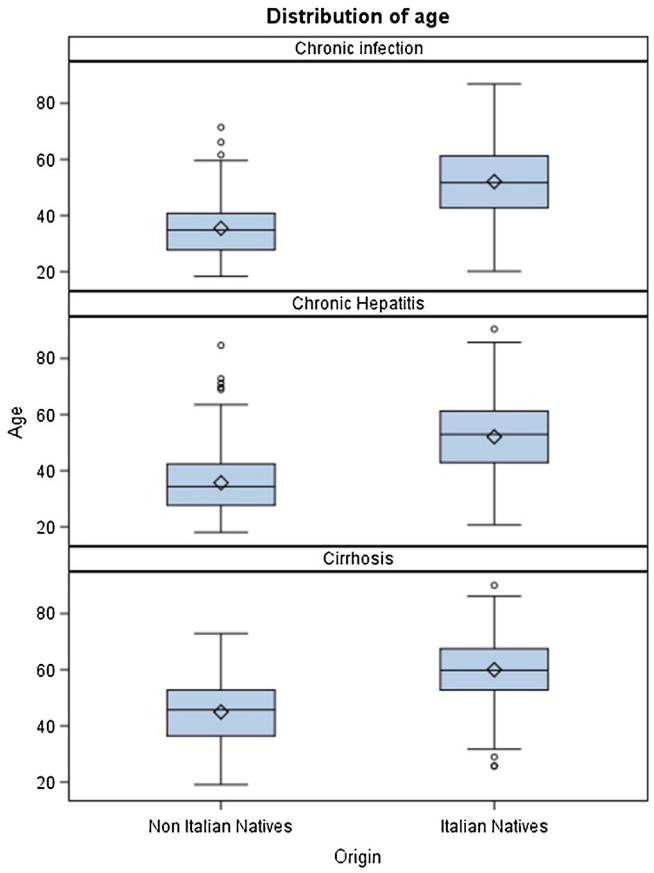

Fig. 1. Age of Italian and non-Italian patients across the different liver disease stages. Age is summarized as whisker box, where the central segment of each box is the median, the lower and upper segments are Q1 and Q3, respectively, and the vertical lines indicate the minimum and maximum values. All comparisons within each disease stage are significant at $p < 0.01$.

1. Methods

An observational, prospective study was launched among Italian centers which adhered to the Italian Association for the Study of the liver (AISF). The minimal requirement for participation was the routine use of PCR methods for HBV DNA quantification; 73 centers throughout Italy agreed to participate; their distribution across the country is in supplemental Fig. 1. All patients with a positive HBsAg in plasma for more than six months, consecutively seen as in– or out–patients, were enrolled irrespective of liver disease status or treatment received; patients with HIV coinfection were excluded. The enrolment started on June, 1st, 2012 and was stopped on February, 2015.

For each patient, an electronic form was filled with demography, clinical and laboratory data; each patient was included only once. Alcohol intake was estimated using a standard questionnaire containing information on the daily intake of various alcoholic beverages and the lifetime duration of alcohol consumption; an excessive alcohol use was >3 units/day for >1 year. Hepatitis B serum markers (HBsAg, HBeAg, anti-HBe), anti-HDV and anti-HCV antibodies were detected by commercial immunoenzymatic assays. Serum HBV DNA levels were assessed in all centers by real time polymerase chain reaction (PCR).

The patients were assessed by the enrolling center and diagnosed as having chronic hepatitis at a liver biopsy taken within 2 years prior to the enrolment or, for those not undergoing liver biopsy, on the basis of persistent or fluctuating abnormal ALT levels and of an HBV DNA level >2000 IU/mL, in the absence of laboratory or ultrasound and/or elastometry signs of cirrhosis; cirrhosis was diagnosed by liver biopsy or by unequivocal laboratory and instrumental features; biopsies demonstrating cirrhosis could be from any time prior to enrollment. Untreated patients with normal ALT values and no laboratory or instrumental evidence of liver disease were classified as having chronic infection [9]. Hepatocellular carcinoma (HCC) was diagnosed according to EASL recommendations [10].

The choice whether to start anti-viral treatment and the type and schedule of treatment were the responsibility of each participating center, in accordance with guidelines. Data were collected anonymously; written or witnessed informed consent was requested. The study protocol was approved by the Ethical Committee of the coordinating center and by the local Ethical Committees.

1.1. Statistical analysis

Continuous variables were summarized by median, first and third quartiles. Histograms were used to describe empirical distributions; kernel density estimates and estimated normal densities were over-imposed in the plots to underline features that might be obscured by the choice of histogram bins and sampling variation. Categorical variables were described by absolute and relative frequencies.

Associations between categorical variables were evaluated by chi-square test; the Fisher exact test was preferred in cases of sparse tables. Continuous covariates were compared by t-test or Wilcoxon rank-sum test when a significant departure from normality was

Table 1
Characteristics of HBsAg positive patients (n = 2877).

Variable	Median or n	(Q1, Q3) or%
Age(years)	49.8	(36.6, 60.1)
Gender (male)	1,973	68.58%
BMI (Kg/m^2)	24.9	(22.7, 27.6)
Origin		
Italian natives	2106	73.30%
Non-Italian Natives (NINs)	767	26.70%
Provenience of NINs		
East Europe	378	49.28%
Asia	193	25.16%
Africa	171	22.29%
Other	25	3.25%
Liver biopsy	1220	41.87%
Disease stage		
Chronic infection	556	19.56%
Chronic Hepatitis	1,657	58.30%
Cirrhosis	629	22.13%
HCC	109	3.8%
HBeAg positive[a]	342	13.09%
Anti-HDV positive[b]	157	8.18%
Anti-HCV positive[c]	80	3.72%

[a] Tested in 2567 patients.
[b] Tested in 1895 patients.
[c] Tested in 2123 patients.

Table 2
Characteristics of Italian native patients vs. non-Italian natives.

Variables	Italian natives (n = 2,106) Median or n	Non-Italian Natives (n = 767) (Q1, Q3) or%	p value
Age (years)	54.5 (44.7, 63.0)	35.2 (28.6, 43.6)	<0.0001
BMI (Kg/m^2)	25.3 (23.1, 27.7)	23.8 (21.9, 26.2)	<0.0001
Gender (male)	1526 (72.46%)	444 (57.89%)	<0.0001
Disease stage			<0.0001
Chronic infection	373 (17.93%)	181 (23.88%)	<0.0001
Chronic hepatitis	1197 (57.55%)	458 (60.42%)	
Cirrhosis	510 (24.52%)	119 (15.70%)	
Liver biopsy	944 (44.93%)	261 (34.03%)	<0.0001

detected. Box and Whisker plots were created to compare empirical distributions.

Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

2. Results

The study enrolled 2877 HBsAg positive individuals. The median age was 49.8 years, (Interquartile range (IQR) 38.6–60.1), 68.6% were males, 27% were non-Italian natives (NIN), mainly coming from East Europe, Asia and sub-Saharian Africa (Table 1). In all, 20% had chronic infection without evidence of liver disease, 58.5% had chronic hepatitis and 21.5% cirrhosis; liver biopsy results were available in 42.1%. HBeAg in serum was detected in 12.1%, anti-HDV in 8.3% and anti-HCV in 3.7%. A daily alcohol intake of more than 3 drinks was reported in 2.9% of the subjects.

Non-Italian natives were younger than Italians (Fig. S2); male gender was less prevalent and liver disease less advanced ($p < 0.0001$ and $p < 0.0001$, respectively); liver biopsy was less frequently available (Table 2). The age difference was maintained across the different stages of liver disease (Fig. 1). HBeAg positive cases were 23.6% among NINs vs 8.3% in Italians ($p < 0.0001$); the prevalence was higher in Asian natives (84/174; 48.3%). HDV coinfection was present in 11.0% vs 7.1% ($p = 0.0056$); the highest prevalence was recorded in patients from East Europe (48/277; 17.3%). HCV coinfection was detected in 2.3% of NINs vs 4.2% ($p = 0.0378$), respectively (Fig. 2).

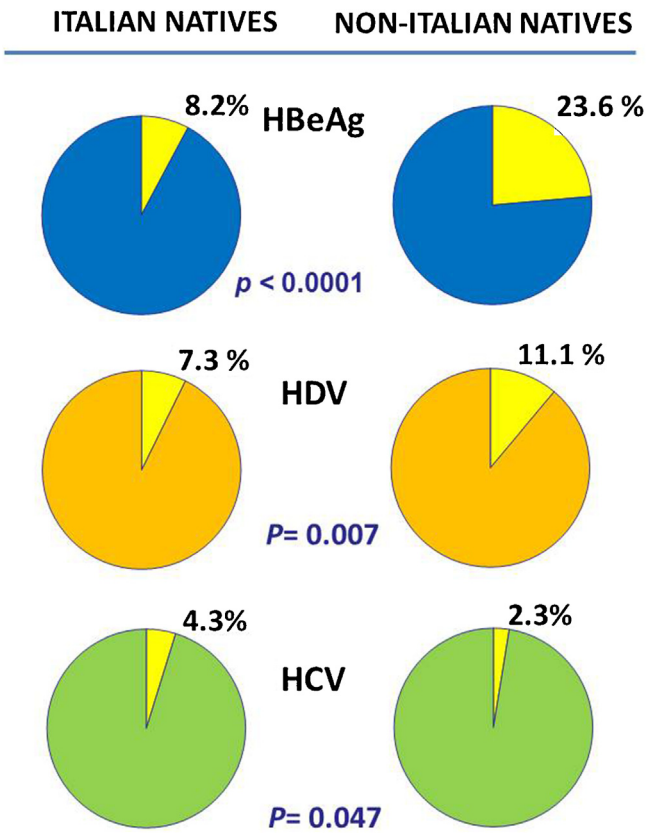

Fig. 2. Prevalences of HBeAg, anti-HDV and anti-HCV among Italian and non-Italian patients.

Comparing patients with cirrhosis with those without cirrhosis, the former were older, more frequently males and Italian natives; anti-HDV or anti-HCV antibodies were more frequently detected in patients with cirrhosis (17.5% vs 5.4% and 6.5% vs 2.9%, respectively). HCC was present in 16% of patients with cirrhosis and in 0.5% in those without cirrhosis (Table 3); prevalence was similar among HDV positive or negative patients; the median age of patients with HCC was 49.1 among NINs and 66.1 among Italians ($p < 0.0001$). Looking at NINs with cirrhosis, 50% of them were aged below 45 years (Fig. 1). At the time of enrolment, 48.3% of Italian patients and 57.1% of NINs with cirrhosis were not undergoing antiviral treatments.

3. Discussion

This study showed an unpredicted high prevalence of non-Italian native persons among subjects with chronic HBV infection attending hospital centers. They were younger and the prevalence of HBeAg positive individuals was three fold higher than in Italian patients, which entails a high potential for transmission. Interestingly, the prevalence of women was higher among NINs, which highlights the potential high risk for vertical transmission from highly viremic mothers [9,11]. At present, the finding of an HBsAg positive is rare among Italian pregnant women and is almost exclusively restricted to immigrant pregnant women [12]. In contrast, the data confirm the continued downtrend of HBeAg positive cases among the Italian population, in line with the data recorded in the last two decades [13]. To date, the Italian population is covered by anti-HBV vaccination for the cohort between 0 and 36 years of age, which abolishes or minimizes the risk of infection. In keeping with

Table 3
Comparison between patients with cirrhosis and those without cirrhosis.

	Patients with cirrhosis N = 629	Patients without cirrhosis N = 2213	p value
Age, median (Q1–Q3)	57.8 (48.5–66.1)	47.3 (36.3–57.3)	<0.0001
Males n (%)	507 (80.6)	1,442 (50.7)	<0.0001
non-Italian natives n (%)	119 (18.9)	639 (28.9)	<0.0001
HBeAg pos[a] n (%)	64 (11.5)	249 (12.4)	0.308
anti-HDV pos[b] n (%)	77(17.5)	79 (5.4)	<0.0001
anti-HCV pos[c] n (%)	33 (6.5)	47 (2.9)	0.0002
HCC n(%)	98 (16.0)	11 (0.5)	<0.0001

[a] Tested in 2567 patients.
[b] Tested in 1895 patients.
[c] Tested in 2123 patients.

this trend, recent epidemiological reports showed that acute hepatitis B cases are limited to adult or aged Italian individuals who are not covered by vaccine and acquired the infection mainly by sexual route [14].

A decrease was confirmed for hepatitis Delta virus coinfection in Italian patients, while the overall prevalence of anti-delta antibodies has stopped decreasing, due to the arrival of co-infected persons from areas where HDV is still endemic. This phenomenon has been described in Europe and seems to be more recent in Italy as in a survey conducted in the years 2006-07, only 7% of HBsAg positive subjects were immigrant [13,15–17]. An opposite trend was noticed for coinfection with HCV, which was more prevalent among Italian individuals.

Cirrhosis was present in 24.5% of Italian patients and in 15.7% of NINs; surprisingly, about half of the patients were not under antiviral treatment at the time of the enrolment. An explanation may be that most cirrhotic patients were enrolled when first seen by the specialist, while being evaluated for therapy; if so, a delay in referral from general practitioners should be suspected. Overall, anti-delta antibodies were present in 17.5% of cirrhotic patients, confirming the ongoing role of hepatitis Delta virus coinfection in this setting. In addition, the data highlighted that about one third of the HBsAg positive patients had not been tested for anti-HDV antibodies at the time of enrollment; this detracting the attention from testing should be counteracted due to the new trend of Delta virus infection in Europe. Missing anti-HCV testing was noted in about 25% of patients. The presence of anti-HCV was higher in patients with cirrhosis; since HCV in curable, testing should be implemented in all HBsAg positive subjects.

Age is an important characteristic of patients with cirrhosis or HCC. Non-Italian patients with cirrhosis or cancer were about a decade younger than Italian patients; thus, while the Italian patients with cirrhosis are getting older and their number will decrease spontaneously in next years, we expect a persisting cirrhosis-related morbidity and mortality due to the wave of younger patients. Not surprisingly, the diagnosis of HCC was made in 16% of patients with cirrhosis and in 0.5% of patients with chronic hepatitis/infection.

Hepatitis B virus is a unique agent due to its property of persisting long-life in the liver of infected individuals as an occult infection [18]. This characteristic extends over the time the impact of HBV infection, since occult HBV has the potential to reactivate in the case of immunodepression or may cooperate with other causes of liver damage to the pathogenesis of hepatocellular carcinoma. In this view, the introduction of a cohort of young subjects with a high rate of exposure to HBV could extend the health consequences over time and adds arguments for dedicated screening and vaccination programs.

A potential limitation of the study is the enrolment in institutional out– or in–patient clinics, which could have over-selected NIN aware of their status or who had overcome cultural barriers towards the health system. However, some participating centers had out-patient clinics dedicated to NIN, which might have minimized the bias. In a US study that compared the effectiveness of HBV screening and linkage to care in foreign-born populations, no difference was found between clinical and nonclinical settings [19]. Further limitation may reside in the high percentage of missing data for some variables, particularly in testing for anti-HDV antibodies; however, comparisons between patients tested and not tested did not show significant differences in their main characteristics, which is against a selection bias (data not shown). Finally, the number of patients with HBV infection and no liver disease might be underestimated due to the enrolment in medical institutions; however, the fact that most patients were seen as out-patients and the length of the study period might have favored the enrolment of patients who accessed out-patients clinics only occasionally, due to their good health status.

In conclusion, the present study offers some insight into the evolving burden of chronic hepatitis B virus infection in the near future and highlights new territories for public health interventions.

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Conflict of interests

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.09.008.

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