EBioMedicine 67 (2021) 103389

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Research Paper

A novel nomogram to predict evident histological liver injury in patients with HBeAg-positive chronic hepatitis B virus infection

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ARTICLE INFO

Article History:
Received 10 February 2021
Revised 22 April 2021
Accepted 22 April 2021
Available online xxx

Keywords:
Chronic hbv infection
Persistent normal of alt
Histological disease
Hepatitis B e Antigen

ABSTRACT

Background: HBeAg-positive chronic infection is a unique phase of chronic hepatitis B virus (HBV) infection. Current guidelines advise against starting antiviral treatment for HBeAg-positive chronic hepatitis B virus (HBV) infection patients, some data suggest treating such patients may reduce the risk of hepatocellular carcinoma. We aimed to explore whether these patients can have evident histological liver injury (EHLI), and develop a non-invasive model for identifying EHLI in such patients.

Method: We assessed whether HBeAg-positive chronic HBV infection patients can have EHLI defined by Ishak fibrosis stage ≥3 and/or histologic activity index ≥ 9 in a prospective multicenter study. Logistic and Lasso regression was used to select the optimal predictors. We used Akaike information criterion, discrimination improvement, net reclassification improvement to develop and validate models predicting EHLI risk in training cohort and two external validation cohorts.

Findings: Of these 336 patients met the inclusion criteria, 181(54%) were HBeAg-positive chronic HBV infection, of whom 60 patients (33%) had EHLI, the proportion of significant fibrosis was higher than that of significant inflammation (33% vs. 8%, $P < 0.001$). Age, liver stiffness measurement, ALT, alkaline phosphatase, and albumin were identified as independent predictors for EHLI and used to develop a nomogram that have been demonstrated having a good performance in predicting EHLI with AUROCs of 0.92(95%CI: 0.86−0.99) in the training cohort (n = 233) and 0.90(95%CI: 0.84−0.95) in validation cohort 1(n = 103), significant correcting current guidelines recommendations overestimating insignificant or significant histological disease. After 72-weeks entecavir treatment for HBeAg-positive chronic HBV infection patients with EHLI identified by nomogram, histological improvement occurred in 40 of 49(82%), 38(78%) had fibrosis reversal, and 35(73%) no longer had EHLI.

Abbreviations: EHLI, Evident histologic liver injury; HBV, Hepatitis B virus; qHBsAg, Quantitative of hepatitis B surface antigen; LSM, Liver stiffness measurement; APRI, AST to platelet index; FIB-4, fibrosis index based on 4 factors; GPR, gamma-glutamyl transpeptidase to platelet ratio; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyltransferase

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https://doi.org/10.1016/j.ebiom.2021.103389

Interpretation: In HBeAg-positive chronic HBV infection patients, 33% has EHLI. The nomogram developed in this study can accurately identify HBeAg-positive chronic HBV infection patients with EHLI, and that responded very well to antiviral therapy.

Funding: This study was funded by the State Key Projects Specialized on Infectious Disease, Chinese Ministry of science and technology (2013ZX10005002; 2018ZX10725506), National Natural Science Foundation of China (81970525) and Beijing Key Research Project of Special Clinical Application (Z151100004015221).

Research in context

Evidence before this study

Immune-tolerant phase represents the classical early phase of HBV infection, but is not well understood. A new nomenclature, called HBeAg-positive chronic HBV infection, has been introduced by international guidelines. Some data suggest treating such patients may reduce the risk of hepatocellular carcinoma. We explore whether these patients can have evident histological liver injury (EHLI), and develop a non-invasive model for identifying EHLI in such patients and initiate anti-HBV treatment.

Added value of this study

We confirmed that 33% of patients with HBeAg-positive chronic HBV infection has EHLI, of whom the frequency of significant fibrosis was higher than that of significant inflammation activity (33% vs. 8%, $P < 0.001$), in particulars, 25% of immune-tolerant CHB patients also had EHLI. We developed and prospectively validated EHLI-nomogram for noninvasive diagnosis of this condition in HBeAg-positive chronic HBV infection, and that responded very well to antiviral therapy.

Implications of all the available evidence

HBeAg-positive chronic HBV infection patients should be further assessed for the presence of EHLI and indication for HBV treatment, and our data strongly support the clinical application of the EHLI-nomogram.

1. Introduction

Chronic hepatitis B virus (HBV) infection is a major human health threat [1]. Globally, at least one-third of cirrhosis can be attributed to chronic hepatitis B (CHB) [2], which also carries significantly increased risk for the development of hepatocellular carcinoma (HCC) [1, 2]. Although HBeAg-negative chronic HBV infection is becoming the predominant type of chronic HBV infection worldwide, serum HBV DNA levels > 20,000 IU/mL in patients with HBeAg-negative chronic HBV infection appear to safely diagnose HBeAg-negative CHB, and must receive antiviral therapy due to all or at least the vast majority of such cases also have persistently or transiently elevated ALT values [3]. However, HBeAg-positive chronic HBV infection, historically called immune-tolerant phase, is a unique phase of chronic HBV infection. Generally, HBeAg-positive chronic HBV infection represents the classical early phase of infection which is associated with high levels of HBV replication and lack of clinical signs of liver inflammation. The HBeAg-positive chronic HBV infection is not well understood till now [4], studies on the natural history of CHB indicated that HBeAg-positive chronic HBV infection could be more heterogeneous than expected. It was reported that 22.5%–49.4% of patients with HBeAg-positive chronic HBV infection has significant histological liver injure [5–8], 8.4% of them having cirrhosis [8]. In addition, untreated HBeAg-positive chronic HBV infection patients were also under high risks for the occurrence of HCC than the treated CHB patients [9]. Therefore, the disease activity in HBeAg-positive chronic HBV infection might be under-represented by assessing HBV DNA and ALT levels. Although recognition that high HBV DNA levels and persistence of HBeAg were associated with an increased risk of HCC had a increasing interest in treating patients with HBeAg-positive chronic HBV infection [2,4], current guidelines only recommends antiviral treatment of HBeAg-positive chronic HBV infection patient with significant histological disease that was determined by liver stiffness measurement (LSM) or liver biopsy [2,4,10]. However, liver biopsy is very limited used in the clinic due to its invasiveness and poor acceptance [11], and the intermediate values of LSM also showed low accuracy [12]. Therefore, there is a great interest in developing better non-invasive method to identify HBeAg-positive chronic HBV infection patients with evident histological liver injury （EHLI, i.e., Ishak fibrosis staging ≥ 3 and/or histologic activity index (HAI) ≥ 9） [2,13] and initiate anti-HBV treatment.

2. Methods

2.1. Patients

This study protocol was approved by the institutional review board at all study sites (Chinese PLA General Hospital the fifth Medical Center Institutional Review Boards, IRB No. 2013145D). Signed informed consent was obtained from all study subjects. Patients were prospectively recruited from 14 centers in China, including Chinese PLA General Hospital the fifth Medical Center, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Yichun People's Hospital of Jiangxi Province, Taihe Traditional Chinese Medicine Hospital of Anhui Province, Fuzhou Infectious Diseases Hospital, Traditional Chinese Medicine Hospital of Chongqing, the 960th Hospital of Chinese PLA, the First Affiliated Hospital of Zhengzhou University, Southwest Hospital of Army Military Medical University, Guangzhou 8th People's Hospital, Beijing Youan Hospital, Shanghai Public Health Clinical Center, Fuyang 2nd People's Hospital, the First Affiliated Hospital of Wenzhou Medical University. They were no related to each other. The training cohort was prospectively recruited from five centers while the external validation cohort 1 involved patients from others nine centers and external validation cohort 2 contained 127 patients who finished entecavir treatment for 72-weeks and underwent the second liver biopsy from all centers. Inclusion criteria: (1) HBsAg-positive for at least 6 months; (2) HBeAg-positive with HBV DNA level greater than 2×10^4 IU/mL; (3) serum ALT less than or equal to the ULN (40 U/L for men or women) for at least 3 determinations in the year prior to baseline liver biopsy; and 4) HBV treatment-naïve. Exclusion criteria were: (1) co-infection with hepatitis *C* virus, hepatitis *D* virus, or human immunodeficiency virus; (2) the presence of substantial alcohol consumption (> 20 g/day for women or > 30 g/day for men); (3) concomitant NAFLD; (4) decompensated cirrhosis or history of any concurrent malignancy; (5) more missing variables.

2.2. Definitions

HBeAg-positive chronic HBV infection would be defined if a patient had at least 3 ALT determinations in the year prior to baseline liver biopsy with all values ≤ 40 U/L, and was HBeAg-positive with HBV DNA load > 10^7 IU/mL [4]. Immune-tolerant CHB would be defined if they had at least 3 ALT determinations in the year prior to baseline liver biopsy with all values ⟨35 U/L in men and < 25 U/L in women, and was HBeAg-positive with HBV DNA load ⟩ 10^7 IU/mL [2]. Patients were also categorized as low-normal ALT (i.e., ALT < 25 U/L for female or < 35 U/L for male) and high-normal ALT (i.e., ALT 25–40 U/L for female or 35–40 U/L for male) [2]. According to the conversion equation suggested by Rozario et al., we have converted the HAI score into approximate METAVIR equivalents such as HAI scores of 0–3 were considered equivalent to METAVIR A0, HAI scores of 4–8 to METAVIR A1, HAI scores of 9–12 to METAVIR A2 and HAI scores of 13–18 to METAVIR A3 [14]. Significant inflammatory activity was defined as at least moderate inflammation (HAI scores of 9–12 or METAVIR A2) [13]. Liver fibrosis was defined as no significant fibrosis (Ishak 0–2), significant fibrosis (Ishak 3–4) or cirrhosis (Ishak 5–6) [15]. Patients were considered to have EHLI if Ishak fibrosis staging ≥ 3 and/or HAI ≥ 9 [2,13].

2.3. Data acquisition

Fasting blood samples were collected and processed independently at each center. Major laboratory tests were performed in the central laboratory. The HBV DNA level was measured using Roche COBAS TaqMan assay (Roche Diagnostics, West Sussex, UK) with a lower limit of quantification 20 IU/mL. HBV genotypic resistance testing was performed using direct sequencing of a 1225-bp-long viral gene fragment (nucleotide (nt) 54–1278) [16]. HBV genomic sequences were deposited in GenBank under accession numbers MK1711258-MK171652. Quantitation of HBsAg (qHBsAg) was determined using the Abbott Architect Assay (Abbott Laboratories, Abbott Park, IL, USA). Quantitation of HBeAg (qHBeAg) was quantified using ARCHITECT i2000SR analyzer by World Health Organization (WHO) HBeAg reference standard (Paul-Ehrlich-Institute, Germany) [17]. The lower limits of quantification of the assays in this study were 0.05 IU/mL for qHBsAg and 0.3 PEIU/mL for qHBeAg. The AST to Platelet Ratio Index (APRI) was calculated by the formula: (AST level / ULN) / platelet count (PLT) (10^9/L) × 100 [18]. The FIB-4 was calculated by the formula: age (years) × AST (U/L)/ (platelets (10^9/L) × (ALT (U/L))$^{1/2}$) [19]. The GPR value was calculated by the formula: (GGT (U/L) / ULN of GGT) / PLT (10^9/L) × 100 [20]. LSM was performed with the FibroScan 502 Touch device (Echosens, France) according to the standard protocol. The LSM result was expressed in kilopascals (kPa) and ranged from 1.5 to 75 kPa. A Quick-cut needle or Menghini needle, 16 G (Allegiance Corporation, McGaw Park, IL, USA) was used for the biopsy. A minimal (20 mm) length of the liver tissue and at least two pieces of liver tissue were collected to ensure that there were at least 11 portal tracts for pathological evaluation. All liver biopsies were reviewed in a central pathology by two liver pathologists blindly. Histological assessment included two major parts: (1) fibrosis stage evaluated by the Ishak fibrosis staging [15]; and (2) the inflammation activity assessed by the Ishak modified HAI grading system. Patients were considered to have EHLI if Ishak fibrosis staging ≥3 and/or histologic activity index ≥ 9[2,13,14].

2.4. Statistical analysis

All statistical analyses were performed in R version 3.5.1 (*R* Foundation for Statistical Computing, Vienna, Austria) or SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Data was expressed as frequencies (percentage) for categorical variables or as a median (interquartile range) for quantitative variables. Comparison between groups of patients was performed using chi-square, or the non-parametric Mann-Whitney *U* Test or Kruskal-Wallis test.

All variables that were shown to be potentially relevant EHLI ($p <$ 0.05 in the univariable logistic regression) were considered for entering into the multivariate logistic regression analysis using forward stepwise selection with the Akaike information criterion (AIC). We fitted the models by the minimum AIC value. The least absolute shrinkage and selection operator (Lasso) regression confirm the variable screened by multivariate logistic analysis. Interactions among

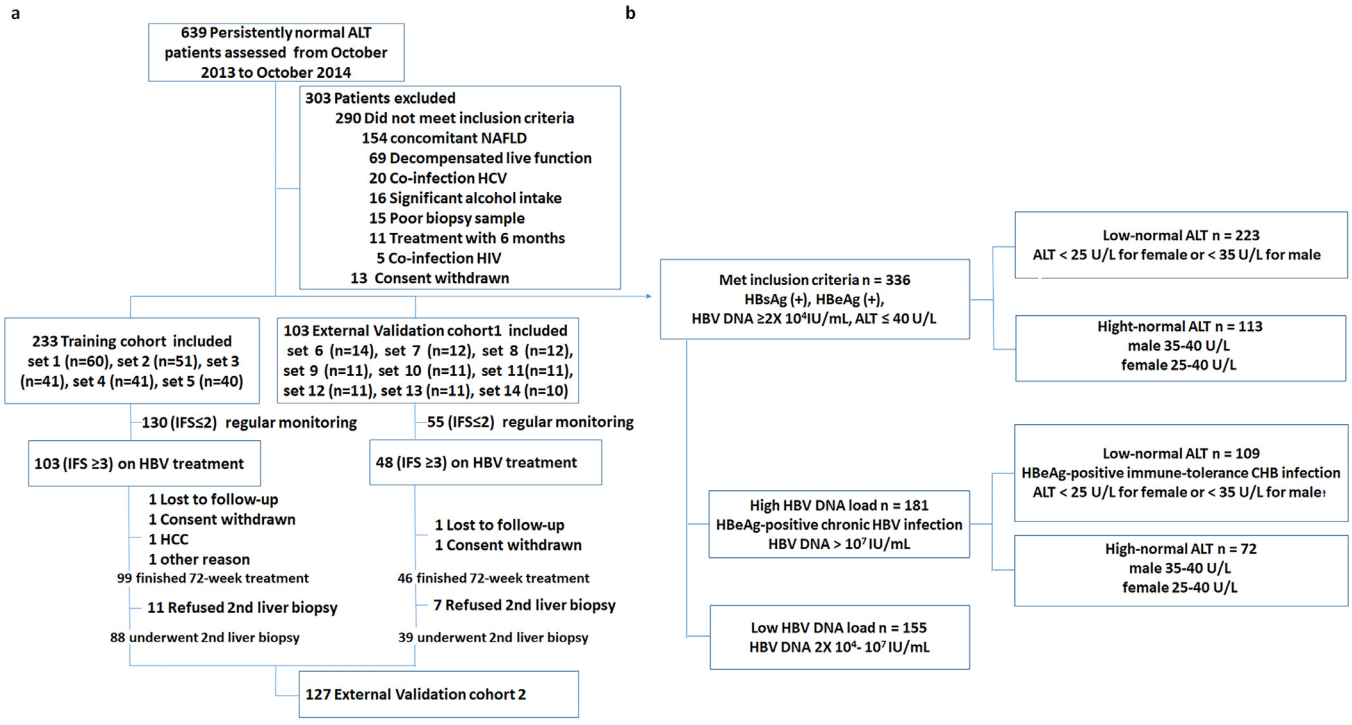

Fig. 1. (a) Flowchart of enrolled patients and(b) met inclusion criteria definitions tree.

the variables in the model were considered. Coefficients of the predictors and Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. We used the regression coefficients for each variable as weights and developed a nomogram and estimated the model performance. The discrimination was evaluated by Harrell's concordance index (*C* index). We calculated R2 values that explained variation where higher values indicate a greater proportion of variation to EHLI is explained by the model. The calibration was assessed by the Hosmer-Lemeshow test, calibration slope and calibration intercept. Additionally, we calculated the cutoff value, specificity, sensitivity, positive/negative predictive value, positive/negative likelihood ratio and accuracy to evaluate the model performance. We calculated integrated discrimination improvement (IDI), net reclassification improvement (NRI), and decision curve analysis (DCA) to compare the diagnostic accuracy and discriminative ability of the nomogram and LSM. The area under the receiver operating characteristic curve (AUROC) analysis and nomogram were conducted using R (version 3.5) with "pROC" and "rms" package. The package "glmnet" was used for Lasso regression. The optimal cut-off was derived using the Youden index, which is defined as sensitivity plus specificity minus one. All statistical tests were two-tailed and $P < 0.05$ was considered as statistically significant.

2.5. Role of funding source

The funder had no any role in study design, data collection, data analysis, interpretation, or writing of report.

3. Results

3.1. Patients

As shown in Fig. 1a and Table 1, 336 patients met the inclusion criteria, 100 (30%) patients had no inflammation on the liver biopsy, 193 (57%) had mild inflammation, and 43 (13%) had moderate inflammation. The number of patients with significant (i.e., at least moderate) inflammation was 0 of 185 patients without significant fibrosis, 26 (24%) of 110 in patients with significant fibrosis and 17 (42%) of 41 among patients with cirrhosis ($P < 0.001$, Fig. S1). Among 336 HBeAg-positive chronic HBV infection patients with persistently normal of ALT, the proportion of significant fibrosis was higher than that of significant inflammation on liver biopsy (45% vs. 13%, $P < 0.001$). All the 151 patients with EHLI received entecavir therapy, 145 (96%) of them finished the 72-weeks of treatment and 127 (84%) had a

Table 1
Characteristics of the population in training set and validation sets.

Variables	Training set	Validation sets	
	Naïve treatment (n = 233)	Naïve treatment (n = 103)	On-treatment (n = 127)
Age (years)	38.0 [16.0]	37.0 [11.5]	43.0 [12.0]
Male (n,%)	145 (62.23)	61(59.22)	76 (59.84)
Body mass index (kg/m^2)	22.82[4.23]	21.42[4.23]	23.00 [4.32]
NSAC (n,%)	22 (9.44)	10 (9.71)	13 (10.24)
Platelet counts (10^9/L)	207 [78.82]	214 [87.00]	184 [87.00]
HBV DNA (log^{10} IU/mL)	7.09 [2.60]	7.00 [2.27]	2.97 [1.02]
$> 10^7$	115 (49.4%)	66 (64.1%)	0 (0%)
$2 \times 10^4 - 10^7$	118 (50.6%)	37 (35.9%)	0 (0%)
$20 - 2 \times 10^4$	0 (0%)	0 (0%)	32 (25.2%)
< 20	0 (0%)	0 (0%)	95 (74.8%)
qHBsAg (log^{10} IU/mL)	5.37 [0.63]	5.42 [0.83]	5.20 [0.45]
qHBeAg ((lg PEIU/mL)	3.52 [0.71]	3.25 [0.58]	2.23 [0.62]
Genotype (n,%)			
B	56 (24.0)	26 (25.2)	31 (24.4)
C	176 (75.5)	76 (73.7)	95 (74.8)
D	1 (0.4)	1 (1.0)	1 (0.8)
LSM (kPa)	7.02 [6.70]	6.84 [7.10]	5.70 [7.40]
CAP (dB/m)	212 [82.0]	201 [60.0]	232 [55.0]
APRI score	0.52 [0.64]	0.56 [0.64]	0.53 [0.23]
FIB-4 score	1.05 [1.19]	1.12 [1.14]	1.28 [1.04]
GPR score	0.40 [0.18]	0.46 [0.22]	0.52 [0.13]
ALT (U/L)	27.0 [13.0]	26.0 [12.5]	22.0 [14.0]
AST (U/L)	26.0 [11.0]	27.0 [13.0]	24.0 [9.50]
Albumin (g/L)	43.0 [4.20]	43.0 [6.00]	44.0 [3.65]
Alkaline phosphatase (U/L)	70.0 [29.0]	72.0 [28.0]	72.0 [32.0]
γ-glutamyltransferase (U/L)	22.0 [27.0]	20.0 [13.0]	20.0 [17.0]
The length of biopsy tissue (mm)	19.0 [3.00]	19.0 [2.00]	19.0 [3.00]
Histologic activity index score	5.00 [4.00]	5.00 [4.00]	3.00 [3.00]
Histologic activity index n (%)			
0–3	69(29.61)	31(30.10)	64(50.39)
4–8	136(58.37)	57(55.34)	60(47.24)
9–12	28(12.02)	15(14.56)	3(2.37)
13–18	0 (0)	0 (0)	0 (0)
Ishak fibrosis stage score	4.00 [3.00]	4.00 [3.00]	3.00 [3.00]
Ishak fibrosis stage n (%)			
1	77(33.04)	30(29.13)	23 (18.11)
2	54(23.18)	24(23.30)	26 (20.47)
3	45(19.31)	23(22.33)	28 (22.05)
4	29(12.45)	13(12.62)	28 (22.05)
5–6	28(12.02)	13(12.62)	22 (17.32)
IFS $\geq$ 3 and/or HAI $\geq$ 9 n (%)	102(43.78)	49 (47.57)	78 (61.42)

NSAC, non-substantial alcohol consumption defined as occasionally alcohol consumption but non-meeting substantial alcohol consumption ($>$ 20 g/day for women or $>$ 30 g/day for men); HBV, hepatitis B virus; qHBsAg, quantitative of hepatitis B surface antigen; qHBeAg, quantitative of hepatitis B e antigen; LSM, liver stiffness measurement; APRI, AST to platelet index; FIB-4, fibrosis index based on 4 factors; GPR, gamma-glutamyl transpeptidase to platelet ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HAI, histologic activity index; IFS, Ishak fibrosis stage.

Table 2
Characteristics for HBeAg-positive chronic hepatitis *B* virus infection.

Variables	High HBV DNA load (n = 181)	Low HBV DNA load (n = 155)	*P* value
Age	37.00 [13.5]	40.00 [12.00]	< 0.001
Male (n,%)	117 (64.64)	89 (57.42)	0.175
NSAC (n,%)	14 (7.73)	18 (11.61)	0.235
Body mass index (kg/m^2)	22.31 [4.23]	23.01 [4.32]	0.079
Platelet counts (10^9/L)	201 [55.31]	169 [82.83]	< 0.001
qHBsAg (log IU/mL)	5.37 [0.89]	4.41 [0.56]	< 0.001
qHBeAg (lg PEIU/mL)	3.72 [1.82]	3.46 [1.41]	0.123
Liver stiffness measurement (kPa)	5.35 [2.38]	8.13 [8.45]	< 0.001
CAP (dB/m)	209 [74.0]	208 [80.3]	0.733
HBV Genotype (n,%)			0.332
B	50 (27.6)	32 (20.6)	
C	130 (71.8)	122 (78.7)	
D	1(0.6)	1 (0.7)	
APRI score	0.33 [0.16]	0.65 [0.40]	< 0.001
FIB-4 score	1.12 [0.69]	1.59 [1.60]	< 0.001
GPR score	0.40 [0.07]	0.58 [0.30]	0.021
Alanine aminotransferase (U/L)	22.00 [13.00]	28.00 [11.00]	0.001
Low-normal ALT	109 (60.22%)	64 (41.29%)	0.001
Aspartate aminotransferase (U/L)	20.50 [10.00]	29.00 [9.00]	< 0.001
Albumin (g/L)	44.22 [4.13]	43.00 [5.38]	0.396
Cholinesterase (U/L)	7140 [2040]	6760 [1730]	0.001
Alkaline phosphatase (U/L)	71.00 [26.82]	88.00 [38.83]	0.027
γ-glutamyltransferase (U/L)	15.00 [10.00]	28.00 [34.00]	< 0.001
Histologic activity index score	4.00 [3.00]	7.00 [4.00]	< 0.001
Histologic activity index			
0–3	63 (34.81%)	37 (23.87%)	0.004
4–8	104 (57.46%)	89 (57.42%)	
9–12	14 (7.74%)	29 (18.71%)	
13–18	0 (0%)	0 (0%)	
Ishak fibrosis staging score	3.00 [3.00]	4.00 [2.75]	< 0.001
Ishak fibrosis staging			< 0.001
1	72 (39.78%)	35 (22.58%)	
2	49 (27.07%)	29 (18.71%)	
3	34 (18.78%)	34 (21.94%)	
4	16 (8.84%)	26 (16.77%)	
5–6	10 (5.53%)	31 (20.00%)	
IFS ≥ 3 and/or HAI ≥ 9	60 (33.15%)	91 (58.71%)	< 0.001

NSAC, non-substantial alcohol consumption defined as occasionally alcohol consumption but non-meeting substantial alcohol consumption (> 20 g/day for women or > 30 g/day for men); CAP, control attenuation parameter; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; qHBsAg, quantitative of hepatitis B surface antigen; qHBeAg, quantitative of hepatitis B e antigen; APRI, AST to platelet index; FIB-4, fibrosis index based on 4 factors; GPR, gamma-glutamyl transpeptidase to platelet ratio; HAI; histologic activity index; IFS, Ishak fibrosis staging.

second liver biopsy. The baseline variables were comparable between training cohort and validation cohort 1 (Table 1).

3.2. Characteristics for patients with HBeAg-positive chronic HBV infection

Fig. 1b displays the distribution of patients according to HBV DNA viral load and ALT level. 181 (54%) patients had high viral load (> 10^7 IU/mL), hence they were labeled HBeAg-positive chronic HBV infection, of whom 109 patients had low-normal ALT, hence they were labeled HBeAg-positive immune-tolerance CHB. As shown in Table 2, HBeAg-positive chronic HBV infection patients were younger while those with low viral loads (2×10^4–10^7 IU/mL) were older (P < 0.001). These patients also showed higher platelet counts (p < 0.001) and qHBsAg (P < 0.001) and lower LSM score (P < 0.001). EHLI was common in both groups, however, the proportion of EHLI was significantly higher in low viral loads group than in high viral loads group (59% vs. 33%, P < 0.001). Furthermore, 25% of HBeAg-positive immune-tolerance CHB patients had significant histologic disease (Table S1).

3.3. Evident histological liver injury predictors

Age, LSM and ALT were strong independent predictors of EHLI (Table 3). The percentage of patients with EHLI significantly increased with age, from 33% (31/95) in < 30yrs to 45% (58/129) in 30–40yrs, to 55% (62/112, P = 0.021, Table S2) in patients >40yrs of age, in particular in those of HBeAg-positive chronic HBV infection patients also from 24% to 31%, to 50% (Table S3). Additionally, the percentage of HBeAg-positive chronic HBV infection patients with EHLI increased from 27% (24/88) in < 6 kPa to 29% (15/52) in 6–9 kPa, to 51% (21/41) in > 9 kPa, (P = 0.020, Table S4). In addition, albumin and ALP were strong, independent predictors of EHLI. We performed the Lasso regression analysis to confirm the results of multivariate logistic analysis (Fig. 2a and b). Nine variables were selected in Lasso regression analysis, which were age, LSM, ALT, ALP, albumin, HBV DNA load, PLT, GGT and sex. Declining in HBV DNA viral load and platelet counts were significant predictors in low (4–7 log) viral load but not HBeAg-positive chronic HBV infection and sex or GGT was a stronger predictor in low viral load than HBeAg-positive chronic HBV infection. However, the associations of HBV DNA viral load with EHLI in patients with HBeAg-positive chronic HBV infection were blunted because presence of HBV DNA viral load > 7 log was used as defining characteristics for HBeAg-positive chronic HBV infection.

Table 3
Univariate and multivariate analysis of the clinical variables associated with evident histological liver injury in HBeAg-positive chronic HBV infection patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	*P* value
Age (≥ 40 vs.< 40) (years)	4.762	2.212–10.251	< 0.001	3.081	1.062–8.853	0.036
LSM (kPa)	1.941	1.534–2.453	< 0.001	1.692	1.301–2.212	< 0.001
ALT (U/L)	1.093	1.051–1.142	< 0.001	1.062	1.013–1.124	0.033
ALP (U/L)	1.054	1.031–1.083	< 0.001	1.054	1.024–1.083	< 0.001
Albumin (g/L)	0.762	0.693–0.854	< 0.001	0.802	0.701–0.923	0.001
AST (U/L)	1.113	1.062–1.171	< 0.001			
GGT (U/L)	1.091	1.054–1.133	< 0.001			
Platelet counts (x10^9/L)	0.983	0.984–0.993	< 0.001			
HBV DNA load (log IU/mL)	0.572	0.452–0.723	< 0.001			
qHBsAg (log IU/mL)	0.443	0.291–0.651	< 0.001			
Body mass index (kg/m^2)	1.082	0.992–1.183	0.103			

HBV, hepatitis *B* virus; LSM, liver stiffness measurement; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; qHBsAg, quantitative of hepatitis *B* surface antigen; OR, odds ratio; CI, confidence interval.

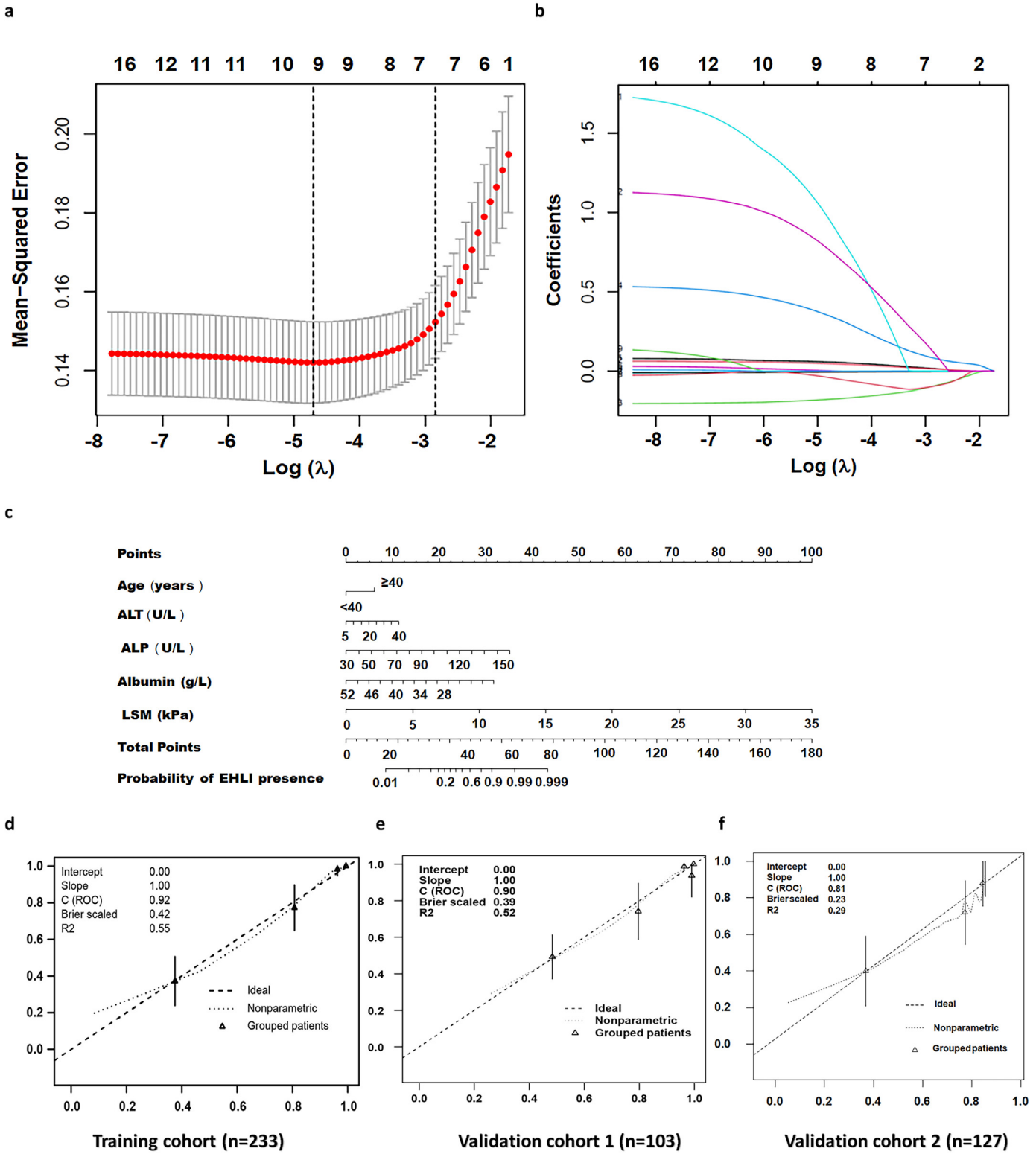

Fig. 2. Nomogram to predict evident histological liver injury risk. (a) Tuning parameter (λ) selection in Lasso model used ten-fold cross-validation via minimum criteria. (b) Lasso coefficient profiles of 16 features. A coefficient profile plot was produced versus the log (λ) sequence. Vertical line was drawn at the value selected where optimal λ resulted in 9 nonzero coefficients. (c) Nomogram to predict the EHLI risk in HBeAg-positive chronic infection. To use the nomogram, find the position of each variable on the corresponding axis, draw a line to the points axis for the number of points, add the points from all of the variables, and draw a line from the total point axis to determine the EHLI probabilities. Validity of the identifying performance of the nomogram in estimating the EHLI risk in the training cohort (d), validation cohort 1 (e), and validation cohort 2 (f). EHLI, evident histological liver injury;AUROC, area under the receiver operator characteristic curve.

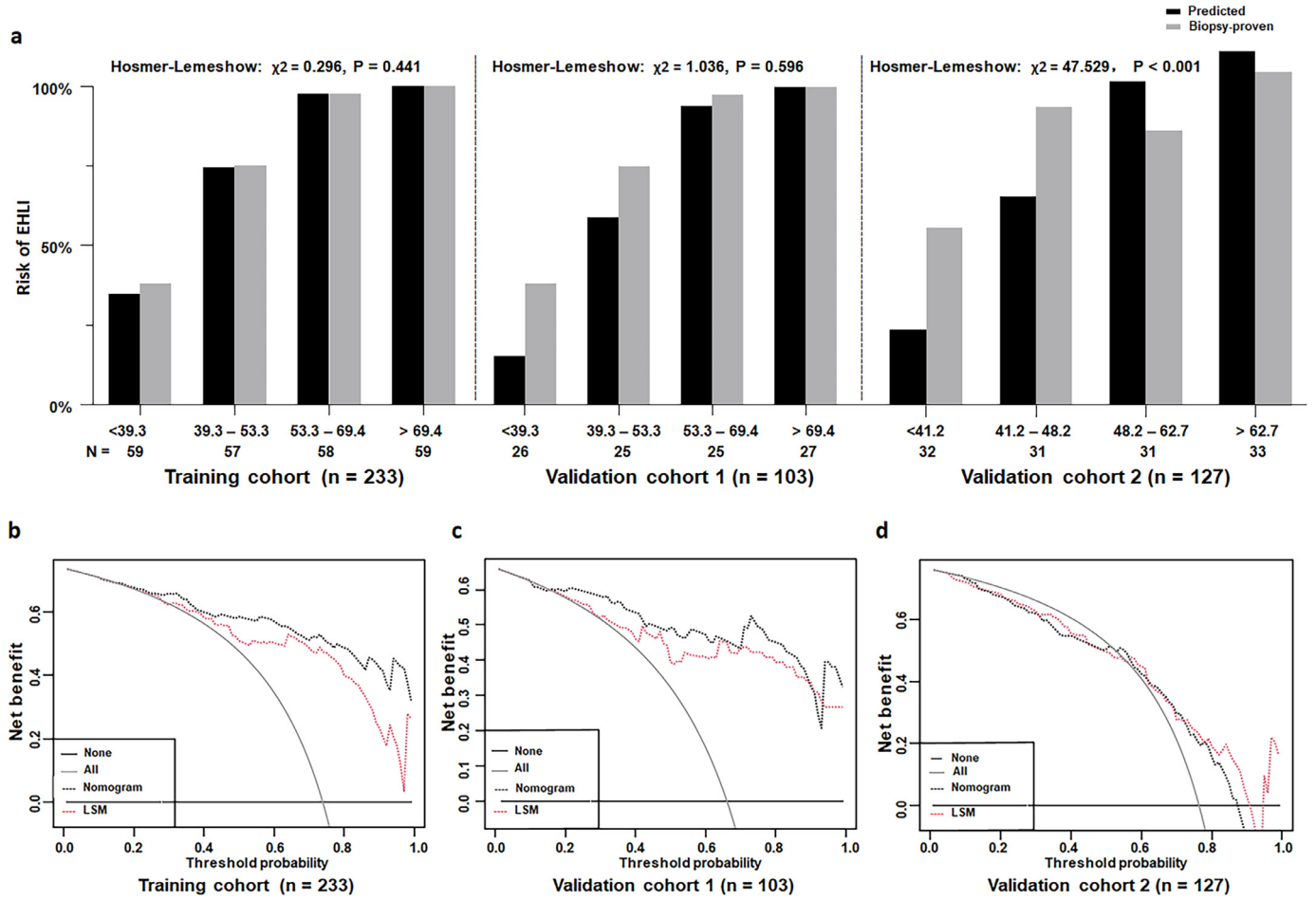

Fig. 3. Performance of the nomogram. (a) Biopsy-proven (black) vs. predicted (gray) presence EHLI rates: according to the approximate quartiles of the EHLI-nomogram score in the HBeAg-positive chronic infection patients included in the training cohort (n = 233, left side), validation cohort 1 (n = 103, middle) and validation cohort 2 (n = 127, right side). The decision curves of net benefit of the nomogram and LSM in training (b), validation cohort 1 (c), and validation cohort 1 (d). Solid gray line, net benefit of all patients; solid black line, net benefit of no patient; dotted lines, net benefit of patients according to the nomogram and LSM.

3.4. Development of nomogram estimating evident histological liver injury risk

Out of the 19 potential predictors that we considered (Table 1), 5 were included in the final models that we developed by the logistic and Lasso regression analysis: age, LSM, ALT, ALP, and albumin (Table 3, Fig. 2a and b). The final formula was as follows.

Logit(P)=2.348+0.525*LSM+0.049*ALP+1.126*Age-0.221*ALB +0.059*ALT. The nomogram, which is named as EHLI-nomogram, was developed based on above 5 independent risk factors associated with the presence of EHLI in HBeAg-positive chronic infection patients. As shown in Fig. 2c, we developed an EHLI-nomogram of the model for an easier use. In the EHLI-nomogram, each factor was ascribed a weighted point total that implied a possibility of EHLI presence. For example, age ≥40yrs was associated with 16 points, whereas 10 kPa of LSM was with 28 points. Each patient with a higher score had a higher risk of the EHLI. The Harrell's C index in the training cohort were 0.92 (95%CI: 0.86–0.99, Fig. 2d), 0.90 (95%CI: 0.84–0.95, Fig. 2e) in validation cohort 1, and 0.81 (95%CI: 72.1–90.5, Fig. 2f) in validation cohort 2. In addition, the EHLI-nomogram explained 55% of variation to the EHLI (R2) in training cohort, 52% in validation cohort 1 and 29% in validation cohort 2. The AUROC in the validation cohort 1 was 0.90, which is considered very good. Furthermore, we calculated the biopsy-proven and predicted EHLI risk by the nomogram quartiles in the training cohort and the validation cohorts. Predicted and biopsy-proven risk of EHLI have a good consistency across the quartiles of the nomogram (Hosmer-Lemeshow: $\chi2$ = 0.296, P = 0.441) in the training cohort and ($\chi2$ = 1.036, P = 0.596, Fig. 3a) in validation cohort 1. But in validation cohort 2, there was an underprediction of EHLI in the two lowest risk groups, and an overprediction of EHLI in the two highest risk groups (Hosmer-Lemeshow χ^2 = 47.529, $P < 0.001$, Fig. 3a).

3.5. Comparison between the nomogram and recommendations by current guidelines in HBeAg-positive chronic HBV infection patients

Analysis focused on HBeAg-positive chronic HBV infection patients with overestimation of insignificant or significant histological diseases using the recommendations by current guidelines [2,4,12]. Overestimation was defined as insignificant histological diseases in HBeAg-positive chronic HBV infection patients with the recommendations by current guidelines for LSM of less than 6 kPa cutoff or age of less than 30yrs, with 27 and 24% of them biopsy-proven having significant histological disease (Table 4), and wrongly classified as having insignificantly histological disease, respectively. Inversely, overestimation was defined as significant histological disease in HBeAg-positive chronic HBV infection patients with the recommendations by current guidelines for LSM of greater than 9 kPa cutoff or age of more than 30yrs, with 52 and 55% of them biopsy-proven to have insignificantly histological disease (Table 4), and wrongly classified as having significantly histological disease, respectively. Some (26/88) of HBeAg-positive chronic HBV infection patients with LSM of less than 6 kPa cutoff had EHLI-nomogram

Table 4
Comparison between EHLI-nomogram and recommendations by current guidelines in HBeAg-positive chronic HBV infection.

	Non-EHLI recommendations by the current guidelines								EHLI recommendations by the current guidelines							
	LSM < 6 kPa (*n* = 88)		Age < 30 (*n* = 62)		HAI < 9 (*n* = 293)		Ishak < 3 (*n* = 185)		LSM > 9 kPa (*n* = 41)		Age ≥ 30 (*n* = 119)		HAI ≥ 9 (*n* = 43)		Ishak ≥ 3 (*n* = 151)	
	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI	Non-EHLI	EHLI
Biopsy-proven n (%)	64(72.7)	24(27.3)	47(75.8)	15(24.2)	185(63.1)	108(36.9)	185(100)	0(0)	20(48.8)	21(51.2)	74(62.2)	45(37.8)	0(0)	43(100)	0(0)	151(100)
EHLI-nomogram																
< cutoff 46.4 (n)	60	2	43	2	175	8	175	0	17	2	69	3	0	1	0	8
≥ cutoff 46.4 (n)	4	22	4	13	10	100	10	0	3	19	5	42	0	42	0	143
AUROC (95% CI)	0.92(0.85–0.97)		0.89(0.78–0.94)		0.91(0.88–0.95)		–		0.90(0.80–0.93)		0.93(0.84–0.97)		–		–	
Sensitivity (%)	91.7(86.5–95.1)		86.7(82.1–91.3)		92.5(88.9–96.7)		–		90.5(83.2–98.4)		93.3(81.2–99.5)		–		–	
Specificity (%)	93.8(78.6–95.4)		91.5(79.4–96.5)		94.5(86.4–97.7)		–		85.3(75.3–93.2)		93.2(79.2–99.4)		–		–	
PPV (%)	84.6(73.4–93.6)		76.5(65.3–89.5)		90.9(85.3–98.9)		–		86.4(70.3–97.4)		89.4(78.2–99.4)		–		–	
NPV (%)	96.7(80.6–99.7)		95.6(82.1–99.8)		95.6(83.6–98.3)		–		89.5(75.4–95.4)		95.8(78.4–99.6)		–		–	
Positive LR	14.79(10.3–18.5)		10.2(7.6–16.5)		16.8(11.8–20.4)		–		6.0(3.5–11.7)		13.8(6.3–18.3)		–		–	
Negative LR	0.09(0.06–0.27)		0.13(0.09–0.22)		0.08(0.05–0.23)		–		0.11(0.07–0.31)		0.07(0.08–0.27)		–		–	
Accuracy (%)	93.2		90.3		94.2		94.6		87.8		93.3		97.7		94.7	

EHLI, evident histologic liver injury; LSM, liver stiffness measurement; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; LR, Likelihood ratio.

scores more than 46.4, EHLI could be correctly predicted 91% of the time, with the average of PPVs of 85% (Table 4). Similarly, 17 out of 66 HBeAg-positive chronic HBV infection patients with age of less than 30yrs had EHLI-nomogram scores more than 46.4, correctly predicting EHLI as 89%, with PPV of 77% (Table 4). Importantly, 21 out of 41HBeAg-positive chronic HBV infection patients with LSM of greater than 9 kPa cutoff had EHLI-nomogram scores more than 46.4 and similarly, 47 out of 119 patients with age of more than 30yrs had EHLI-nomogram scores more than 46.4, the correctly predicting EHLI as 87% and 92%, respectively (Table 4). Among 293 patients with HAI < 9, full agreement between EHLI-nomogram predictive histological disease and liver biopsy reached 94.2%, while 43 with HAI ≥ 9 reached 97.7% (42 of 43, Table 4). Further, among 185 patients with Ishak ≤ 2 and 151 patients with Ishak ≥ 9, the concordance of EHLI-nomogram predictive histological disease and liver biopsy reached almost perfect, 94.6% and 94.7%, respectively (Table 4).

3.6. Compare the performance of the nomogram with LSM, APRI, FIB-4 and GRP

As our EHLI-nomogram contains LSM as a parameter, we want to answer how assessed the performance of EHLI-nomogram with other reported models in the treatment-naïve (validation cohort 1, $n = 103$) and on-treatment (validation cohort 2, $n = 127$; Table 1). In validation cohort 1, the AUROC of the EHLI-nomogram predicting EHLI risk (0.90, 95% CI 0.84–0.95) was higher than that of LSM (0.79, 95% CI 0.70–0.89, $P = 0.048$), GRP (0.75, 95% CI 0.669–0.889, $P = 0.005$), APRI (0.70, 95% CI 0.63–0.83, $P = 0.043$), and FIB-4 (0.68, 95% CI 0.62–0.84, $P = 0.009$ Table 5). Using the optimal cut-off value determined in the validation cohort 1(46.4), the sensitivity and specificity of EHLI-nomogram to identifying EHLI risk was 83% and 96%, respectively (Table 5). In validation cohort 2, the AUROC of the EHLI-nomogram predicting EHLI risk (0.81, 95% CI 0.72–0.91) was higher than that of GRP (0.64, 95% CI 0.54–0.71, $P = 0.038$), LSM (0.69, 95% CI 0.59–0.80, $P = 0.046$), APRI (0.59, 95% CI 0.44–0.71, $P = 0.021$), and FIB-4 (0.61, 95% CI 0.51–0.73, $P = 0.016$). Using the optimal cut-off value determined in the validation cohort 2 (46), the sensitivity and specificity of EHLI-nomogram to identifying EHLI risk was 81% and 73%, respectively (Table 5). Compared to the diagnostic accuracy and discriminative ability of the EHLI-nomogram and LSM predicting EHLI risk, we calculated NRI and IDI in the training cohort（NRI 1.070 (95%CI: 0.838–1.303, $P < 0.001$) and IDI 0.141 (95%CI: 0.088- 0.195, $P < 0.001$), Fig. 3b）, in the validation cohort 1（NRI: 0.989 (95%CI: 0.563–1.416, $p < 0.001$) and IDI: 0.163 (95%CI: 0.068 - 0.258, $P < 0.001$), Fig. 3c）, and in the validation cohort 2（NRI 0.223 (95%CI:0.172 - 0.617, $P = 0.268$ and IDI −0.012 (95%CI−0.111 - 0.087, $P = 0.816$), Fig. 3d）. The EHLI-nomogram had a higher overall net benefit compared to LSM in training cohort and validation cohort 1, but not better in validation cohort 2.

3.7. Patients with EHLI identified by EHLI-nomogram responded to HBV treatment

Applying the optimal cut-off (46.4) for 181 patients with HBeAg-positive chronic HBV infection, 60 (33.15%) patients had more than 46.4, hence they were identified the HBeAg-positive chronic HBV infection patients with EHLI and labeled antiviral treatment while biopsy confirmed that only 55 (92%) patients were qualified for EHLI (Table 6). Histologic improvement after 72-week of entecavir treatment occurred in 40 of 49 HBeAg-positive chronic HBV infection patients (82%). Additionally, entecavir treatment also resulted in significant higher the proportion of fibrosis regression in the HBeAg-positive chronic HBV infection group than that of the low HBV DNA viral group (78% vs. 55%, $P = 0.015$). The proportion of no longer having EHLI was significantly higher in HBeAg-positive chronic HBV infection group than

Table 5
Diagnostic performances of EHLI-nomogram, GPR, LSM, APRI and FIB-4 in the training set and in validation sets.

Variables	Training set (absence vs. presence EHLI)	Validation sets (absence vs. presence EHLI)	
	Treatment-naïve (n = 223)	Treatment-naïve (n = 103)	On-treatment (n = 127)
EHLI-nomogram			
AUROC (95% CI)	92.4 (86.4–98.7)	90.2 (84.2–95.1)	81.3 (72.1–90.5)
Cutoff value	46.4	46.4	46
Sensitivity/specificity (%)	94.3/86.2	83.0/95.8	80.6/72.8
Accuracy (%)	89.2	87.3	78.2
PPV/NPV (%)	97.2/79.4	97.5/74.2	90.5/70.2
Positive/negative LR	12.02/0.22	19.9/0.18	7.9/0.28
GPR			
AUROC (95% CI)	77.2 (77.1–83.3)	75.2 (65.9–87.9)	63.7 (53.7–70.5)
Cutoff value	0.32	0.30	0.32
Sensitivity/specificity (%)	81.6/53.1	55.3/80.6	70.6/50.1
Accuracy (%)	68.6	63.7	49.6
PPV/NPV (%)	64.5/70.6	81.8/51.2	60.5/70.3
Positive/negative LR	8.05/0.5	6.64/0.49	5.36/0.53
LSM			
AUROC (95% CI)	81.3 (71.0–90.7)	79.4 (70.5–89.2)	68.8 (58.9–80.4)
Cutoff value	5.8	5.8	5.8
Sensitivity/specificity (%)	79.4/86.9	73.2/85.4	74.3/66.7
Accuracy (%)	80.3	71.4	68.7
PPV/NPV (%)	84.6/59.6	85.7/63.9	77.1/46.5
Positive/negative LR	6.05/0.34	15.6/0.29	2.29/0.36
APRI			
AUROC (95% CI)	78.2 (73.3–85.0)	70.2 (63.1–83.3)	58.7 (43.7–70.8)
Cutoff value	0.46	0.46	0.46
Sensitivity/specificity (%)	73.4/46.5	45.8/78.6	47.7/58.2
Accuracy (%)	66.5	60.3	57.5
PPV/NPV (%)	73.4/46.5	70.1/61.8	53.5/34.6
Positive/negative LR	3.0/0.35	4.47/0.38	3.04/0.58
FIB-4			
AUROC (95% CI)	71.6 (61.7–87.6)	68.3 (62.2–84.3)	60.7 (50.8–72.7)
Cutoff value	0.91	0.91	0.91
Sensitivity/specificity (%)	73.7/75.4	70.8/63.3	60.8/70.0
Accuracy (%)	71.2	68.9	60.2
PPV/NPV (%)	71.6/50.0	68.7/72.3	78.8/38.7
Positive/negative LR	3.0/0.35	4.47/0.31	3.04/0.49
Comparison of AUROC			
EHLI-nomogram and GRP	$P < 0.001$	$P = 0.005$	$P = 0.038$
EHLI-nomogram and LSM	$P = 0.006$	$P = 0.048$	$P = 0.046$
EHLI-nomogram and APRI	$P < 0.001$	$P = 0.043$	$P = 0.021$
EHLI-nomogram and FIB-4	$P < 0.001$	$P = 0.009$	$P = 0.016$

EHLI, evident histological liver injury; GPR, gamma-glutamyl transpeptidase to platelet ratio; LSM, liver stiffness measurement; APRI, AST to platelet index; FIB-4, fibrosis index based on 4 factors; AUROC, area under the receiver operator characteristic curve; CI, confidence intervals, PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

in low viral loads group (73% vs. 32%, P <0.001). However, the proportion of patients who achieved undetectable HBV DNA were significant lower in the HBeAg-positive chronic HBV infection group than in the low viral load group (39/58(67%) vs. 78/87 (90%), P = 0.001; Table 6). Compared to baseline tests, conserved-site changes with or without polymorphic-site changes were observed in 8 of 145 (5.5%) patients. None of these 8 patients experienced clinical virologic breakthrough (Table S5).

Table 6
Summary of the treatment efficacy of HBeAg-positive chronic HBV infection patients with EHLI identified by nomogram.

Response	High HBV DNA Load (n = 181)	Low HBV DNA Load (n = 155)	P values
Nomogram identified EHLI	60 (33.15%)	94 (60.65%)	< 0.001
Biopsy-proven EHLI	55/60 (91.67%)	88/94 (93.62%)	0.921
Entecavir therapy	60	91	
Change in HBV DNA from baseline (log IU/mL)	−7.18[2.57]	−5.25[1.71]	< 0.001
Undetectable HBV DNA	39/57 (68.42%)	80/88 (90.91%)	0.001
Change in qHBsAg from baseline (log IU/mL)	−0.10 [0.48]	−0.12[0.68]	0.495
HBeAg seroconversion	4/58 (6.89%)	8/87 (9.19%)	0.854
[a]Histological improvement	40/49 (81.63%)	47/78 (60.26%)	0.012
[b]Regression of fibrosis	38/49 (77.55%)	44/78 (56.41%)	0.015
[c]Worsening of fibrosis	1/49(2.04%)	7/78 (8.97%)	0.234
HAI ≤ 8 and IFS ≤ 2	35/49 (72.73%)	25/78 (32.05%)	< 0.001

[a] Histological improvement: ≥ 2 point reduction in Knodell necroinflammatory score with no worsening of fibrosis at treatment week 72;.
[b] Regression of Fibrosis: ≥ 1 point reduction by Ishak fibrosis stage system at treatment week 72;.
[c] Worsening Ishak fibrosis score: increased at least 1 point by Ishak fibrosis stage system at treatment week 72; EHLI, evident histological liver injury; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; IFS, Ishak fibrosis staging; HAI, histologic activity index.

4. Discussion

This study provides compelling evidence that significant histological disease is indeed common (33%) in HBeAg-positive chronic HBV infection patients while exclusion biopsy-proven concomitant NAFLD. More importantly, we found the frequency of significant fibrosis in HBeAg-positive chronic HBV infection patients with persistently normal of ALT was significantly higher than that of significant inflammation activity. Especially, among HBeAg-positive chronic HBV infection patients without significant fibrosis, the probability of significant inflammatory activity in patients with persistent normal ALT is very low, almost none. Notably, we also found that even among HBeAg-positive chronic HBV infection, those < 30yrs old or with LSM < 6 kPa, who are often thought to have absent or minimal significant histology [2,4,12], but 24% were found to have frequency of significant histological disease in present study. Our finding reconfirmed some previous reports that HBeAg-positive chronic HBV infection patients may have significant histology[5−8]. Therefore, identifying patients with EHLI is more important than defining HBeAg-positive chronic HBV infection. Our results confirmed that HBeAg-positive chronic HBV infection patients are a heterogeneous group. Although the frequency of significant histological disease was a higher in HBeAg-positive patients with normal ALT and low viral load than in HBeAg-positive chronic HBV infection patients, both groups had > 33% patients with significant histology, i.e., active CHB. These data support that all of HBeAg-positive patients with normal ALT should be further assessed for the presence of histological disease and indication for HBV treatment.

We also found that 25% of immune-tolerant CHB patients had EHLI. Significantly, 22% of those < 30yrs old had biopsy-proven significant disease. These results emphasize the importance of further assessment in immune-tolerant CHB patients even when under 30 years old. Although liver biopsy has been the gold standard for determination of histological disease, its invasiveness and complications have limited its application [11]. In order to develop a noninvasive mathematical model to replace liver biopsy to determine EHLI in HBeAg-positive chronic HBV infection patients, we developed and validated EHLI-nomogram that accurately predict EHLI risk in HBeAg-positive chronic HBV infection patients according to 5 routine available variables. The concordance of EHLI-nomogram predictive histological disease and liver biopsy reached almost perfect in HBeAg-positive chronic HBV infection. The management of HBeAg-positive chronic HBV infection strategies based on EHLI risk estimates derived from our EHLI-nomogram resulted in greater predicted net benefit that of current guidelines [2,4,12]. Notably, based on EHLI risk estimates for HBeAg-positive chronic HBV infection, our EHLI-nomogram can be used to significantly reduce the risk of patients missing antiviral therapy compared to the recommendations by current guidelines [2,4,12]. Moreover, the EHLI-nomogram had a high diagnostic performance for identifying patients with EHLI and that its diagnostic performance was better than other established models and methods (i.e., APRI, FIB-4, LSM alone, and GPR). A potential explanation for the better diagnostic performance of the EHLI-nomogram might be an effective combination of five variables from various directions. First, age, an important demographic variable, is positively associated with progression of chronic liver diseases [6,7]. Second, LSM is rapid and easy to perform in clinical practice [12]. Notably, 71% of LSM 6−9 kPa and 49% of LSM > 9 kPa in HBeAg-positive chronic HBV infection patients also showed minimal histology disease in our study. These data support the intermediate values of LSM have low accuracy [12,20]. Third, although ALT indicated liver injury [2,4], the disease activity in HBeAg-positive chronic HBV infection might be under-reported by assessing ALT [4−8]. Fourth, serum albumin indicated hepatic synthetic function, and ALP for liver function [21,22]. That might explain why the lower sensitivity of APRI, FIB-4, LSM alone and GPR to identify significant inflammation and fibrosis, as previously reported [12,20,23] and as observed in our study. These data reconfirmed that LSM alone, GRP, APRI and FIB-4 scores are not suitable for use in clinical practice in HBeAg-positive chronic HBV infection patients for assessment of the disease activity, especially in gaging improvements in histological disease following anti-virial therapy [23].

Another striking feature found in this study was in regards to using paired liver biopsy assessment in HBeAg-positive chronic HBV infection patients with EHLI identified by EHLI-nomogram responded to HBV treatment excellent, that significantly improves their outcomes. The majority of HBeAg-positive chronic HBV infection patients with EHLI who achieved an improvement in fibrosis stage also achieved a reduction in proportion of patients with EHLI, supporting findings for the treatment of the HBeAg-positive chronic HBV patients with EHLI offers the potential to control HBV replication and to arrest or halt the progression of liver disease. Notably, despite the high antiviral potency of entecavir, the proportion of HBeAg-positive chronic HBV infection patients who achieved undetectable HBV DNA was not as high as expected due to these patients with very high viral load in baseline. Despite patients with ongoing low levels of viral replication, no confirmed entecavir resistance could be found by viral genotypic or phenotypic analyses. These results further confirmed that EHLI-nomogram can reliably identify 95% of HBeAg-positive chronic HBV infection patients with significant disease to provide a unique opportunity of targeted early HBV treatment, and reduce the risk of liver-related complications, especially in high HBV endemic countries and regions, but with limited resource.

It should be noted that our study is limited by lacking non-Asian CHB patients. In addition, the EHLI-nomogram has limited accuracy in monitoring fibrosis change in response to therapy due to underprediction or overprediction probability of presence EHLI on-treatment patients. With ongoing antiviral therapy, improvement of liver inflammation activity rapidly resulted in reduction in liver elasticity and blood biomarkers. Consequently, EHLI-nomogram tend to improve independently of fibrosis regression leading to a tendency to underestimate fibrosis stage, thereby reducing the utility of available EHLI-nomogram for assessment of short-term fibrosis response to treatment. Despite its limitations, our study has several strengths such as its large sample size, as well as well-characterized cohort of HBeAg-positive chronic HBV infection patients with a wide range of Ishak fibrosis stages, and follow-up liver biopsy data after 72-weeks of antiviral therapy.

In conclusion, the present study demonstrated among HBeAg-positive chronic HBV infection patients, 33% had significant histology, of whom the frequency of significant fibrosis was higher than that of significant inflammation activity. An EHLI-nomogram developed in this study is superior to some other non-invasive, correcting current guidelines recommendations overestimating insignificant or significant histological disease, in providing a non-invasive, convenient, and highly reliable diagnostic approach to identify HBeAg-positive chronic HBV infection patients with EHLI. We also found that good response to HBV treatment was achievable. This would provide a unique opportunity in targeted, early HBV treatment in urgently needed patients to reduce the HBV-related complications. Our data strongly support the clinical application of the EHLI-nomogram.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to acknowledge the work of all our clinical colleagues at every center participated in the study. This study was funded by the State Key Projects Specialized on Infectious Disease, Chinese Ministry of science and technology (2013ZX10005002; 2018ZX10725506), National Natural Science Foundation of China (81970525) and Beijing Key Research Project of Special Clinical Application (Z151100004015221).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103389.

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