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Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels

Aslı Ormeci [a], Yucel Aydın [a], Abdullah Sumnu [b], Bulent Baran [c], Ozlem Mutluay Soyer [a], Binnur Pınarbasi [a], Suut Gokturk [a], Mine Gulluoglu [d], Derya Onel [e], Selim Badur [e], Filiz Akyuz [a], Cetin Karaca [a], Kadir Demir [a], Fatih Besisik [a], Sabahattin Kaymakoglu [a,*]

[a] Department of Gastroenterohepatology, Istanbul Faculty of Medicine, Istanbul University, Capa, 34390, Istanbul, Turkey
[b] Medipol University, Department of Nephrology, Istanbul, Turkey
[c] Department of Gastroenterology, Koç University Hospital, Istanbul, Turkey
[d] Department of Pathology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
[e] Department of Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

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SUMMARY

Objectives: Serum alanine aminotransferase (ALT) is a controversial marker for disease monitoring in hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients. The aim of this study was to determine the fibrosis stage and histological activity index (HAI) in HBeAg-negative CHB patients with persistently normal ALT (PNALT) and high serum HBV DNA (≥2000 IU/ml) and to investigate clinical risk factors for the requirement of treatment through the examination of liver biopsy specimens.

Methods: HBeAg-negative CHB patients with PNALT (≤40 IU/l) and high serum HBV DNA (≥2000 IU/ml) were included. HBV fibrosis stage and HAI were scored according to the Ishak system. Multivariate logistic regression analysis was used to estimate the independent risk factors for fibrosis stage ≥2 and/or HAI ≥6. Receiver operating characteristic curve analysis was used to determine an optimal age cut-off for liver biopsy.

Results: A total 120 patients were enrolled. These patients had a mean HBV DNA level of 123 680 ± 494 500 IU/ml; the HBV DNA load was 2000–20 000 IU/ml in 68 patients (56.6%) and ≥20 000 IU/ml in 52 (43.4%). Eighteen patients (15%) had moderate-to-severe histological activity (HAI ≥6). Forty-three patients (35.9%) had a fibrosis stage ≥2. Forty-eight patients (40%) had a fibrosis stage ≥2 and/or HAI ≥6. On multivariate logistic regression analysis, independent variables associated with fibrosis stage ≥2 and/or HAI ≥6 included age and HBV DNA viral load. Patients with HBV DNA 2000–20 000 IU/ml were more likely to require treatment compared to those with a viral load ≥20 000 IU/ml. The optimal age cut-off to predict fibrosis stage ≥2 and/or HAI ≥6 was 46 years.

Conclusions: Significant liver damage was detected in 40% of CHB patients with PNALT and high HBV DNA upon biopsy. Age and HBV DNA viral load were independent predictors of significant liver damage. A biopsy to determine the degree of liver damage is advisable for CHB patients older than 46 years.

1. Introduction

Hepatitis B virus (HBV) infection remains an important public health concern; approximately 400 million people are infected worldwide.[1] Chronic hepatitis B (CHB) has a wide clinical spectrum, ranging from asymptomatic carrier status to cirrhosis and hepatocellular carcinoma.[2,3] Proper management of the disease is important to prevent mortality by reducing HBV-related complications.

The predominant type of CHB infection is hepatitis B e antigen (HBeAg)-negative. This develops after the loss of HBeAg and can subsequently remain in a low/non-replicative phase (inactive chronic HBV carrier status) or progress to an active phase.[4,5] The

* Corresponding author. Tel.: +90 212 4142000 (32671–31140); fax: +90 212 6319743.
E-mail address: kaymakoglus@hotmail.com (S. Kaymakoglu).

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distinction between these two conditions is crucial. Observation without treatment can be sufficient for HBV carriers; however, HBeAg-negative CHB infections require treatment.

HBeAg-negative CHB is generally differentiated from the inactive carrier state by serial measurement of serum alanine aminotransferase (ALT) and HBV DNA levels. Positivity for hepatitis B surface antigen (HBsAg), negative HBeAg, and elevated ALT and serum HBV DNA levels are diagnostic of HBeAg-negative CHB.[6] Serum levels of ALT, an enzyme released by hepatocytes during liver injury, usually reflect the degree of liver damage; however, not every HBeAg-negative CHB-infected patient exhibits elevated ALT. HBV DNA viral load and ALT levels can fluctuate in cases of HBeAg-negative CHB infection. A single measurement of ALT or HBV DNA viral load is insufficient to determine the current phase of the disease. A proportion of HBeAg-negative CHB patients can have persistently normal ALT (PNALT) levels for an extended period.[7]

The European Association for the Study of the Liver (EASL) define PNALT as an ALT below 40 IU/l when checked every 3–4 months in a single year.[8]

According to the latest EASL guidelines, HBeAg-negative CHB patients with PNALT and HBV DNA levels between 2000 and 20 000 IU/ml, and who have no evidence of liver disease, do not require immediate liver biopsy or treatment. However, it is recommended that they receive careful follow-up, with ALT assessments every 3 months, as well as HBV DNA load measurements every 6–12 months, for at least 3 years.[8]

However, there have been reports of histological injury in patients with PNALT. Furthermore, more recent studies have shown liver damage in CHB patients with PNALT who have viral loads above 2000 IU/ml.[9,10] Liver biopsy can be used to assess the severity of necrosis and inflammation in addition to fibrosis, and can rule out other causes of liver disease; hence, biopsy is regarded as the best method to assess the severity of inflammatory activity and fibrosis.[7]

It was hypothesized that a significant proportion of HBeAg-negative HBV-infected patients with high HBV DNA levels may have significant histological liver abnormalities despite PNALT. This prospective study was therefore performed to determine the fibrotic stage and histological activity index (HAI) in HBeAg-negative CHB patients with PNALT and high serum HBV DNA ($\geq$2000 IU/ml) viral loads. The clinical risk factors associated with significant histological abnormalities in liver biopsy specimens were also investigated.

2. Methods

2.1. Study design and setting

The study was designed as a single-center, prospective study in the Gastroenterohepatology Department of Istanbul Faculty of Medicine, Istanbul University. The study protocols abided by the ethical guidelines as stated in the 1975 Declaration of Helsinki and were approved by the local institutional review board. Written informed consent for participation in the study was obtained from each patient.

2.2. Patients

A total 120 patients with CHB admitted to the university hospital gastroenterology department between April 2009 and December 2012 were included in this study. Patients presenting directly to the gastroenterology department or referred from other clinical centers to the department clinic as a tertiary healthcare institution were recruited; they were included according to the date at first presentation to the department clinic. Inclusion criteria were age $\geq$18 years, diagnosis with CHB infection (defined as positive HBsAg for more than 6 months), HBV DNA load $\geq$2000 IU/ml at least twice within the 6–12-month interval checks in the past year, PNALT (according to at least four values obtained at 3-month intervals in the past year), and no previous or concomitant anti-HBV therapy. Patients with liver comorbidities including hepatitis delta virus (HDV) infection, hepatitis C virus (HCV) co-infection, chronic alcohol consumption (>30 g of pure alcohol per day), Wilson's disease, HIV co-infection, autoimmune hepatitis, present or past evidence of any symptoms related to chronic liver disease, and imaging or laboratory results that indicated cirrhosis were excluded from the study, as were those with evidence of immune suppression.

2.3. PNALT definition

PNALT was defined according to the EASL guidelines; i.e., ALT remaining below 40 IU/l when checked every 3–4 months within a single year.[8] Serum levels of ALT were measured every 3 months for at least 1 year before liver biopsy.

2.4. Serum markers

All of the patients underwent serum biochemistry tests, including for ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, and alpha-fetoprotein, in addition to complete blood counts. ELISAs were used to measure HBsAg, HBeAg, antibodies to HBeAg (anti-HBe), antibodies to HCV (anti-HCV), HDV IgG antibodies (anti-HDV), and HIV. Quantitative HBV DNA testing was performed using the COBAS AmpliPrep/COBAS Taqman 96 system (Roche, Branchburg, NJ, USA). The dynamic measurement level of the kit ranges between 20 and 170 000 000 IU/ml.

2.5. Liver biopsy

All patients underwent a percutaneous liver biopsy guided by ultrasonography. Liver biopsies were performed using 18-gauge biopsy needles. The specimens obtained were fixed, paraffin-embedded, and stained with hematoxylin–eosin. Appropriate diagnosis of a biopsy specimen included the observation of at least six portal areas in the sample. To avoid differences and the bias that can occur between examiners, all data were examined and evaluated by a single experienced pathologist who was blinded to the clinical data. Fibrosis and the HAI were scored using the Ishak scoring system.[11] Stages of fibrosis ranged from 0 (no fibrosis) to 6 (cirrhosis; probable or definite).

2.6. Treatment indication

Treatment indication (significant histological abnormalities) was defined as the presence of HAI $\geq$6 and/or the presence of stage $\geq$2 fibrosis in liver biopsy specimens. The optimal age cut-off to detect treatment indication was determined through receiver operating characteristic (ROC) curve analysis. The optimal cut-off point was calculated using the ROC curve coordinates for treatment indication (point nearest to the top left corner, yielding the best relationship between sensitivity and specificity).

2.7. Statistical analysis

NCSS (Number Cruncher Statistical System, 2007) and PASS (Power Analysis and Sample Size, 2008) statistical software were used for the statistical analysis (NCSS LLC, Kaysville, UT, USA). Descriptive statistics such as the mean, standard deviation, frequency, and rate were used. Furthermore, the Student *t*-test

was used to compare parameters with a normal spread between the groups. Diagnostic screening tests and area under the ROC curve (AUC) analysis were used to define the cut-off point for age. The 95% confidence intervals (CIs) were determined for all results; $p < 0.05$ was considered significant.

3. Results

A total 120 patients were included in the study, of whom 58 (48.3%) were male. The mean age of the patients was 42.88 ± 11.32 years (range 20–72 years).

All patients were anti-HBe-positive with PNALT. None of the patients were positive for anti-HCV or anti-HDV antibodies. All patients underwent liver biopsies after 1 year of follow-up. The mean HAI score was 2.89 ± 2.30 (median 3, range 0–8), while the mean stage was 1.1 ± 0.95 (median 3, range 0–18) according to the Ishak scoring system. The baseline demographic and laboratory characteristics of the 120 patients are summarized in Table 1.

The histopathological findings on liver biopsy are shown in Table 2. No patient had liver disease above stage 4. There were nine patients (7.5%) with a HAI score of 6, seven (5.8%) with a HAI score of 7, and two (1.7%) with a HAI score of 8; 40% of the patients had HAI ≥6 or stage ≥2 disease, or both, which indicated treatment.

The HBV DNA load of all patients was above 2000 IU/ml at first examination and during the follow-up period. The mean HBV DNA load was 123 680 ± 494 500 IU/ml (5.09 ± 5.69 $\log_{10}$ IU/ml). The median HBV DNA value was 18 000 IU/ml (4.2, range 3.3–6.65 $\log_{10}$ IU/ml). With regard to viral load distribution, 68 patients (56.6%) had an HBV DNA load in the range 2000–20 000 IU/ml and 43 (35.9%) had an HBV DNA load in the range 20 000–200 000 IU/ml. Nine patients (7.5%) had an HBV DNA load higher than 200 000 IU/ml.

Table 1
Baseline demographic and laboratory characteristics of the 120 study patients

	Patients (N = 120)
Age, years, mean ± SD	42.8 ± 11.32
Sex, *n* (%)	
Male	58 (48.3)
Female	62 (51.7)
AST (IU/l)	
Mean ± SD	23 ± 6.06
Median (range)	22 (12–40)
ALT (IU/l)	
Mean ± SD	25 ± 8.25
Median (range)	24 (12–40)
ALP (IU/l)	
Mean ± SD	126.32 ± 55.23
Median (range)	120 (34–332)
GGT (IU/l)	
Mean ± SD	22.64 ± 14.84
Median (range)	20 (7–131)
Total bilirubin, mg/dl	
Mean ± SD	0.64 ± 0.33
Median (range)	0.6 (0.2–1.2)
Alpha-fetoprotein, ng/ml	
Mean ± SD	3.02 ± 2.95
Median (range)	2 (0.10–15.0)
HBV DNA ($\log_{10}$IU/ml)	
Mean ± SD	5.09 ± 5.69
Median (range)	4.2 (3.3–6.65)
Stage	
Mean ± SD	1.1 ± 0.95
Median (range)	1 (0–6)
HAI	
Mean ± SD	2.89 ± 2.30
Median (range)	3 (0–18)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HAI, histological activity index; HBV, hepatitis B virus; SD, standard deviation.

Table 2
Histopathological findings in biopsy specimens of chronic hepatitis B patients with persistently normal alanine aminotransferase, according to the Ishak scoring system

Liver biopsy findings	*n* (%)
Stage	
0	38 (31.7%)
1	39 (32.5%)
2	38 (31.7%)
3	2 (1.7%)
4	3 (2.5%)
HAI	
<6	102 (85%)
≥6	18 (15%)
HAI ≥6 or stage ≥2	
Negative	72 (60.0%)
Positive	48 (40.0%)

HAI, histological activity index.

3.1. Analysis of factors associated with significant necroinflammatory activity and fibrosis

The effect of each variable on significant necroinflammatory activity was assessed. Univariate analysis indicated that ALT, AST, age, and male sex were associated with significant necroinflammatory activity (HAI ≥6) in HBeAg-negative patients with PNALT (Table 3). Moreover, age was associated with significant fibrosis in these patients (Table 4).

Table 3
Univariate analysis of factors associated with significant necroinflammatory activity in HBeAg-negative patients with persistently normal alanine aminotransferase

	Necroinflammatory activity		*p*-Value
	HAI <6	HAI ≥6	
Age, years	*n* = 102	*n* = 18	
Mean ± SD	41.86 ± 11.29	48.67 ± 9.98	0.018[c*]
Median (range)	41.5 (20–72)	49 (30–68)	
Sex			0.003[b**]
Male	43	15	
Female	59	3	
AST, IU/l	*n* = 102	*n* = 18	
Mean ± SD	22.64 ± 5.86	25.83 ± 6.63	0.038[c*]
Median (range)	22 (12–40)	25.5 (14–37)	
ALT, IU/l	*n* = 102	*n* = 18	
Mean ± SD	24.24 ± 8.05	29.33 ± 8.24	0.015[c*]
Median (range)	23 (11–40)	29.5 (12–40)	
ALP, IU/l	*n* = 102	*n* = 18	
Mean ± SD	123.53 ± 55.48	145.62 ± 51.4	0.076[a]
Median (range)	120 (46–332)	150 (34–210)	
GGT, IU/l	*n* = 102	*n* = 18	
Mean ± SD	22.51 ± 15.57	23.5 ± 9.14	0.261[a]
Median (range)	20 (7–131)	23 (8–48)	
Total bilirubin, mg/dl	*n* = 102	*n* = 18	
Mean ± SD	0.64 ± 0.32	0.7 ± 0.4	0.766[a]
Median (range)	0.6 (0–2.19)	0.67 (0.31–1.78)	
Alpha-fetoprotein ng/ml	*n* = 102	*n* = 18	
Mean ± SD	3.18 ± 3.12	2.23 ± 1.76	0.434[a]
Median (range)	2.2 (0.1–15)	1.95 (0.15–5)	
Platelet count, $\times 10^9$/l	*n* = 102	*n* = 18	
Mean ± SD	243.5 ± 64.4	234.2 ± 49.0	0.560[c]
Median (range)	233.5 (95–421)	226 (166–350)	
HBV DNA, $\log_{10}$ IU/ml	*n* = 102	*n* = 18	
Mean ± SD	5.13 ± 5.72	4.72 ± 5.03	0.763[a]
Median (range)	4.2 (3.3–6.6)	4.06 (3.34–5.64)	

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; SD, standard deviation.
[a]Mann–Whitney *U*-test; [b]Yates continuity correction test; [c]Student *t*-test; $^*p < 0.05$, $^{**}p < 0.01$.

Table 4
Univariate analysis of factors associated with significant fibrosis in HBeAg-negative patients with persistently normal alanine aminotransferase

	Stage		*p*-Value
	Stage <2	Stage ≥2	
Age, years	*n* = 77	*n* = 43	
Mean ± SD	41.25 ± 10.68	45.81 ± 11.98	0.034[c*]
Median (range)	41 (20–68)	47 (23–72)	
Sex			
Male	36 (46.8%)	22 (51.2%)	0.785[b]
Female	41 (53.2%)	21 (48.8%)	
AST, IU/l	*n* = 77	*n* = 43	
Mean ± SD	22.91 ± 6.25	23.49 ± 5.75	0.617[c]
Median (range)	22 (14–40)	23 (12–37)	
ALT, IU/l	*n* = 77	*n* = 43	
Mean ± SD	24.78 ± 8.25	25.4 ± 8.32	0.696[c]
Median (range)	24 (11–40)	24 (14–40)	
ALP, IU/l	*n* = 77	*n* = 43	
Mean ± SD	133.59 ± 56.49	111.56 ± 50.19	0.081[a]
Median (range)	120 (48–332)	110 (34–218)	
GGT, IU/l	*n* = 77	*n* = 43	
Mean ± SD	23.44 ± 16.81	21.03 ± 9.81	0.749[a]
Median (range)	20 (7–131)	20 (7–58)	
Total bilirubin, mg/dl	*n* = 77	*n* = 43	
Mean ± SD	0.66 ± 0.34	0.61 ± 0.31	0.384[a]
Median (range)	0.6 (0–2.19)	0.6 (0.2–1.78)	
Alpha-fetoprotein, ng/ml	*n* = 77	*n* = 43	
Mean ± SD	3.21 ± 3.12	2.75 ± 2.69	0.564[a]
Median (range)	2.2 (0.1–15)	2.05 (0.13–14)	
Platelet count, $\times 10^9$/l	*n* = 77	*n* = 43	
Mean ± SD	248.7 ± 63.6	230.2 ± 58.4	0.119[c]
Median (range)	239 (138–421)	230 (95–369)	
HBV DNA, $\log_{10}$ IU/ml	*n* = 77	*n* = 43	
Mean ± SD	5.16 ± 7.75	4.92 ± 5.53	0.164[a]
Median (range)	4.3 (3.3–6.6)	4.04 (3.3–6.3)	

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; SD, standard deviation.
[a]Mann–Whitney *U*-test; [b]Yates continuity correction test; [c]Student *t*-test; *$p < 0.05$.

3.2. Univariate and multivariate analyses of factors associated with significant fibrosis and/or necroinflammation

The effect of each variable on treatment indication was assessed in HBeAg-negative patients with PNALT. When patients were divided into two groups according to their viral load (2000–20 000 IU/ml vs. ≥20 000 IU/ml), univariate analysis indicated that age and HBV DNA viral load (2000–20 000 IU/ml) were associated with significant fibrosis and/or necroinflammatory activity (Table 5).

Histopathological findings and referral for treatment (HAI ≥6 and/or stage ≥2) according to the HBV DNA viral load group are reported in Table 6. The results showed that an HBV DNA viral load of 2000–20 000 IU/ml (odds ratio 3.098, 95% CI 1.228–7.814, $p = 0.017$) and older age (odds ratio 1.055, 95% CI 1.010–1.103, $p = 0.017$) were risk factors for requiring treatment (HAI ≥6 and/or fibrosis ≥2) on multivariate analysis (Table 7).

3.3. AUC for the association of age with treatment indication

Age was used to predict the probability of being diagnosed with significant histological abnormalities in HBeAg-negative patients with PNALT. After assessment of the frequency of treatment indication in all patients, the cut-off point for patient age was determined to be 46 years and above. The AUC of the age associated with requiring a treatment intervention in these patients was 0.71 (95% CI 0.580–0.847; sensitivity = 84.62%, specificity = 59.81%, $p = 0.012$) (Figure 1).

Table 5
Univariate analysis of factors associated with significant fibrosis and/or necroinflammation in HBeAg-negative patients with persistently normal alanine aminotransferase

	Treatment indication (HAI ≥6 and/or stage ≥2)		*p*-Value
	Negative	Positive	
Age, years	*n* = 72	*n* = 48	
Mean ± SD	40.82 ± 10.39	45.98 ± 12.06	0.014[c*]
Median (range)	41 (20–64)	46.5 (23–72)	
Sex			
Male	34 (45.8)	24 (52.1)	0.628[b]
Female	39 (54.2)	23 (47.9)	
AST, IU/l	*n* = 72	*n* = 48	
Mean ± SD	23.08 ± 6.16	23.17 ± 5.96	0.942[c]
Median (range)	22 (14–40)	22 (12–37)	
ALT, IU/l	*n* = 72	*n* = 48	
Mean ± SD	24.88 ± 8.20	25.19 ± 8.40	0.840[c]
Median (range)	24 (11–40)	24 (12–40)	
ALP, IU/l	*n* = 72	*n* = 48	
Mean ± SD	132.77 ± 56.88	115.74 ± 51.39	0.130[c]
Median (range)	120 (48–332)	115 (34–218)	
GGT, IU/l	*n* = 72	*n* = 48	
Mean ± SD	23.29 ± 17.06	21.58 ± 10.38	0.971[a]
Median (range)	20 (7–131)	20 (7–58)	
Total bilirubin, mg/dl	*n* = 72	*n* = 48	
Mean ± SD	0.66 ± 0.35	0.62 ± 0.31	0.503[a]
Median (range)	0.6 (0–2.19)	0.6 (0.2–1.78)	
Alpha-fetoprotein, ng/ml	*n* = 72	*n* = 48	
Mean ± SD	3.28 ± 3.22	2.72 ± 2.60	0.509[a]
Median (range)	2.3 (0.1–15)	2 (0.13–14)	
Platelet count, $\times 10^9$/l	*n* = 72	*n* = 48	
Mean ± SD	250.8 ± 65.2	229.08 ± 55.5	0.060[c]
Median (range)	239.5 (138–421)	226 (95–369)	
HBV DNA, $\log_{10}$ IU/ml	*n* = 72	*n* = 48	
Mean ± SD	5.18 ± 5.76	4.88 ± 5.51	0.085[a]
Median (range)	4.30 (3.30–6.65)	4.01 (3.30–6.35)	
HBV DNA, 3.30–4.30 vs. ≥4.30 $\log_{10}$ IU/ml	34 vs. 38	34 vs. 14	0.008[a**]

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; SD, standard deviation.
[a]Mann–Whitney *U*-test; [b]Yates continuity correction test; [c]Student *t*-test; *$p < 0.05$, **$p < 0.01$.

4. Discussion

In the present study, liver histology characteristics in HBeAg-negative patients with PNALT and HBV DNA ≥2000 IU/ml, in addition to risk factors for advanced fibrosis and necroinflammatory activity, were assessed.

HBV DNA viral load, ALT, and liver histology play important roles in defining the severity of liver disease in CHB patients.[8] The

Table 6
Histopathological findings and referral for treatment in different HBV DNA viral load groups

	HBV DNA		*p*-Value
	3.30–4.30 $\log_{10}$ IU/ml (*n* = 68)[a]	≥4.30 $\log_{10}$ IU/ml (*n* = 52)[b]	
Stage <2	38 (55.9%)	39 (75%)	0.024*
Stage ≥2	30 (44.1%)	13 (25%)	
HAI <6	56 (82.4%)	46 (88.5%)	0.253
HAI ≥6	12 (17.6%)	6 (11.5%)	
Treatment indicated	34 (50%)	14 (26.9%)	0.008**
Treatment not indicated	34 (50%)	38 (73.1%)	

HBV, hepatitis B virus; HAI, histological activity index.
[a] HBV DNA 2000–20 000 IU/ml.
[b] HBV DNA ≥20 000 IU/ml.
*$p < 0.05$; **$p < 0.01$.

Table 7
Multivariate analysis for treatment indication

HAI ≥6 and/or stage ≥2	Multivariate analysis		
	OR	95% CI	*p*-Value
Age, years	1.055	1.010–1.103	0.017*
Platelet count, $\times 10^9$/l	1.000	1.000–1.000	0.055
HBV DNA (3.30–4.30 $\log_{10}$ IU/ml)[a]	3.098	1.228–7.814	0.017*
Sex, male	1.025	0.405–2.594	0.958

CI, confidence interval; HAI, histological activity index; HBV, hepatitis B virus; OR, odds ratio.
[a] HBV DNA = 2000–20 000 IU/ml.
*$p < 0.05$.

ALT level is commonly used to assess the activity of liver disease and to identify patients who require treatment. However, ALT may be influenced by various factors, making it an imperfect surrogate marker.

EASL guidelines recommend non-invasive follow-up for liver fibrosis in PNALT patients with HBV DNA ≥2000 IU/ml with 3-month ALT assessment intervals.[8] Liver biopsy is not recommended urgently, especially in PNALT patients with HBV DNA levels between 2000 and 20 000 IU/ml. However, many studies have reported significant histological damage to the livers of HBeAg-negative CHB patients with PNALT but high HBV DNA viral loads.[9,10,12,13] Kumar et al. detected stage 2 and above advanced fibrosis in over 40% of HBeAg-negative CHB patients with PNALT.[9] In the present study, the top ALT limit of 40 IU/l was used in accordance with the EASL guidelines, yet stage 2 and above fibrosis was detected in 35.9% ($n = 43$) of patients; moreover, significant necroinflammatory (HAI ≥6) activity was detected in 15% ($n = 18$) of patients. These data indicate that a sizable proportion of patients with PNALT had developed significant liver damage, as seen on liver biopsy.

According to the EASL guidelines, high HBV DNA levels are a risk factor for liver damage.[8] HBV DNA is also a predictive factor for hepatocellular carcinoma and cirrhosis.[14–16] Papatheodoridis et al. performed a systematic review and concluded that PNALT patients with HBV DNA loads of 2000–20 000 IU/ml are indicated for ALT assessment every 6 months, HBV DNA load measurement every year, and follow-up with transient elastography and liver biopsy in patients with elevated ALT levels.[17] They suggest close follow-up of patients who have HBV DNA loads of 2000–20 000 IU/ml. Conversely, when patients were separated into two groups according to their viral load (2000–20 000 IU/ml vs. ≥20 000 IU/ml) in the present study, referral for treatment was significantly higher in the former group. An HBV DNA viral load of 2000–20 000 IU/ml was determined to be a predictive factor for requiring treatment intervention on univariate and multivariate analysis (odds ratio 3.098, 95% CI 1.228–7.814, $p = 0.017$). It was also found that histologically significant liver disease is not rare in HBeAg-negative patients with PNALT and with serum HBV DNA viral loads of 2000–20 000 IU/ml. In patients infected later in life, CHB may be indolent owing to immunoclearance; this results in HBeAg seroconversion and a relatively low HBV DNA viral load.[18,19] These are the patients in whom low HBV DNA loads with normal ALT levels are likely (in those who are HBeAg-negative). As the present data show, while this situation is complex, histologically active liver disease can be suspected in patients with HBV DNA viral loads of 2000–20 000 IU/ml. In order to assess the phase of infection in CHB, serial HBV DNA and ALT measurements are required, as the nature of CHB infection necessitates these tests. Patients with HBV DNA levels between 2000 and 20 000 IU/ml and normal ALT are considered to be within the 'gray zone'. The follow-up period in this group of patients is very important in order to assess the phase of infection.

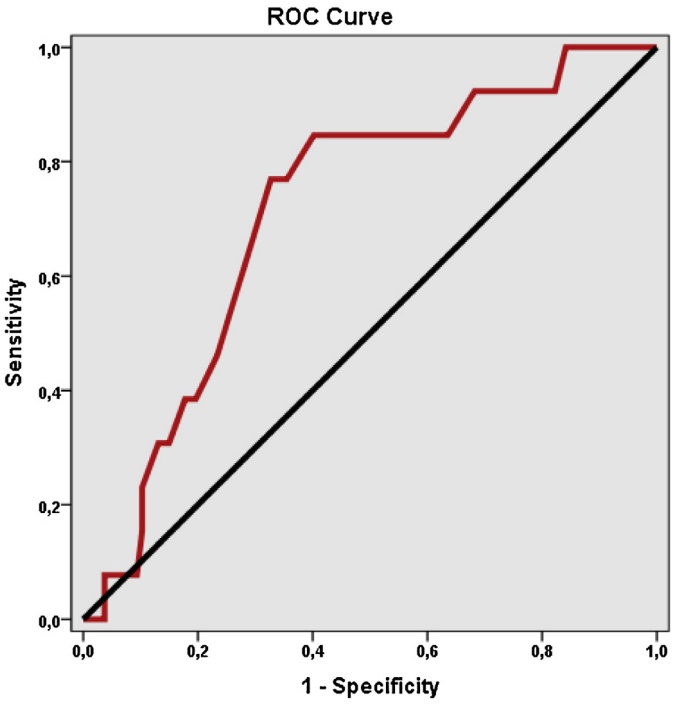

Figure 1. Area under the curve (AUC) of age associated with treatment indication in HBeAg-negative persistently normal ALT patients (AUC = 0.71, 95% CI 0.580–0.847; sensitivity = 84.62%, specificity = 59.81%, $p = 0.012$).

In this study, patients with HBV DNA values between 2000 and 20 000 IU/ml and with persistently normal aminotransferase profiles were followed for 1 year. The short follow-up period might a limitation of our study with regard to the definition of persistently normal ALT. A longer follow-up period would be beneficial for understanding the aforementioned 'gray zone' patients.

The duration of disease is another factor eliciting liver damage. Patients with PNALT who were infected perinatally have significantly worse liver damage than those infected in adulthood, since the damage of fibrosis increases with time. Patients who were infected prenatally, during birth, or in their first or second year of life may have more extensive liver damage due to the fact that they have a relatively longer immune-tolerance phase followed by an equally prolonged immune-clearance phase.[20–22] In the present patient group, this translates into the probability that those with a long disease duration may have significant histological liver damage.

Since HBV tends to exhibit a varying course, a single high HBV DNA load at a single point in time is not sufficient for an appropriate assessment of the disease.[7] In the present study, the HBV DNA viral load was measured twice before liver biopsy, which was performed 1 month after the last measurement. If any of the HBV DNA viral load measurements were below 2000 IU/ml, that patient was excluded from the study.

Age is also one of the most important factors that affect histological activity and the stage of disease.[23] All guidelines recommend that patient age should be considered in making treatment decisions, and patients over 40 years old (over 30 years old according to the EASL; over 40 years old according to the American Association for the Study of Liver Diseases and the Asian Pacific Association for the Study of the Liver) are currently considered at risk.[7,8,23] In the present study, age was used to predict the probability of requiring treatment in HBeAg-negative patients with PNALT. Following liver histopathology assessment, the age cut-off was determined to be 46 years and over. Treatment was indicated in 50% of patients older than 46 years and 33.3% of

patients who were younger. The AUC of the age associated with significant liver histopathology in HBeAg-negative PNALT patients was 0.71 (95% CI 0.580–0.847; sensitivity = 84.62%, specificity = 59.81%, $p = 0.012$).

A limitation of the present study is that the patients' HBV genotypes were not investigated, since HBV genotyping is not routinely performed in clinical practice.

In this cohort, treatment necessity in the HBeAg-negative CHB infection patients with HBV DNA levels between 2000 and 20 000 IU/ml was significantly greater than found in previous studies in the literature. As mentioned before, longer and more detailed follow-up of these 'gray zone' patients, or re-evaluation of the patients with non-invasive methods (such as the AST-to-platelet ratio index (APRI), fibrosis 4 score, FibroTest, and vibration-controlled transient elastography), is recommended.

In conclusion, significant liver damage requiring treatment was detected in 40% of CHB patients with PNALT and a high HBV DNA load (≥2000 IU/ml) after undergoing liver biopsy. Furthermore, HBeAg-negative CHB patients with PNALT do not have a benign prognosis, and patients with an HBV DNA viral load of 2000–20 000 IU/ml who are ≥46 years old are more likely to be candidates for treatment.

Ethical approval: The study protocol was prepared in accordance with the 1975 version of the Declaration of Helsinki and was approved by the Istanbul Medical Faculty Ethics Committee.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Conflict of interest: The authors declare that they have no conflicts of interest.

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