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Prevalence of undiagnosed hepatitis B virus infection in patients with COVID-19

A single center retrospective study

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Abstract

At its onset, the coronavirus disease 2019 (COVID-19) pandemic brought significant challenges to healthcare systems, changing the focus of medical care on acute illness. Disruptions in medical service provision have impacted the field of viral hepatitis, with screening programs paused throughout much of 2020 and 2021. We performed a retrospective study on consecutive outpatients with COVID-19 during the second and third wave of COVID-19 in Romania, from November 2020 to April 2021, aiming to characterize the prevalence of undiagnosed hepatitis B virus (HBV) infection among patients presenting with acute illness. Overall, 522 patients had available records during the study timespan. Their mean ± standard deviation age was 51 ± 13 years; 274 (52.5%) were male. We identified 16 (3.1%) cases of active HBV infection; only six of these patients were aware of their HBV status, and 3 of the newly diagnosed cases were identified as candidates for HBV treatment. A total of 96 patients (18.4%) had serological markers suggestive for prior HBV vaccination. A large proportion of patients (n = 120, 23.0%) had positive HBV core antibodies; among these, 90 (17.2%) had cleared a previous HBV infection (being positive for HBV surface antibodies and HBV core antibodies). We identified the following parameters that were significantly more frequent in patients with a history of HBV infection: older age ($P < .001$), hypoalbuminemia ($P = .015$), thrombocytopenia ($P < .001$), thrombocytopenia followed by thrombocytosis ($P = .041$), increased blood urea nitrogen ($P < .001$) and increased creatinine ($P = .011$). In conclusion, the COVID-19 pandemic has taught us essential lessons about the importance of maintaining access to screening programs and of ensuring active monitoring of patients with chronic infections such as hepatitis B, even during a medical crisis.

Abbreviations: 95% CI = 95% confidence interval, ALB = albumin, ALT = alanine aminotransferase, anti-HBc = hepatitis B virus core antibodies, anti-HBs = hepatitis B virus surface antibodies, BIL = total bilirubin, BUN = blood urea nitrogen, COVID-19 = coronavirus disease 2019, GGT = gamma-glutamyl transferase, HBsAg = hepatitis B virus surface antigen, HBV = hepatitis B virus, HCV = hepatitis C virus, HDL-chol = high-density lipoprotein cholesterol, HIV = human immunodeficiency virus, OR = odds ratio, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Keywords: cascade of care, COVID-19, hepatitis B virus, undiagnosed fraction

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has brought significant challenges to healthcare systems worldwide and has, for the largest part of the past two years, changed the main focus of medical care on acute illness, while non-essential medical checkups were postponed indefinitely, at least in the beginning of the pandemic. While 2021 and 2022 have seen tentative strategies for resuming preexisting programs related to endemic pathogens, much work is still needed in order to resume non-acute care at pre-pandemic levels.[1]

The disruption in medical service provision has particularly impacted the field of viral hepatitis, where screening programs were virtually put on hold throughout much of 2020 and 2021, halting the progress towards the World Health Organization's 2030 global elimination targets for viral hepatitis B and C.[1] This disruption of screening programs was a result of multiple

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This study was approved with a waiver for informed consent by the Bioethics Committee and by the Institutional Review Board of the National Institute for Infectious Diseases "Prof Dr Matei Balș".

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concurrent factors, such as: lockdown/stay-at-home orders that temporarily limited patient movement to essential medical care only, during the first months of the pandemic; temporary bans on public gatherings making it impossible to deploy pre-planned on-site screening campaigns for viral hepatitis; and staff, resources and laboratory priorities provisionally shifted to COVID-19.

The National Institute for Infectious Diseases "Prof Dr Matei Balș," Bucharest, Romania is a tertiary care hospital and the main reference center for infectious diseases from the Southern region of Romania. For many years, it has also been the main driver of hepatitis B virus (HBV) screening and opt-in testing programs nationwide. However, in March 2020, the hospital was transformed into a COVID-only hospital, along with the entire network of infectious diseases hospitals throughout the country. As a consequence, throughout 2020 to 2021 the institute's activity was mostly focused on cases of COVID-19. However, even during pandemic times, each patient's interaction with the healthcare system can be used as opportunity to perform screening for essential chronic viral infections. For this reason, as part of routine clinical practice, we offered testing for human immunodeficiency virus (HIV), hepatitis B and hepatitis C to all patients who presented to our institute for diagnosis, monitoring and treatment of COVID-19.

Through the current data analysis, we aimed to characterize the prevalence of undiagnosed HBV infection among patients presenting with acute severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and to evaluate the evolution of liver-related laboratory abnormalities during the course of COVID-19 in patients with and without evidence of prior HBV infection. This analysis aimed to fill a gap in current knowledge, as data is still scarce on liver-related outcomes among patients with COVID-19 who also have a history of prior resolved viral hepatitis. The current study also provides novel data highlighting the fact that, in countries such as Romania, with medium prevalence of HBV infection, screening for viral hepatitis among patients presenting for acute care can also help narrow the gap and reach the undiagnosed fraction of patients living with chronic HBV infection.

2. Methods

We conducted a retrospective study on outpatients who presented for COVID-19 at the National Institute for Infectious Diseases "Prof Dr Matei Balș," Bucharest, Romania over a time span of 6 months, from November 2020 to April 2021, during the second and third wave of COVID-19 in Romania.

Outpatients who presented with symptoms of acute COVID-like illness and who were confirmed through a positive SARS-CoV-2 reverse transcription polymerase chain reaction or European Commission-recognized rapid antigen test were clinically examined and biologically evaluated with complete blood count, chemistry, and urine analysis. They were also screened for HBV surface antigen (HBsAg) and antibody (anti-HBs), HBV core antibody (anti-HBc), hepatitis C virus (HCV) antibodies (confirmed by HCV ribonucleic acid, if positive) and HIV enzyme-linked immunosorbent assay serology. The clinical evolution was followed for 28 days, and they underwent regular laboratory investigations every 7 days (at baseline and at days: 7, 14, and 28). All patients who progressed to severe COVID-19 were hospitalized and received specific care.

In this analysis we included all consecutive adult outpatients (18 years and over) positive for SARS-CoV-2 during the study period who had available results for screening against HBV, HCV, HIV, as well as complete results of weekly monitoring of laboratory parameters. We report here the patterns of liver-related laboratory abnormalities in patients with and without evidence of prior HBV infection (defined based on positive or negative status of anti-HBc) as well as the overall trend for the entire study group.

2.1. Statistical analysis

We checked the distribution of all continuous variables with the Shapiro–Wilk test. For normally distributed variables we report the mean and standard deviation and for non-parametrical variables we report the median and the interquartile range, presented as 25th and 75th percentile; continuous data were compared with the two-tailed independent samples *t* test for parametric variables and with Mann–Whitney *U* test for non-parametric variables. For categorical values, we report the frequency and percentage, and the results of the chi-square test, along with odds ratio (OR) and 95% confidence interval (95% CI). To adjust for potential confounding factors, we performed binomial logistic regression by computing all parameters that had an initial P value < .05. The statistical analysis was performed with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY).

2.2. Ethics approval

This study was approved with a waiver for informed consent by the Bioethics Committee and by the Institutional Review Board of the National Institute for Infectious Diseases "Prof Dr Matei Balș."

3. Results

3.1. Baseline study population information

A total number of 522 patients had available medical records during the study time span. Their mean age ± standard deviation were 51 ± 13 years; 274 (52.5%) were male. All outpatients included in this study presented to our hospital in the first week of illness.

3.2. Viral seromarkers

A large proportion of the patients (n = 120, 23.0%) had positive anti-HBc. Among these, 16 (13.3%) had active HBV infection and were also positive for HBsAg, some other 90 patients (17.2%) had cleared a past HBV infection (positive anti-HBc plus positive anti-HBs) and 14 patients (11.7%) had isolated anti-HBc.

Among our study participants, 96 patients (18.4%) had been vaccinated against HBV, displaying anti-HBs alone. We also identified 16 (3.1%) cases of active HBV infection (positive HBsAg); only 6 of these patients were already aware of their HBV status and were engaged in care for chronic HBV infection. Data on liver fibrosis staging and necro-inflammatory activity was available for 5 patients (4–F0-F1 and 1–F2; 4–A0-A1 and 1–A3). Data on HBV viral load assessment was available for 8 patients (2—undetectable, 3—below 1000 IU/mL, 2—between 2000–2999 IU/mL, and 1–5 × 10^6 IU/mL). Based on this preliminary evaluation, three of the ten newly diagnosed patients (30%) were identified as candidates for HBV treatment.

Fifteen (2.9%) patients were positive for anti-HCV, but only one patient (0.2%) was viremic, indicating active infection. None of the patients tested positive for HIV infection.

3.3. Risk factors

When looking specifically at patients with positive HBsAg, we found no particularities of the laboratory parameters assessed (all P > .05). Analyzing the data from patients with positive anti-HBc, we found that patients with a prior history of HBV infection were more likely to have a cumulus of risk factors, such as older age and higher prevalence of cardiovascular disease, obesity and other types of chronic liver disease apart from viral infection. However, after adjusting for age, COVID-19 severity at the first contact with the hospital, and the presence of chronic conditions (cardiovascular disease, liver disease, obesity), the only factors that remained significantly associated with prior HBV exposure were: advanced age, chronic liver disease, and

COVID-19 baseline severity (Table 1). Furthermore, during COVID-19, patients with prior HBV exposure displayed the following parameters significantly more frequently: presence of hypoalbuminemia ($P = .015$), thrombocytopenia ($P < .001$), or thrombocytopenia followed by thrombocytosis by day 14 ($P = .041$), increased lactate dehydrogenase ($P = .045$), increased blood urea nitrogen (BUN) ($P < .001$) and increased creatinine ($P = .011$; Table 2; Fig. 1). However, after adjusting for confounding patient characteristics, none of the laboratory changes retained statistical significance, suggesting that they could more likely be explained by other underlying patient characteristics rather than the prior HBV exposure per se.

3.4. Liver function tests and association to COVID-19

Over the course of COVID-19, most patients displayed an upward trend in liver-related parameters such as alanine aminotransferase (ALT), aspartate aminotransferase, and total bilirubin (BIL), and a downward trend in total proteins and albumin (ALB) throughout the second week of disease, with a return to baseline levels by day 14 or day 28, at the latest (Figs. 2 and 3; Table 3). This was seen both in patients with and without evidence of prior HBV infection, but those with evidence of prior HBV infection displayed particular liver-related abnormalities, such as higher baseline values of ALT, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase (GGT), BIL, and BUN, and lower levels of total proteins, ALB, platelets, total cholesterol and high-density lipoprotein cholesterol. This difference remained present throughout the 28-day study period, as can be seen from Figures 2 to 4, and the statistical analysis revealed significant differences in patients with evidence of prior HBV infection, such as: a slower return to normal of ALT levels, with significantly higher ALT mean ranks at day 28 ($P = .048$, $r = -0.1$), higher alkaline phosphatase levels at baseline and day 28 ($P = .014$, $r = -0.1$ and $P = .043$, $r = -0.1$, respectively), higher GGT levels at day 14 ($P = .035$, $r = -0.1$), persistently lower ALB levels and persistently higher BUN levels ($P < .05$ at all evaluated time points; Figs. 2 and 4). Platelet levels displayed an interesting trend, comparable in both groups of patients, with reactive thrombocytosis seen at day 14, followed by a return to normal by day 28 in patients without evidence of prior HBV infection and a marked decrease by day 28 in those with evidence of prior HBV infection ($P = .006$, $r = -0.1$; Table 3).

We also performed an analysis of the impact of patient sex on the occurrence of laboratory abnormalities during the course of COVID-19. Regardless of the status of prior exposure to HBV, we found that males were more likely than females to develop hypo- but not hypercholesterolemia (OR = 2.0, 95% CI: 0.9–4.5, $P = .055$ in anti-HBc-positive patients and OR = 1.6, 95% CI: 1.1–2.3, $P = .013$ in anti-HBc-negative patients), as well as decreased levels of high-density lipoprotein (OR = 2.3, 95% CI: 1.6–3.5, $P < .001$ and OR = 2.3, 95% CI: 1.8–2.9, $P < .001$), and increased levels of BUN (OR = 1.6, 95% CI: 1.1–2.4, $P = .045$ and OR = 2.0, 95% CI: 1.5–2.7, $P < .001$). While the occurrence of liver cytolysis or cholestasis was not significantly different in males versus females with prior HBV exposure, among patients with no such prior exposure, males were more likely than females to develop liver cytolysis (OR = 1.5, 95% CI: 1.2–1.9, $P < .001$) and liver cholestasis during the course of COVID-19, presenting a higher occurrence of increased GGT levels (OR = 1.6, 95% CI: 1.2–2.1, $P < .001$) and increased BIL levels (OR = 1.9, 95% CI: 1.1–3.5, $P = .025$).

Throughout the course of disease, 17 patients (3.3%) progressed to severe COVID-19, requiring hospitalization, oxygen supplementation, along with specific intravenous treatment with remdesivir, tocilizumab and dexamethasone. The rate of progression towards severe disease was 6.6-fold higher (n = 11, 9.2%) in patients with prior exposure to HBV than in those without (n = 6, 1.5%), $P < .001$.

Table 1

Clinical characteristics of patients with COVID-19 with and without prior HBV infection.

Patient characteristic	Positive anti-HBc (n = 120)	Negative anti-HBc (n = 402)	Total (n = 522)	Statistical analysis (crude odds ratios)	Statistical analysis (adjusted odds ratios)
Demographics					
Gender (male)	68 (56.7%)	206 (51.2%)	274 (52.5%)	$P = .300$	
Age (yr), mean ± standard deviation*,†	58.4 ± 12.4	48.8 ± 12.5	51.0 ± 13.1	$t(520) = -7.4$, $P < .001$, $d = 0.8$	aOR = 1.05, 95% CI: 1.03–1.07, $P < .001$
Clinical characteristics					
Body mass index (kg/m^2), mean ± standard deviation*	28.6 ± 4.6	26.6 ± 4.5	27.1 ± 4.6	$t(517) = -1.9$, $P < .001$, $d = 0.4$	aOR = 1.04, 95% CI: 0.9–1.1, $P = .344$
Cardiovascular disease*	76 (63.3%)	143 (35.6%)	219 (42.0%)	OR = 3.1, 95% CI: 2.0–4.8, $P < .001$	aOR = 1.5, 95% CI: 0.9–2.6, $P = .122$
Diabetes	13 (10.8%)	24 (6.0%)	37 (7.1%)	$P = .058$	–
Obesity*	40 (33.3%)	82 (20.4%)	122 (6.1%)	OR = 2.0, 95% CI: 1.2–3.1, $P = .005$	aOR = 0.9, 95% CI: 0.5–2.2, $P = .977$
Chronic lung disease	5 (4.2%)	14 (3.5%)	19 (3.6%)	$P = .454$	–
Chronic kidney disease	4 (3.3%)	11 (2.7%)	15 (2.9%)	$P = .467$	–
Chronic liver disease*,† (previously diagnosed)	17 (14.2%)	15 (3.7%)	32 (6.1%)	OR = 4.4, 95% CI: 2.1–8.8, $P < .001$	aOR = 4.1, 95% CI: 1.8–9.1, $P = .001$
Baseline disease severity*,†					
Mild COVID-19	24 (20.0%)	148 (36.8%)	172 (33.0%)	OR = 2.3, 95% CI: 1.4–3.7, $P = .001$	aOR = 6.4, 95% CI: 1.9–21.7, $P = .003$
Moderate COVID-19	96 (80.0%)	254 (63.2%)	350 (67.0%)		
Disease evolution					
Progression to severe COVID-19*	11 (9.2%)	6 (1.5%)	17 (3.3%)	OR = 6.6, 95% CI: 2.4–18.3, $P < .001$	N/A

Baseline disease severity was defined according to the US National Institutes of Health standards for laboratory-confirmed cases of COVID-19, as: mild (symptomatic patients, without dyspnea, without pneumonia seen on chest CT or X-ray), moderate (symptomatic patients, with pneumonia seen on chest CT or X-ray), severe (symptomatic patients, with pneumonia, requiring oxygen supplementation with ambient air peripheral oxygen saturation < 94%), critical (patients requiring management in the intensive care unit for COVID-19 with respiratory failure, septic shock, and/or multiple organ dysfunction).[2]
95% CI = 95% confidence interval, aOR = adjusted odds ratio, COVID-19 = coronavirus disease 2019, HBV = hepatitis B virus, OR = odds ratio, N/A = not applicable.
*Statistically significant by chi-square test or two-tailed independent samples *t* test.
†Statistically significant by logistic regression after adjusting for age, COVID-19 baseline severity, and preexisting cardiovascular disease, liver disease, or obesity.

Table 2

Results of 28-day routine laboratory assessment in patients with COVID-19 with and without prior HBV infection.

Patient characteristic	Positive anti-HBc (n = 120)	Negative anti-HBc (n = 402)	Total (n = 522)	Statistical analysis (crude odds ratios)	Statistical analysis (adjusted odds ratios)
Liver cytolysis (increased transaminases)	63 (52.5%)	202 (50.2%)	265 (50.8%)	$P = .602$	–
Hypoproteinemia	10 (8.3%)	20 (5.0%)	30 (5.7%)	$P = .181$	–
Hypoalbuminemia*	8 (6.7%)	8 (2.0%)	16 (3.1%)	OR = 3.5, 95% CI: 1.3–9.9, $P = .015$	aOR = 0.6, 95% CI: 0.2–2.5, $P = .500$
Increased alkaline phosphatase	7 (5.8%)	13 (3.2%)	20 (3.8%)	$P = .187$	–
Increased gamma glutamyl transferase	44 (36.7%)	137 (34.1%)	181 (34.7%)	$P = .583$	–
Increased total bilirubinemia	3 (2.5%)	26 (6.5%)	29 (5.6%)	$P = .114$	–
Increased direct bilirubinemia	5 (4.2%)	23 (5.7%)	28 (5.4%)	$P = .647$	–
Thrombocytopenia*	45 (37.5%)	80 (19.9%)	125 (23.9%)	OR = 2.5, 95% CI: 1.6–4.0, $P < .001$	aOR = 0.6, 95% CI: 0.3–1.1, $P = .064$
Thrombocytosis	43 (35.8%)	113 (28.1%)	156 (29.9%)	$P = .085$	–
Initial thrombocytopenia followed by thrombocytosis*	10 (8.3%)	14 (3.5%)	24 (4.6%)	OR = 2.6, 95% CI: 1.1–6.0, $P = .041$	aOR = 0.7, 95% CI: 0.3–1.9, $P = .502$
Increased creatine phosphokinase	24 (20.0%)	79 (19.7%)	103 (19.7%)	$P = .896$	–
Increased lactate dehydrogenase*	71 (59.2%)	202 (50.2%)	273 (52.3%)	OR = 1.6, 95% CI: 1.01–2.4, $P = .045$	aOR = 1.1, 95% CI: 0.7–1.9, $P = .553$
Hypocholesterolemia	21 (17.5%)	55 (13.7%)	76 (14.6%)	$P = .304$	–
Hypercholesterolemia	79 (65.8%)	243 (60.4%)	322 (61.7%)	$P = .336$	–
Decreased HDL cholesterol	80 (66.7%)	233 (58.0%)	313 (60.0%)	$P = .091$	–
Increased blood urea nitrogen*	59 (49.2%)	121 (30.1%)	180 (34.5%)	OR = 2.3, 95% CI: 1.5–3.4, $P < .001$	aOR = 1.1, 95% CI: 0.6–1.7, $P = .878$
Increased creatinine*	4 (3.3%)	1 (0.2%)	5 (1.0%)	OR = 13.8, 95% CI: 1.5–124.9, $P = .011$	aOR = 0.2, 95% CI: 0.1–1.4, $P = .092$
Proteinuria	8 (6.7%)	37 (9.2%)	45 (8.6%)	$P = .461$	–

Normal ranges for laboratory tests are given below. Transaminases: alanine aminotransferase (normal range: 10–33 U/L), aspartate aminotransferase (normal range: 10–36 U/L); total serum proteins (normal range: 6.0–8.0 g/dL); serum albumin (normal range: 3.5–5.5 g/dL); alkaline phosphatase (normal range: 43–115 U/L), gamma-glutamyl transferase (normal range: 5–32 U/L); total bilirubin (normal range: 0–1.1 mg/dL); direct bilirubin (normal range: 0–0.4 mg/dL); platelet count (normal range: 163–375 × 10^3/μL); creatine phosphokinase (normal range: 24–169 U/L); lactate dehydrogenase (normal range: 135–281 U/L); total cholesterol (normal range: 125–200 mg/dL); HDL cholesterol (normal range: 40–60 mg/dL); blood urea nitrogen (normal range: 0.7–1.4 mg/dL); creatinine (normal range: 0.7 1.4 mg/dL); urine protein (normal range: negative).

95% CI = 95% confidence interval, anti-HBc = hepatitis B virus core antibodies, aOR = adjusted odds ratio, COVID-19 = coronavirus disease 2019, HBV = hepatitis B virus, HDL = high-density lipoprotein.

*Statistically significant by chi-square test or two-tailed independent samples *t* test, but not by logistic regression adjusting for age, COVID-19 baseline severity, and preexisting cardiovascular disease, liver disease, or obesity.

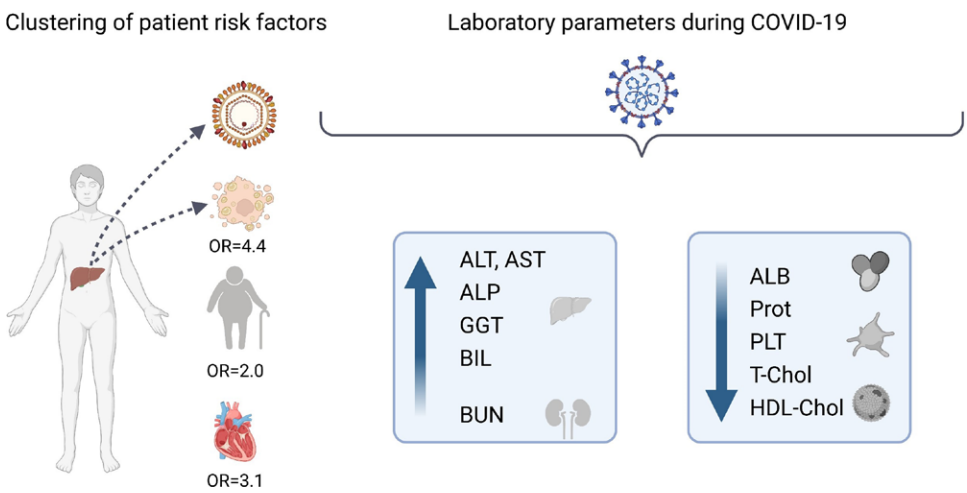

Figure 1. COVID-19 risk profile of patients with prior HBV infection. Patients with prior HBV infection (anti-HBc positive) present a clustering of risk factors such as preexisting liver disease, obesity, higher age, cardiovascular disease. During COVID-19, patients with prior HBV infection display a specific pattern of liver-related abnormalities. ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, anti-HBc = hepatitis B virus core antibodies, AST = aspartate aminotransferase, BIL = total bilirubin, BUN = blood urea nitrogen, GGT = gamma-glutamyl transferase, HBV = hepatitis B virus, HDL-Chol = high-density lipoprotein cholesterol, OR = odds ratio, PLT = platelets, Prot = total proteins, T-chol = total cholesterol. Created with BioRender.com.

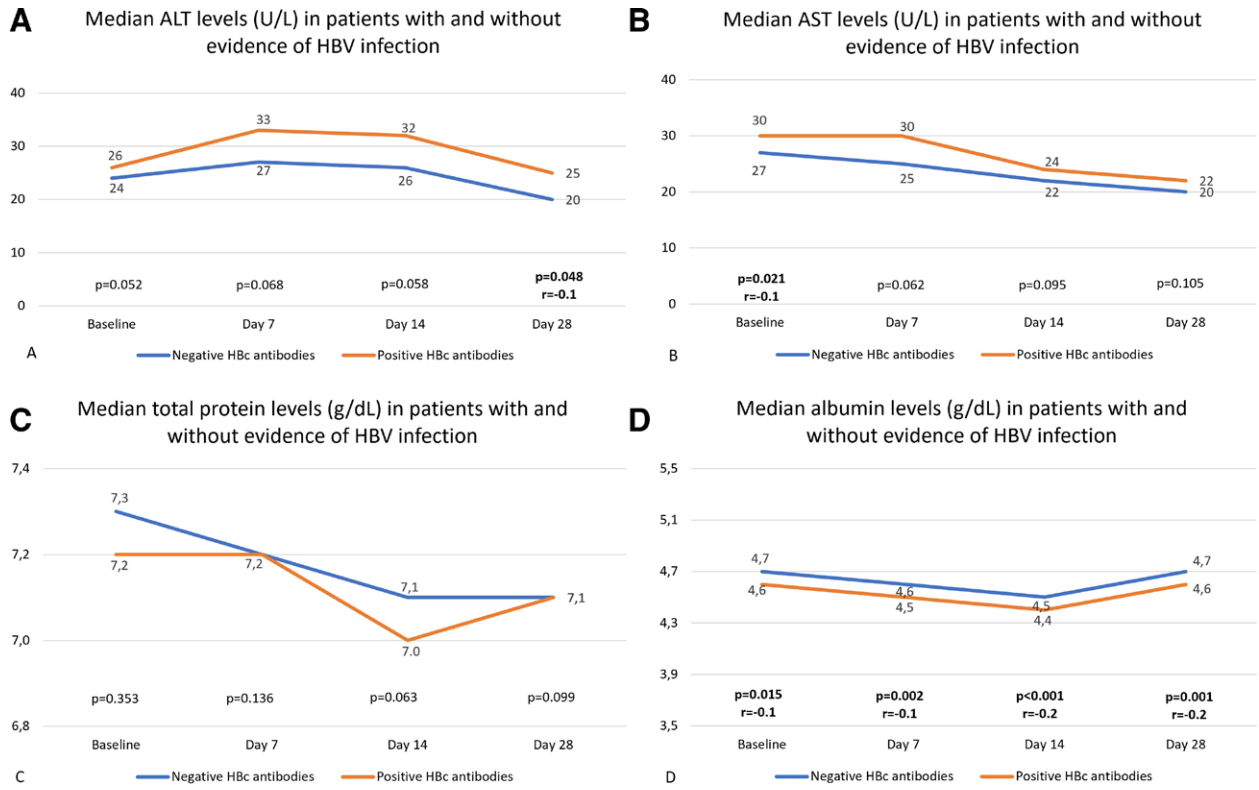

Figure 2. Dynamics of liver-related laboratory parameters in patients with COVID-19 with and without evidence of prior HBV infection. (A) ALT (normal range: 10–33 U/L); (B) AST (normal range: 10–36 U/L); (C) total serum proteins (normal range: 6.0–8.0 g/dL); (D) serum albumin (normal range: 3.5–5.5 g/dL). The statistical analysis between the two patient groups at each time point was performed with the Mann–Whitney *U* test for non-parametric continuous variables. ALT = alanine aminotransferase, AST = aspartate aminotransferase, HBV = hepatitis B virus.

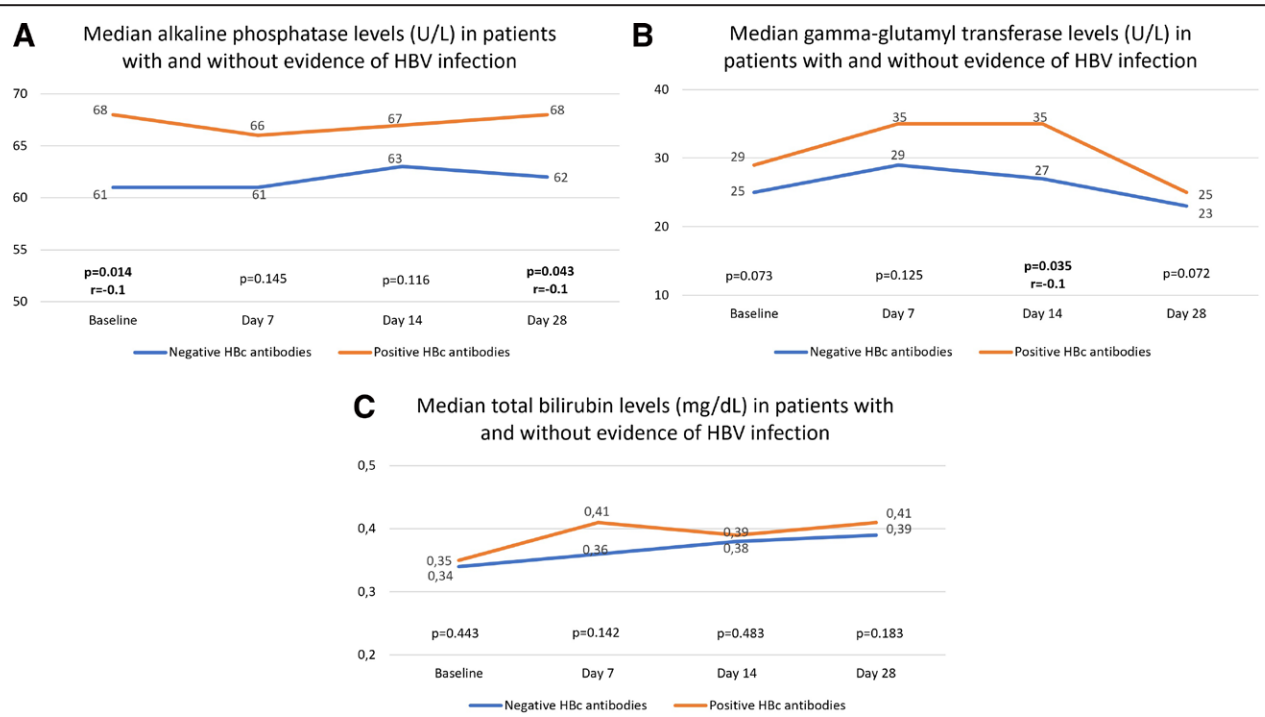

Figure 3. Dynamics of cholestasis-related laboratory parameters in patients with COVID-19 with and without evidence of prior HBV infection. (A) Alkaline phosphatase (normal range: 43–115 U/L); (B) gamma-glutamyl transferase (normal range: 5–32 U/L); (C) total bilirubin (normal range: 0–1.1 mg/dL). The statistical analysis between the two patient groups at each time point was performed with the Mann–Whitney *U* test for non-parametric continuous variables. HBV = hepatitis B virus.

Table 3

Statistical comparison of 28-day routine laboratory assessments in patients with COVID-19 with and without prior HBV infection.

Laboratory assessment	Positive anti-HBc (n = 120)	Negative anti-HBc (n = 402)	Statistical analysis
Alanine aminotransferase (normal range: 10–33 U/L)			
Baseline	26 (18–45)	24 (15–36)	$P = .052$, $U = 21{,}031$, $r = -0.1$
Day 7	33 (19–61)	27 (16–52)	$P = .068$, $U = 18{,}096$, $r = -0.1$
Day 14	32 (19–57)	26 (17–49)	$P = .058$, $U = 17{,}451$, $r = -0.1$
Day 28*	25 (16–37)	20 (14–34)	$P = .048$, $U = 18{,}389$, $r = -0.1$
Aspartate aminotransferase (normal range: 10–36 U/L)			
Baseline*	30 (22–41)	27 (21–35)	$P = .021$, $U = 20{,}514$, $r = -0.1$
Day 7	30 (20–41)	25 (19–37)	$P = .062$, $U = 18{,}262$, $r = -0.1$
Day 14	24 (18–33)	22 (18–31)	$P = .095$, $U = 17{,}737$, $r = -0.1$
Day 28*	22 (18–27)	20 (17–26)	$P = .105$, $U = 18{,}857$, $r = -0.1$
Total proteins (normal range: 6.0–8.0 g/dL)			
Baseline	7.2 (6.9–7.6)	7.3 (6.9–7.6)	$P = .353$, $U = 22{,}547$, $r = 0.0$
Day 7	7.2 (6.8–7.4)	7.2 (6.8–7.5)	$P = .136$, $U = 19{,}042$, $r = -0.1$
Day 14	7.0 (6.7–7.3)	7.1 (6.7–7.4)	$P = .063$, $U = 17{,}685$, $r = -0.1$
Day 28	7.1 (6.8–7.3)	7.1 (6.8–7.4)	$P = .099$, $U = 18{,}825$, $r = -0.1$
Albumin (normal range: 3.5–5.5 g/dL)			
Baseline*	4.6 (4.3–4.8)	4.7 (4.4–4.9)	$P = .015$, $U = 20{,}569$, $r = -0.1$
Day 7*	4.5 (4.2–4.7)	4.6 (4.3 = 4.8)	$P = .002$, $U = 17{,}417$, $r = -0.1$
Day 14*	4.4 (4.2–4.6)	4.5 (4.3–4.7)	$P < .001$, $U = 16{,}007$, $r = -0.2$
Day 28*	4.6 (4.4–4.7)	4.7 (4.5–4.8)	$P = .001$, $U = 16{,}486$, $r = -0.2$
Alkaline phosphatase (normal range: 43–115 U/L)			
Baseline*	68 (56–78)	61 (51–76)	$P = .014$, $U = 20{,}404$, $r = -0.1$
Day 7	66 (52–79)	61 (51–75)	$P = .145$, $U = 19{,}265$, $r = -0.1$
Day 14	67 (53–78)	63 (52–75)	$P = .116$, $U = 18{,}050$, $r = -0.1$
Day 28*	68 (54–80)	62 (52–73)	$P = .043$, $U = 18{,}335$, $r = -0.1$
Gamma-glutamyl transferase (normal range: 5–32 U/L)			
Baseline	29 (17–49)	25 (14–42)	$P = .073$, $U = 21{,}362$, $r = -0.1$
Day 7	35 (20–57)	29 (16–50)	$P = .125$, $U = 19{,}163$, $r = -0.1$
Day 14*	35 (18–59)	27 (15–50)	$P = .035$, $U = 17{,}356$, $r = -0.1$
Day 28	25 (17–41)	23 (14–36)	$P = .072$, $U = 18{,}622$, $r = -0.1$
Total bilirubin (normal range: 0–1.1 mg/dL)			
Baseline	0.35 (0.25–0.46)	0.34 (0.23–0.47)	$P = .443$, $U = 22{,}836$, $r = 0.0$
Day 7	0.41 (0.29–0.55)	0.36 (0.26–0.50)	$P = .142$, $U = 19{,}067$, $r = -0.1$
Day 14	0.39 (0.29–0.54)	0.38 (0.27–0.53)	$P = .483$, $U = 19{,}160$, $r = 0.0$
Day 28	0.41 (0.32–0.56)	0.39 (0.28–0.54)	$P = .183$, $U = 19{,}237$, $r = -0.1$
Total cholesterol (normal range: 125–200 mg/dL)			
Baseline	170 (143–204)	182 (154–211)	$P = .399$, $U = 22{,}724$, $r = 0.0$
Day 7	177 (148–210)	183 (158–207)	$P = .478$, $U = 20{,}447$, $r = 0.0$
Day 14	196 (165–223)	197 (170–223)	$P = .538$, $U = 19{,}269$, $r = 0.0$
Day 28	213 (177–238)	207 (176–240)	$P = .677$, $U = 20{,}434$, $r = 0.0$
HDL-cholesterol (normal range: 40–60 mg/dL)			
Baseline	40 (35–51)	44 (34–57)	$P = .442$, $U = 22{,}775$, $r = 0.0$
Day 7	36 (31–44)	40 (32–50)	$P = .221$, $U = 19{,}391$, $r = -0.1$
Day 14*	41 (34–50)	44 (35–53)	$P = .030$, $U = 17{,}286$, $r = -0.1$
Day 28	47 (39–55)	49 (40–60)	$P = .138$, $U = 19{,}041$, $r = -0.1$
Platelets (normal range: 163–375 × 10^3/μL)			
Baseline	204 (154–246)	210 (181–267)	$P = .107$, $U = 16{,}403$, $r = -0.1$
Day 7	266 (206–346)	279 (224–338)	$P = .447$, $U = 16{,}568$, $r = 0.0$
Day 14	302 (241–357)	304 (257–386)	$P = .225$, $U = 16{,}514$, $r = -0.1$
Day 28*	235 (190–269)	249 (220–298)	$P = .006$, $U = 15{,}257$, $r = -0.1$
Blood urea nitrogen (normal range: 5–20 mg/dL)			
Baseline*	14 (11–19)	13 (10–16)	$P = .004$, $U = 19{,}777$, $r = -0.1$
Day 7*	16 (12–20)	14 (11–18)	$P = .001$, $U = 16{,}805$, $r = -0.1$
Day 14*	16 (13–20)	13 (11–17)	$P < .001$, $U = 14{,}688$, $r = -0.2$
Day 28*	14 (12–18)	13 (11–16)	$P = .006$, $U = 17{,}359$, $r = -0.1$

Data are presented as median (interquartile range, i.e., 25th–75th percentile).

COVID-19 = coronavirus disease 2019, anti-HBc = hepatitis B virus core antibodies, HBV = hepatitis B virus, HDL = high-density lipoprotein.

*Statistically significant by Mann–Whitney *U* test.

4. Discussion

Healthcare systems around the world were taken by surprise by the emergence and fast spread of SARS-CoV-2. Rapid adaptation was required, and most resources were initially directed towards the management of patients with COVID-19 and towards limiting SARS-CoV-2 transmission.[3] All these measures taken at the onset of the pandemic together with the fear of possible exposure to the novel coronavirus drastically reduced Emergency Department visits as well as hospital visits for chronic diseases.[4] In addition, in most countries, screening programmes were suspended or conducted with reduced coverage.[5–8] The effects of these behaviors and decisions are likely to have had negative consequences on non-COVID-19 patients, particularly those with chronic diseases and should be followed carefully in the future to identify and address them early.

Patients with chronic infectious diseases, especially chronic hepatitis B or C and HIV, have suffered from difficult access

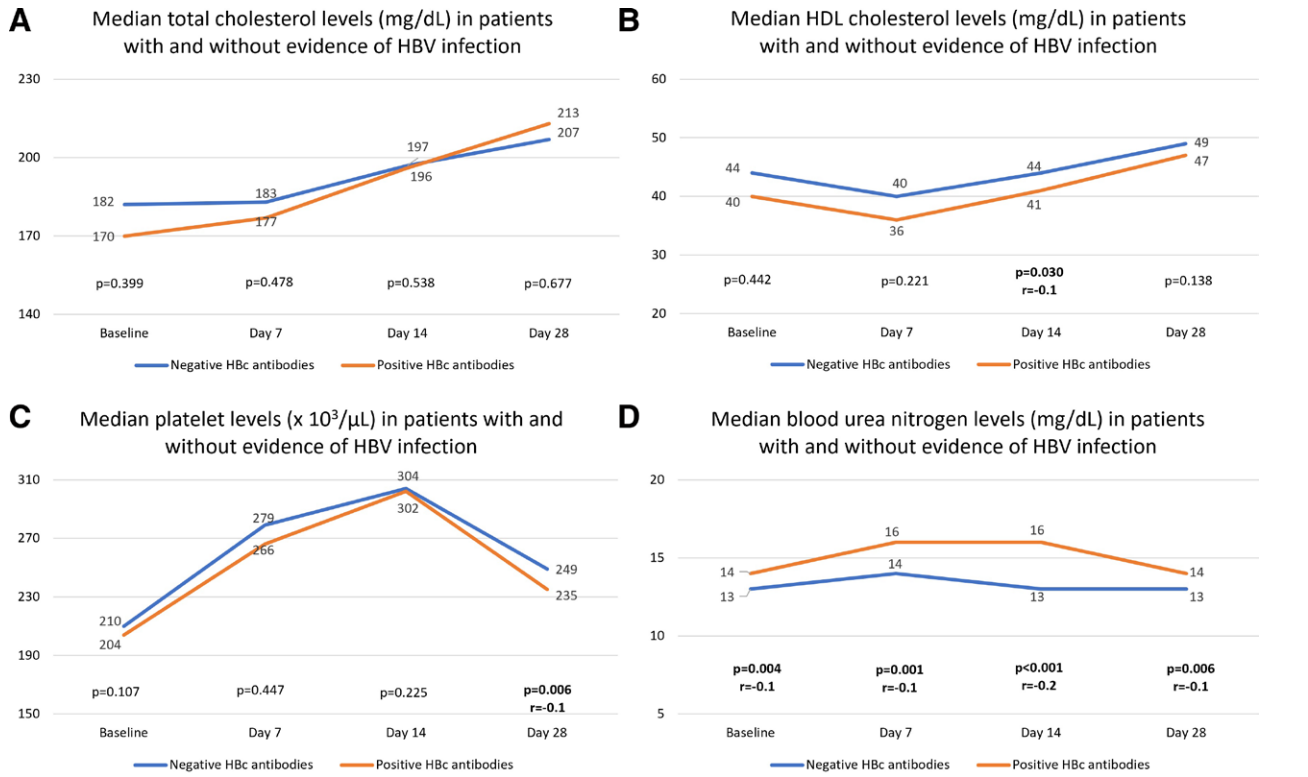

Figure 4. Dynamics of other laboratory parameters in patients with COVID-19 with and without evidence of prior HBV infection. (A) Total cholesterol (normal range: 125–200 mg/dL); (B) HDL cholesterol (normal range: 40–60 mg/dL); (C) platelet count (normal range: 163–375 × 10^3/μL). (D) Blood urea nitrogen (normal range: 0.7–1.4 mg/dL). The statistical analysis between the two patient groups at each time point was performed with the Mann–Whitney *U* test for non-parametric continuous variables. HBV = hepatitis B virus, HDL = high-density lipoprotein.

to medication and regular reassessments.[8–10] In addition, most infectious disease hospitals, including ours, used to have the necessary structure for national hepatitis and HIV screening programs, but their conversion to COVID-19-only hospitals abruptly reduced surveillance of these infectious diseases.[8,10] Following the transformation of our institute into a COVID-19-only hospital in March 2020, the epidemiological circuits had to be reorganized to respond to the novel identified needs, including separate areas for patients and for healthcare workers, necessary to ensure the safety of the medical personnel. This also led to a temporary disruption in the provision of care for patients with chronic infections such as hepatitis B, as all non-urgent medical care was postponed during the beginning of the pandemic. Even after the resumption of outpatient services for non-COVID patients, in many cases patients with chronic viral infections chose to delay their scheduled in-person medical visits for fear of cross-contamination.

Thus, under the conditions imposed by the pandemic, it was necessary to adapt. The management of the patient with COVID-19 has to be carried out taking into account the particularities of the patient, and particularly his/her known and unknown chronic conditions, the latter needing to be investigated. In addition, given the large number of COVID-19 cases in our country in the period November 2020 to April 2021, some of the patients who referred to our hospital were evaluated as outpatients and followed up on a case-by-case basis in order to limit and timely identify any progression to severe forms of disease, to ensure early intervention. Thus, all patients were evaluated clinically and by laboratory investigations (including viral serologies), and in this analysis we included only those who had complete follow-up for 28 days in order to provide an extensive picture of the impact of the pandemic among patients with or without hepatitis B exposure.

The prevalence of 3.1% HBsAg that we found in our study of outpatients presenting with COVID-19 at a tertiary care hospital in Romania is in line with the data previously reported for Romania, which placed the country at intermediate prevalence, with 4.4% of the general population testing positive for HBsAg,[11] and higher than that reported from a comparable study performed in Italy in a slightly smaller patient group, where 0.5% of 372 patients tested positive for HBsAg.[12] In our study, only six of our 16 HBsAg-positive patients were aware of their HBV status, and the overall rate of new HBV diagnoses was 1.9% in the entire patient cohort, suggesting that there might be quite a large proportion of undiagnosed patients that we still need to actively identify and link to care. Importantly, one third of these newly diagnosed cases of HBV infection also met the criteria for starting antiviral treatment, highlighting the need for earlier access to screening, to allow timely therapeutic intervention. None of the routine laboratory parameters assessed was associated significantly with active HBV infection, reinforcing what is already known, that is, that HBV infection can be silent, requiring active screening programs for timely detection, diagnosis, and treatment.

Only a small fraction of the study population had serological evidence of having been vaccinated against HBV (18.4%), which can be partly explained by the mean age of the study population (51 years old), as in Romania vaccination of newborns against HBV was first implemented in 1995, with subsequent catch-up campaigns for all age cohorts dating back to 1986, that is, younger patients than most of those presenting with COVID-19 during the second and third wave, as seen in our study group.

Furthermore, we found a high prevalence (23%) of anti-HBc, indicative of past HBV infection, higher than the prevalence of 17% reported among 320 subjects from a similar patient population tested in Italy.[12] The following patient characteristics

were considered to be highly suggestive for history of prior HBV infection and should trigger an in-depth liver and viral hepatitis workup: older age, hypoalbuminemia, decreased platelet counts either alone or followed by increased platelet counts at day 14, and increased lactate dehydrogenase. We further found that patients with evidence of prior HBV infection displayed particular dynamics of liver-related laboratory parameters over the course of COVID-19, specifically: higher liver cytolysis (increased transaminases) and cholestasis (increased bilirubin), lower ALB levels, and lower platelet counts, compared to patients without evidence of prior HBV infection; they also displayed higher BUN levels, indicating the need to closely follow the kidney function in this subset of patients. However, while these changes were evident between the two patient groups, those with and without evidence of prior HBV infection, the laboratory values in themselves were not markedly outside the laboratory normal ranges, suggesting that, in most patients, no clinically significant liver injury occurred in our studied population of mainly mild to moderate cases of COVID-19. Furthermore, these laboratory abnormalities were most likely explained by other baseline patient characteristics, as seen from the adjusted statistical analysis. This observation is in line with data coming from field literature, that have shown that for patients with active HBV infection, worse COVID-19 outcomes can be seen, with 2-fold[13] or 3-fold higher mortality[14]; however, these do not remain significantly different when adjusting for patient characteristics such as age, sex and comorbidities, and, particularly, the underlying degree of liver damage,[13] suggesting that liver function abnormalities mediate the increased risk of poor COVID-19 prognosis in patients with HBV infection.[14] However, ours is the first such report specifically for this study population, that is, patients with prior history of HBV infection at the time when they developed COVID-19, and our data is therefore adding to the current literature by highlighting certain clinical and laboratory abnormalities that should trigger an in-depth liver workup and screening for viral hepatitis.

Dexamethasone and tocilizumab are part of the standard of care for some patients with severe COVID-19, to prevent further progression of pneumonia.[15,16] Given the high anti-HBc seroprevalence found in our study, we could raise a theoretical concern related to the use of these agents and the risk of HBV reactivation in the absence of antiviral prophylaxis, as previously shown.[17,18] One particular reported case of HBV reactivation in an HBsAg-negative anti-HBc-positive patient receiving cortico-steroids and tocilizumab for rheumatoid arthritis[17] seems to suggests that silent viral reactivation can occur, in the absence of any warning signs on routine laboratory assessments. In this reported case, reactivation occurred after 18 months of tocilizumab treatment,[17] while for COVID-19 a single dose of 400 to 800 mg based on body weight is generally administered, with different local practices recommending as many as three doses. However, subclinical reactivation of HBV has also been reported in patients with rheumatoid arthritis after only 1 to 3 doses of tocilizumab, in patients with positive HBsAg.[19]

Because of the observational retrospective nature of the study, with no predefined outcomes, other than a patient testing positive or negative for viral hepatitis markers, and because all consecutive patients with complete medical records from the respective COVID-19 waves were included in our study, no formal sample size calculation was performed. A post hoc sample size analysis considering a rate of HBsAg positivity estimated at 4.4% (as reported by the European Center for Disease Control[11]), a standard conventional margin of error of 0.05, a 95% confidence interval, and an unlimited population size, indicated that a sample size of at least 65 patients should be included in the analysis, and this is much below the actual sample size of 522 patients that had available records for analysis in this study.

Our retrospective study comes with a set of limitations. Due to the fact that this data was collected from routine clinical practice during two of the largest waves of COVID-19 that had occurred in Romania up to that point, we were unable to further investigate patients with positive anti-HBc. Each of these patients received the recommendation to perform an HBV-DNA test, a liver ultrasound and a fibrosis assessment, but because the largest part of the medical system was focused mostly on emergencies and essential medical care during that time of the pandemic, this was hard to achieve. The surprisingly high (23%) prevalence of anti-HBc highlights the need for further in-depth investigation into this patient population. Other limitations include the retrospective nature of the study, the fact that it was performed in a single center, and that this data derived from an outpatient population cannot be generalized to other patients, that is, those requiring hospitalization, who may be older or may present a different set of risk factors and are therefore underrepresented in this outpatient-based study, where only 17 patients (3.3%) required hospitalization and administration of remdesivir, tocilizumab and corticosteroids. Furthermore, the absence of an electronic HBV vaccine registry at the time of our study made it impossible to double-check the patients' vaccination history.

The inclusion of a hepatitis screening workup in the management of patients with COVID-19 could allow us to better understand the prevalence of undiagnosed HBV infection and to allow tailored therapeutic decisions in this patient population, particularly in settings where the seroprevalence of HBV is intermediate to high.

5. Conclusions

In conclusion, the COVID-19 pandemic has taught us essential lessons about the importance of maintaining access to screening programs and of ensuring active monitoring of patients with chronic infections such as hepatitis B, even during a medical crisis.

Author contributions

All authors had equal contributions and were involved in study conception and design, collection, interpretation, and analysis of data, drafting the manuscript and/or revising the manuscript critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

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