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Research Article

Keywords: chronic hepatitis B, MASLD, hepatic steatosis, pegylated interferon, HBsAg clearance, metabolic burden

Posted Date: September 4th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10628850/v1>

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Severe MASLD and Metabolic Burden Are Associated with Reduced HBsAg Clearance During Pegylated Interferon Therapy in HBeAg-Negative Chronic Hepatitis B

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Abstract

Background and aims: The impact of metabolic dysfunction-associated steatotic liver disease (MASLD) on pegylated interferon-alpha (PEG-IFN α)-induced HBsAg clearance remains uncertain. We investigated whether MASLD severity and cumulative metabolic burden were associated with HBsAg clearance in HBeAg-negative chronic hepatitis B (CHB) patients with low baseline HBsAg.

Methods: This multicenter cohort study included HBeAg-negative CHB adults with low baseline HBsAg (≤ 1500 IU/mL) and suppressed/low-level HBV replication completing 48 weeks of PEG-IFN α therapy. Patients were classified as having CHB without steatosis or with MASLD (mild, n=68; moderate, n=40; severe, n=99) based on controlled attenuation parameter, metabolic risk factors, and alcohol exclusion. The primary endpoint was week-48 HBsAg clearance.

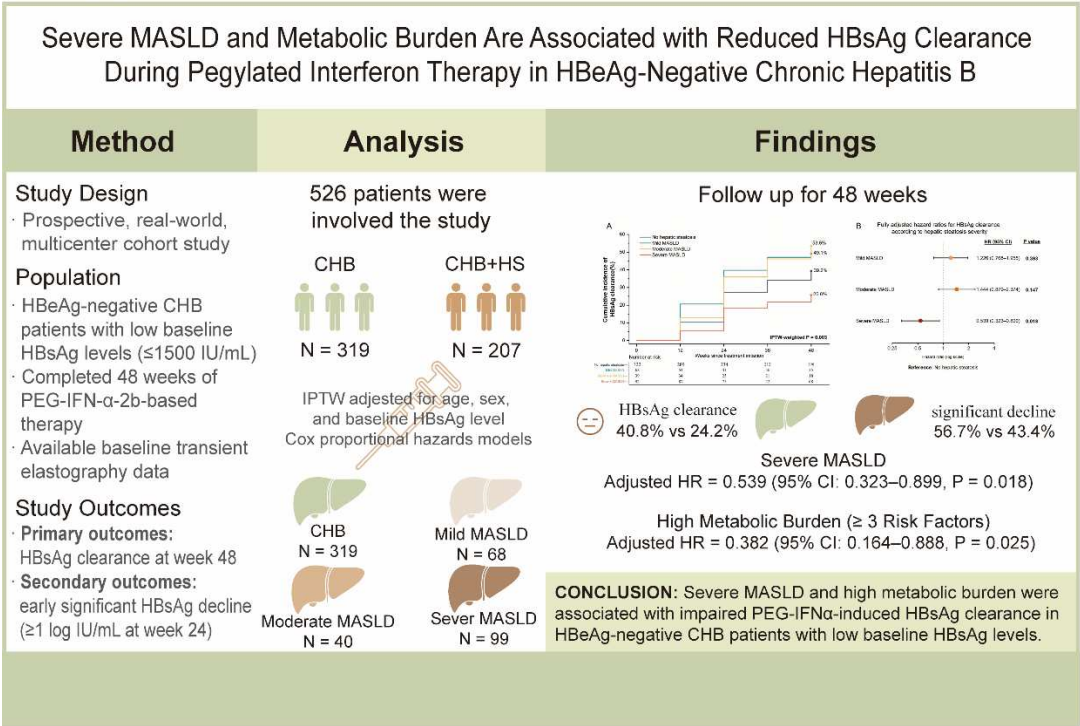
Results: Among 526 patients, 206 (39.2%) achieved HBsAg clearance by week 48. While overall clearance did not differ between non-steatosis and MASLD groups (40.8% vs. 36.7%, $P=0.354$). However, severe MASLD was associated with a lower HBsAg clearance rate than no hepatic steatosis (24.2% vs. 40.8%, adjusted $P = 0.018$), whereas clearance rates were 44.1% and 55.0% in patients with mild and moderate MASLD, respectively. After inverse probability of treatment weighting and multivariable Cox adjustment, severe MASLD remained independently associated with reduced HBsAg clearance (adjusted HR=0.539, 95% CI: 0.323–0.899, $P = 0.018$). Moreover, ≥ 3 cardiometabolic risk factors predicted lower clearance (adjusted HR=0.382, 95% CI: 0.164–0.888, $P=0.025$).

Conclusions: Severe MASLD and high cumulative metabolic burden, rather than MASLD per se, were associated with impaired PEG-IFN α -induced HBsAg clearance in

HBeAg-negative CHB patients with low baseline HBsAg, aiding selection for interferon-based functional cure strategies.

Keywords: chronic hepatitis B; MASLD; hepatic steatosis; pegylated interferon; HBsAg clearance; metabolic burden

GRAPHICAL ABSTRACT



1. Introduction

Chronic hepatitis B virus (HBV) infection remains a major global health burden and a leading cause of cirrhosis and hepatocellular carcinoma [1]. Although nucleos(t)ide analogues and pegylated interferon-alpha (PEG-IFN α) improve viral suppression, achieving functional cure, defined as sustained hepatitis B surface antigen (HBsAg) loss with undetectable HBV DNA, remains challenging [2–4]. HBsAg clearance is a key endpoint in HBV cure strategies and is associated with improved long-term clinical outcomes [5–7]. However, even among HBeAg-negative patients with low baseline HBsAg levels, who are considered favorable candidates for PEG-IFN α therapy, treatment responses remain heterogeneous [8,9].

Metabolic dysfunction-associated steatotic liver disease (MASLD) frequently coexists with chronic hepatitis B (CHB) and represents an increasingly recognized metabolic comorbidity in this population [10–11]. Previous studies have reported inconsistent associations between hepatic steatosis and HBV treatment outcomes, with some suggesting increased HBsAg seroclearance among patients with steatosis, whereas others reported impaired antiviral responses [12–15]. These discrepancies may reflect differences in patient characteristics, treatment modalities, baseline viral activity, and definitions or assessment methods of steatosis [16–17].

Importantly, previous studies have rarely evaluated whether the severity of hepatic steatosis or cumulative metabolic burden influences PEG-IFN α -induced functional cure. Therefore, using prospectively collected real-world data from two clinical cohorts, we investigated the associations of MASLD severity and metabolic burden with HBsAg clearance in HBeAg-negative CHB patients with low baseline HBsAg receiving PEG-IFN α -based therapy. We hypothesized that hepatic steatosis per se may not uniformly affect treatment response, whereas severe steatosis and greater metabolic burden may identify patients with impaired functional cure probability.

2. Methods

2.1. Study Design And Participants

This prospective, real-world multicenter cohort study pooled data from two PEG-IFN α -based functional cure trials: the STARHB Project (ChiCTR2200061541) and the Everest Project (ChiCTR1800020369). Eligible patients, enrolled between January 2021 and December 2025 at participating centers including Beijing Youan Hospital, were HBeAg-negative CHB adults with low baseline HBsAg (STARHB: <1000 IU/mL; Everest: $\leq$ 1500 IU/mL) and low-level or suppressed HBV replication (STARHB: HBV DNA <2000 IU/mL; Everest: undetectable under NA therapy).

Inclusion criteria were: 1) serum HBsAg positivity for at least 6 months; 2) HBeAg negativity at baseline; 3) baseline ALT $\leq$ 2 times the upper limit of normal; 4) no use of immunosuppressive agents within 6 months before enrollment; 5) no interferon therapy within 6 months before enrollment; and 6) available baseline transient elastography data. Prior or ongoing NA treatment was allowed. The exclusion criteria were: 1) autoimmune disease; 2) coinfection with HIV, HAV, HCV, HDV, or HEV; 3) decompensated cirrhosis or a history of decompensated liver disease; 4) liver cancer, suspected malignancy, or other active cancer; 5) severe cardiac disease, severe infection, uncontrolled diabetes, thyroid disease, retinopathy, or other contraindications to PEG-IFN α therapy; and 6) missing baseline transient elastography data. Baseline demographic, clinical, laboratory, treatment-related, and transient elastography data were extracted from standardized case report forms and electronic medical records.

2.2. Treatment And Follow-up

Patients received PEG-IFN α -based therapy (monotherapy or combined with NAs) for at least 48 weeks. Initial regimens depended on baseline HBV DNA, prior NA history, and clinical indications. Concomitant NAs included entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate. Follow-up occurred every 12 weeks ($\pm$ 14 days) through week 48. At each visit (baseline, weeks 12, 24, 36, and 48), quantitative HBsAg, HBV DNA, liver biochemistry, hematological parameters, and adverse events were assessed.

2.3 Definitions And Outcome Measures

Patients were classified at baseline as CHB without hepatic steatosis or CHB with MASLD(CHB+MASLD). MASLD was defined as hepatic steatosis plus at least one cardiometabolic risk factor, in the absence of harmful alcohol intake or other secondary causes of hepatic steatosis, according to the updated MASLD nomenclature and clinical practice guidelines [11]. MASLD was defined as hepatic steatosis (controlled attenuation parameter [CAP] ≥ 248 dB/m) plus at least one of five cardiometabolic risk factors, in the absence of secondary causes of steatosis. Detailed definitions and numerical thresholds for these cardiometabolic factors are provided in the Supplementary Methods. Metabolic burden was assessed categorically, with high metabolic burden defined as three or more risk factors.

Baseline hepatic steatosis and liver fibrosis were assessed using transient elastography. Within the CHB+MASLD group, steatosis severity was categorized as mild, 248–267 dB/m; moderate, 268–279 dB/m; or severe, ≥ 280 dB/m. Liver fibrosis was categorized using CHB-specific LSM cutoffs: F0–F1, ≤ 7.0 kPa; F2, > 7.0 to ≤ 8.0 kPa; F3, > 8.0 to ≤ 11.0 kPa; and F4, > 11.0 kPa. The primary outcome was HBsAg clearance at week 48, defined as serum HBsAg < 0.05 IU/mL with HBV DNA < 10 IU/mL. The secondary outcome was early significant HBsAg decline, defined as a ≥ 1 log₁₀ reduction in quantitative HBsAg from baseline to week 24. Changes in quantitative HBsAg from baseline to weeks 12, 24, 36, and 48 were also assessed.

2.4 Statistical Analysis

Continuous variables are presented as medians (interquartile ranges, IQRs) and categorical variables as counts (percentages). Group comparisons utilized the Mann-Whitney U test, Kruskal-Wallis test, chi-square test, or Fisher's exact test, with Bonferroni correction for post-hoc analyses. Time to HBsAg clearance was calculated from treatment initiation, with non-responders censored at their last available assessment up to week 48. The cumulative probability of HBsAg clearance was estimated using Kaplan-Meier curves, and weighted curve differences were assessed via inverse probability of treatment weighting (IPTW)-weighted Cox models.

Cox proportional hazards models evaluated the association between steatosis severity and HBsAg clearance, reporting hazard ratios (HRs) and 95% confidence intervals (CIs). To minimize baseline imbalances in age, sex, and HBsAg levels across steatosis groups, stabilized IPTW based on generalized propensity scores (estimated via multinomial logistic regression) was applied. Balance was confirmed by standardized mean differences < 0.10 . Sequentially adjusted IPTW-weighted Cox models were constructed: Model 1 included stabilized IPTW only; Model 2 further adjusted for ALT, AST, fibrosis stage, and treatment regimen; Model 3 was additionally adjusted for baseline HBV DNA status and comprehensive laboratory parameters (listed in Table 2). The proportional hazards assumption was verified using Schoenfeld residuals (all $P > 0.05$). Additionally,

an exploratory post-hoc analysis evaluated the impact of cumulative metabolic burden (≥ 3 risk factors) on HBsAg clearance. Sensitivity analyses truncated stabilized weights at the 1st and 99th percentiles. Analyses were performed using available data without imputation. A two-sided $P < 0.05$ indicated statistical significance.

2.5 Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki. The STARHB Project was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University (Approval No. LL-2022-067-K). The Everest Project was approved by the relevant institutional ethics committees before patient enrollment, with local ethics approval at Beijing Youan Hospital (Approval No. LL-2018-055-K). Written informed consent was obtained from all participants before data collection and treatment initiation. All patient data were anonymized before analysis to protect patient privacy.

3. Results

3.1 Baseline characteristics

A total of 1,062 HBeAg-negative CHB patients with HBsAg $\leq 1,500$ IU/mL and low or suppressed HBV DNA were screened. After excluding 328 patients lacking baseline transient elastography data, 734 patients with baseline CAP/LSM measurements were evaluated. Based on eligibility criteria, 136 patients were further excluded, leaving 598 eligible patients enrolled to receive PEG-IFN α -2b-based therapy (363 without hepatic steatosis and 235 with MASLD). During the 48-week follow-up, 72 patients were excluded due to treatment discontinuation or loss to follow-up ($n = 53$) or missing key follow-up data ($n = 19$). Ultimately, 526 patients were included in the final analysis (Fig. 1). Among them, 207 patients were classified as having CHB with MASLD and 319 as CHB without hepatic steatosis. In the CHB+MASLD group, 68 patients had mild steatosis, 40 had moderate steatosis, and 99 had severe steatosis, accounting for 32.9%, 19.3%, and 47.8%, respectively.

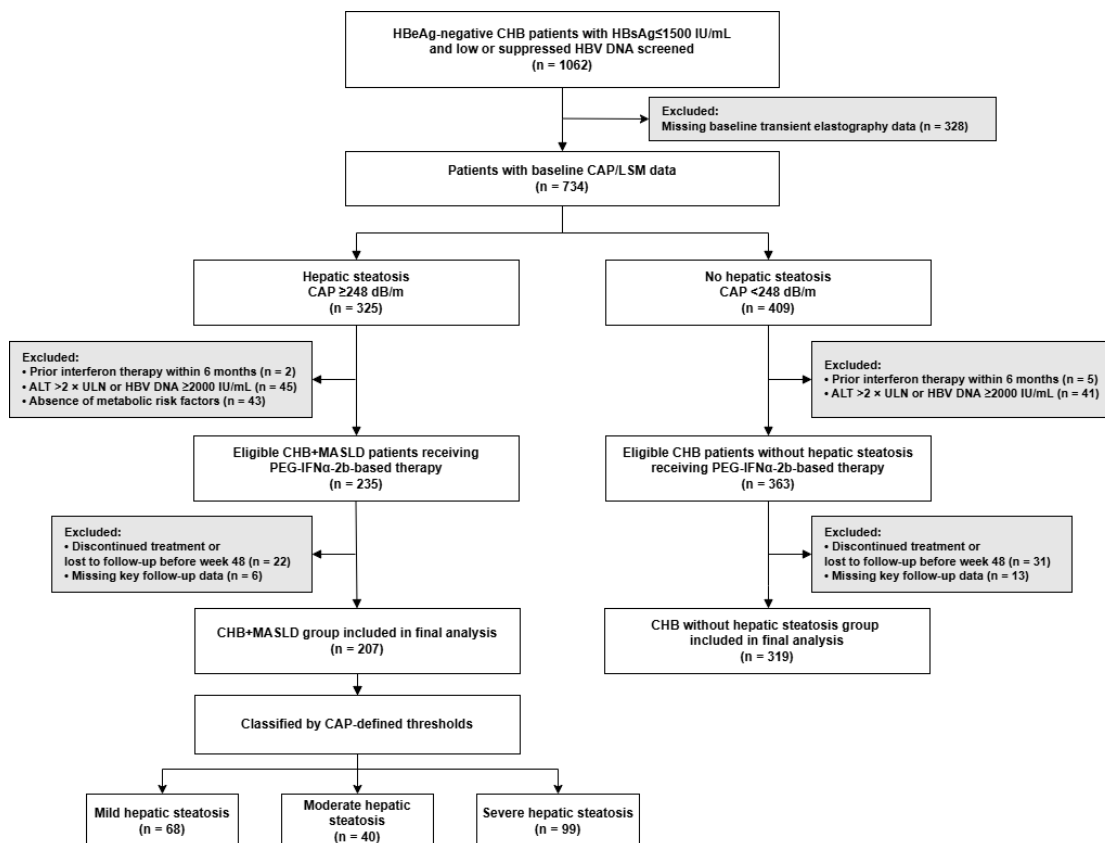


Fig. 1. Flowchart of patient selection.

Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PEG-IFN α -2b, pegylated interferon alpha-2b; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; MASLD, metabolic dysfunction-associated steatotic liver disease; TE, transient elastography; ULN, upper limit of normal.

Baseline characteristics are summarized in Table 1. The median age of the overall cohort was 41 years, and 319 patients (60.6%) were male. Baseline HBsAg levels were comparable between patients with and without MASLD [115.00 (12.45, 447.10) vs. 131.20 (17.80, 430.70) IU/mL; $P = 0.733$], as were baseline HBV DNA levels and the proportion of patients with undetectable HBV DNA. Compared with patients without hepatic steatosis, those with CHB+MASLD were older, more frequently male, and had higher levels of ALT, AST, fasting blood glucose, triglycerides, total cholesterol, and low-density lipoprotein cholesterol, but lower high-density lipoprotein cholesterol levels. CAP and LSM values were higher in the CHB+MASLD group, and fibrosis stage distribution differed modestly between groups. Treatment regimens were similar between the two groups. Baseline characteristics according to hepatic steatosis severity are presented in Supplementary Table 1. Across patients without hepatic steatosis and those with mild, moderate, or severe steatosis, baseline HBsAg levels, HBV DNA levels, baseline HBV DNA status, cohort source, and treatment regimens were comparable, whereas age, sex

distribution, BMI, metabolic parameters, CAP, LSM, and fibrosis stage differed significantly across groups.

Table 1. Baseline characteristics of patients with CHB according to the presence of MASLD.

Variables	Overall cohort (n = 526)	CHB without hepatic steatosis (n = 319)	CHB+MASLD (n = 207)	P value
Age, years	41 (35, 47)	39 (34, 45)	43 (38, 49)	<0.001
Male sex, n (%)	319 (60.6%)	161 (50.5%)	158 (76.3%)	<0.001
Baseline HBsAg, IU/ml	129.00 (15.20, 439.50)	131.20 (17.80, 430.70)	115.00 (12.45, 447.10)	0.733
HBsAg <1000 IU/ml, n (%)	503 (95.6%)	308 (96.6%)	195 (94.2%)	0.198
Baseline HBV DNA , IU/ml	0 (0, 93)	0 (0, 124)	0 (0, 66.5)	0.451
Undetectable HBV DNA, n(%)	241 (45.8%)	145 (45.5%)	96 (46.4%)	0.836
Hemoglobin, g/L	151 (137, 160)	145 (133, 157)	155 (146, 163)	<0.001
WBC($\times 10^9$ /L)	5.42 (4.59, 6.62)	5.25 (4.46, 6.35)	5.70 (4.80, 6.95)	<0.001
ANC($\times 10^9$ /L)	3.13 (2.54, 3.96)	3.00 (2.42, 3.69)	3.34 (2.65, 4.19)	0.003
PLT($\times 10^9$ /L)	214 (175, 255)	210 (171, 253)	218 (185, 258)	0.081
ALT(U/L)	21 (16, 28)	19 (14, 25)	24 (19, 34)	<0.001
AST(U/L)	23 (19, 27)	22 (18, 26)	24 (21, 28)	<0.001
TBIL(μ mol/L)	15.9 (11.8, 20.9)	15.7 (11.8, 20.6)	16.5 (11.9, 20.9)	0.427
TP(g/L)	76.0 (73.0, 78.8)	75.7(72.6, 78.4)	76.3(73.4, 79.3)	0.076
ALB(g/L)	46.9 (44.9, 48.8)	46.5 (44.6, 48.6)	47.4 (45.6, 49.2)	<0.001
BUN(mmol/L)	4.79 (3.99, 5.61)	4.70 (3.91, 5.45)	4.93 (4.26, 5.68)	0.021
Cr(μ mol/L)	64 (54, 74)	63 (52, 74)	66 (59, 75)	0.005
FBG(mmol/L)	5.30 (4.92, 5.71)	5.12 (4.87, 5.51)	5.48 (5.11, 5.89)	<0.001
TG(mmol/L)	1.18 (0.85, 1.68)	0.90 (0.72, 1.14)	1.44 (1.19, 1.91)	<0.001
TC(mmol/L)	4.67 (4.28, 5.26)	4.54 (4.08, 5.12)	4.85 (4.39, 5.43)	0.047
HDL(mmol/L)	1.11 (0.92, 1.33)	1.25 (1.06, 1.50)	1.00 (0.88, 1.15)	<0.001
LDL(mmol/L)	3.11 (2.54, 3.62)	2.80 (2.32, 3.35)	3.29 (2.69, 3.78)	0.004
CAP, dB/m	238 (209, 275)	211 (193, 229)	279 (264, 315)	<0.001
LSM (kPa)	5.1 (4.2, 6.2)	4.9 (4.1, 5.9)	5.3 (4.3, 6.7)	0.001
Fibrosis stage, n(%)				0.035
F0/F1	455 (86.5%)	287 (90.0%)	168 (81.2%)	
F2	33 (6.3%)	14 (4.4%)	19 (9.2%)	
F3	20 (3.8%)	9 (2.8%)	11 (5.3%)	
F4	18 (3.4%)	9 (2.8%)	9 (4.3%)	
Number of metabolic risk factors, n (%)				<0.001
0	183 (34.8%)	183 (57.4%)	0 (0%)	
1	207 (39.3%)	95 (29.8%)	112 (54.1%)	
2	91 (17.3%)	31 (9.7%)	60 (29.0%)	
≥ 3	45 (8.6%)	10 (3.1%)	35 (16.9%)	
Treatment, n(%)				0.331
ETV+IFN	214 (40.7%)	126 (39.5%)	88 (42.5%)	
TDF/TAF+IFN	217 (41.3%)	129 (40.4%)	88 (42.5%)	
IFN	95 (18.1%)	64 (20.1%)	31 (15.0%)	

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAP, controlled attenuation parameter; CHB, chronic hepatitis B; Cr, creatinine; ETV, entecavir; FBG, fasting blood glucose; HB, hemoglobin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDL, high-density lipoprotein; IFN, interferon; LDL, low-density lipoprotein; LSM, liver stiffness

measurement; PLT, platelet count; TAF, tenofovir alafenamide fumarate; TBIL, total bilirubin; TC, total cholesterol; TDF, tenofovir disoproxil fumarate; TG, triglyceride; TP, total protein; WBC, white blood cell count.

3.2. Impact of hepatic steatosis on serological responses during PEG-IFN α -based therapy

As shown in Figure 2A, HBsAg clearance at week 48 was achieved in 206 of 526 patients (39.2%) in the overall cohort. The rate of HBsAg clearance was 40.8% (130/319) in CHB patients without hepatic steatosis and 36.7% (76/207) in patients with CHB+MASLD, with no significant difference between the two groups ($P = 0.354$). Early significant HBsAg decline, defined as ≥ 1 log₁₀ reduction in quantitative HBsAg from baseline to week 24, was observed in 285 patients (54.2%) overall. The corresponding rates were 56.7% (181/319) in CHB patients without hepatic steatosis and 50.2% (104/207) in patients with CHB+MASLD, again with no significant between-group difference ($P=0.144$; Figure 2B).

Dynamic changes in quantitative HBsAg during treatment are shown in Figure 2C and Supplementary Table 3. HBsAg levels declined progressively from baseline to week 48 in both groups. Although absolute HBsAg levels at each follow-up visit were comparable between the two groups (Supplementary Table 2), patients with CHB+MASLD showed a smaller median decline in log₁₀ HBsAg from baseline, with significant differences at weeks 12, 36, and 48 ($P=0.022$, 0.028 , and 0.028 , respectively), although the difference at week 24 did not reach statistical significance ($P=0.060$). These data indicate a modestly attenuated on-treatment HBsAg decline in the MASLD group, while rates of early significant HBsAg decline and week-48 HBsAg clearance remained comparable.

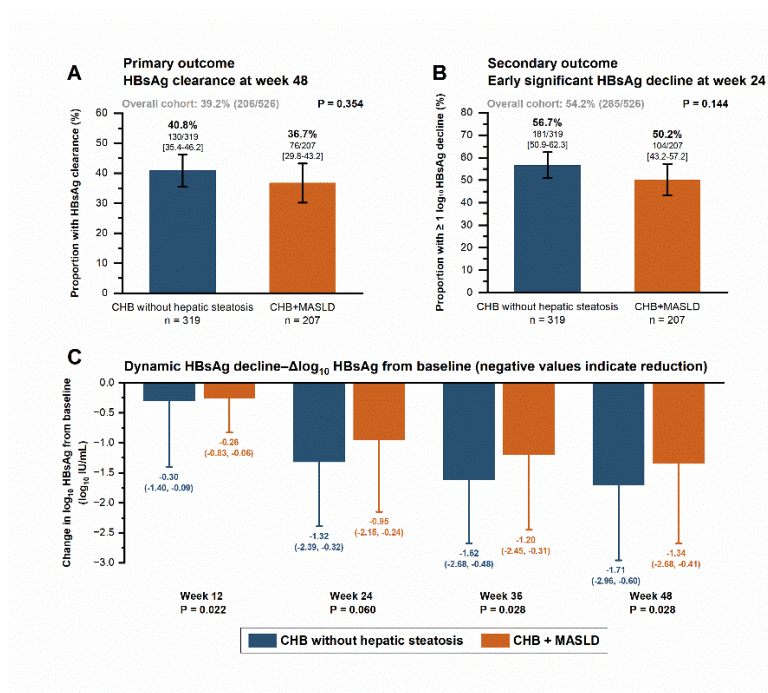


Figure 2. Serological responses during PEG-IFN α -2b-based therapy according to hepatic steatosis status.

(A) Proportions of patients achieving HBsAg clearance at week 48. (B) Proportions of patients achieving early significant HBsAg decline at week 24, defined as a ≥ 1 log₁₀ reduction in quantitative HBsAg from baseline. (C) Dynamic changes in log₁₀-transformed HBsAg levels from baseline to weeks 12, 24, 36, and 48. Changes were calculated as log₁₀ HBsAg at each follow-up time point minus log₁₀ HBsAg at baseline; negative values indicate reductions from baseline. Columns in panels A and B represent proportions, with 95% confidence intervals shown as error bars. Columns in panel C represent median Δ log₁₀ HBsAg values, with interquartile ranges shown as error bars. P values indicate between-group comparisons between patients without hepatic steatosis and those with CHB+MASLD. Categorical outcomes were compared using the chi-square test or Fisher's exact test, as appropriate; changes in log₁₀ HBsAg were compared using the Mann–Whitney U test. HBsAg, hepatitis B surface antigen; MASLD, metabolic dysfunction-associated steatotic liver disease; PEG-IFN α -2b, pegylated interferon alpha-2b.

3.3 Impact of hepatic steatosis severity on serological response to PEG-IFN α -based therapy

Patients with CHB+MASLD were further stratified according to CAP-defined steatosis severity into mild (68/207, 32.9%), moderate (40/207, 19.3%), and severe (99/207, 47.8%) steatosis subgroups. As shown in Figure 3A, week-48 HBsAg clearance rates differed significantly among patients without hepatic steatosis and those with mild, moderate, or severe MASLD (overall $P = 0.003$). The corresponding rates were 40.8% (130/319), 44.1% (30/68), 55.0% (22/40), and 24.2% (24/99), respectively. In Bonferroni-adjusted pairwise comparisons, patients with severe MASLD had significantly lower HBsAg clearance rates than patients without hepatic steatosis and those with mild or moderate MASLD, whereas no significant differences were observed among the other three groups (Supplementary Table 4).

The proportion of patients achieving an early significant HBsAg decline at week 24 differed among the four groups (Figure 3B), with the lowest response rate observed in patients with severe MASLD. The corresponding rates were 56.7% (181/319), 63.2% (43/68), 45.0% (18/40), and 43.4% (43/99) in patients without hepatic steatosis and those with mild, moderate, and severe MASLD, respectively (overall $P = 0.029$). Nevertheless, after Bonferroni adjustment for multiple comparisons, none of the pairwise comparisons reached statistical significance (Supplementary Table 4).

Longitudinal analysis of HBsAg kinetics further showed significant differences in HBsAg decline across the four groups at weeks 12, 24, 36, and 48 (overall $P = 0.032$, 0.012, 0.002, and 0.015, respectively; Figure 3C and Supplementary Table 5). Patients with severe MASLD showed the smallest median decline in log₁₀ HBsAg from baseline throughout treatment. Absolute HBsAg levels also differed significantly among the four groups from week 24 onward (Supplementary Table 6), with patients with severe MASLD generally maintaining higher residual HBsAg levels during follow-up. Collectively, these findings suggest that the unfavorable association between hepatic steatosis and PEG-IFN α treatment response was mainly observed in patients with severe steatosis, whereas patients with mild or moderate MASLD had treatment responses broadly comparable to

those without hepatic steatosis.

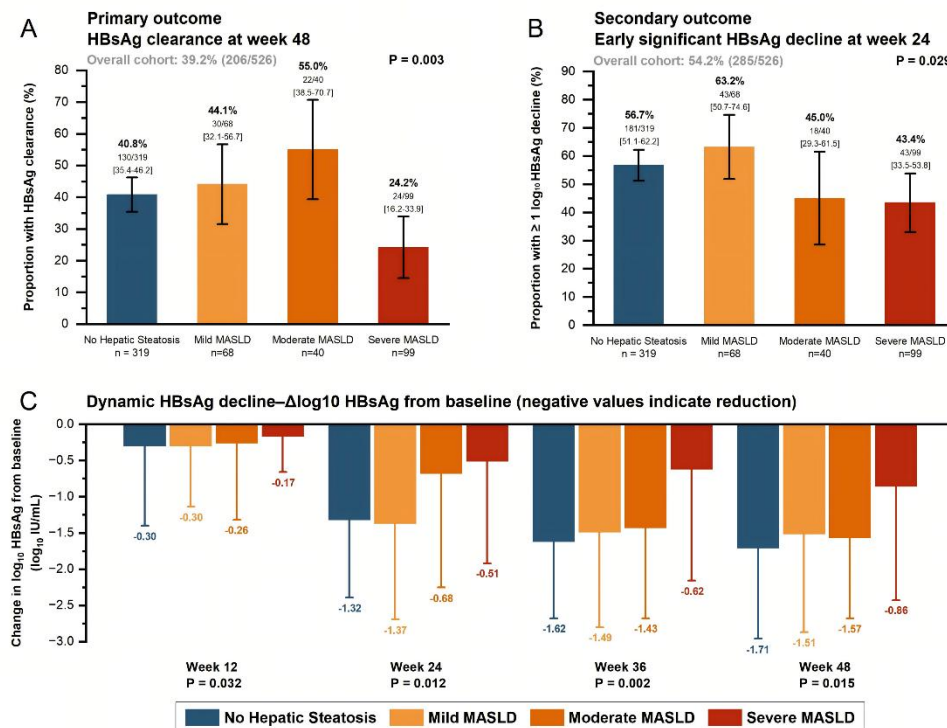


Figure 3. Serological responses according to hepatic steatosis severity during PEG-IFN α -2b-based therapy. (A) Proportions of patients achieving HBsAg clearance at week 48 among patients without hepatic steatosis and those with mild, moderate, or severe MASLD. (B) Proportions of patients achieving early significant HBsAg decline at week 24 according to hepatic steatosis severity. Early significant HBsAg decline was defined as a $\geq 1 \log_{10}$ reduction in quantitative HBsAg from baseline to week 24. (C) Dynamic changes in $\log_{10}$ -transformed HBsAg levels from baseline to weeks 12, 24, 36, and 48 according to hepatic steatosis severity. Changes were calculated as $\log_{10}$ HBsAg at each follow-up time point minus $\log_{10}$ HBsAg at baseline; therefore, negative values indicate reductions from baseline. In panels A and B, columns represent proportions and error bars indicate 95% confidence intervals. In panel C, columns represent median $\Delta \log_{10}$ HBsAg values and error bars indicate interquartile ranges. Overall P values indicate between-group comparisons. Pairwise comparisons were adjusted using the Bonferroni correction. Different letters indicate statistically significant differences after Bonferroni correction. HBsAg, hepatitis B surface antigen; MASLD, metabolic dysfunction-associated steatotic liver disease; PEG-IFN α , pegylated interferon alpha.

3.4 Severe hepatic steatosis is an unfavorable factor for HBsAg clearance at 48 weeks

After balancing age, sex, and baseline HBsAg levels using IPTW, the cumulative probability of HBsAg clearance differed significantly according to hepatic steatosis severity during the 48-week treatment period (IPTW-weighted log-rank test, $P=0.003$; Figure 4A). Patients with severe MASLD showed the lowest cumulative probability of HBsAg clearance. The balance of baseline covariates before and after IPTW is

presented in Supplementary Table 7. Consistent with the weighted cumulative incidence analysis, IPTW-weighted Cox regression identified severe MASLD as the only steatosis category independently associated with a lower likelihood of HBsAg clearance.

Compared with patients without hepatic steatosis, severe MASLD was associated with a significantly reduced likelihood of HBsAg clearance in the IPTW-weighted model without additional covariate adjustment (Model 1: HR=0.597, 95% CI: 0.375–0.951, P= 0.030).

This association remained significant after additional adjustment for HBV DNA, ALT, AST, fibrosis stage, and treatment regimen (Model 2: HR=0.602, 95% CI: 0.365–0.992, P=0.046), and after further adjustment for hemoglobin and other laboratory parameters (Model 3: HR=0.539, 95% CI: 0.323–0.899, P=0.018; Figure 4B and Table 2). In contrast, neither mild nor moderate MASLD was significantly associated with HBsAg clearance in any of the three models. In a supplementary analysis, patients with three or more metabolic risk factors had a significantly lower likelihood of achieving HBsAg clearance than those with fewer than three metabolic risk factors after multivariable adjustment (adjusted HR=0.382, 95% CI: 0.164–0.888, P=0.025; Supplementary Figure 1).

Table 2. IPTW-weighted Cox regression analyses for HBsAg clearance over 48 weeks according to hepatic steatosis severity

Characteristic	Model 1		Model 2		Model 3	
	HR(95% CI)	P-value	HR(95% CI)	P-value	HR(95% CI)	P-value
Mild MASLD	1.434 (0.933–2.204)	0.100	1.485 (0.960–2.295)	0.075	1.226 (0.768–1.955)	0.393
Moderate MASLD	1.491 (0.926–2.400)	0.100	1.445 (0.880–2.372)	0.146	1.444 (0.879–2.374)	0.147
Severe MASLD	0.597 (0.375–0.951)	0.030	0.602 (0.365–0.992)	0.046	0.539 (0.323–0.899)	0.018

Patients without hepatic steatosis served as the reference group. Model 1 was an IPTW-weighted Cox regression model without additional covariate adjustment. Model 2 was additionally adjusted for HBV DNA, ALT, AST, fibrosis stage, and treatment regimen. Model 3 was further adjusted for hemoglobin, white blood cell count, absolute neutrophil count, platelet count, total bilirubin, total protein, albumin, blood urea nitrogen and creatinine. IPTW was constructed using generalized propensity scores from a multinomial logistic regression model based on age, sex, and baseline HBsAg level.

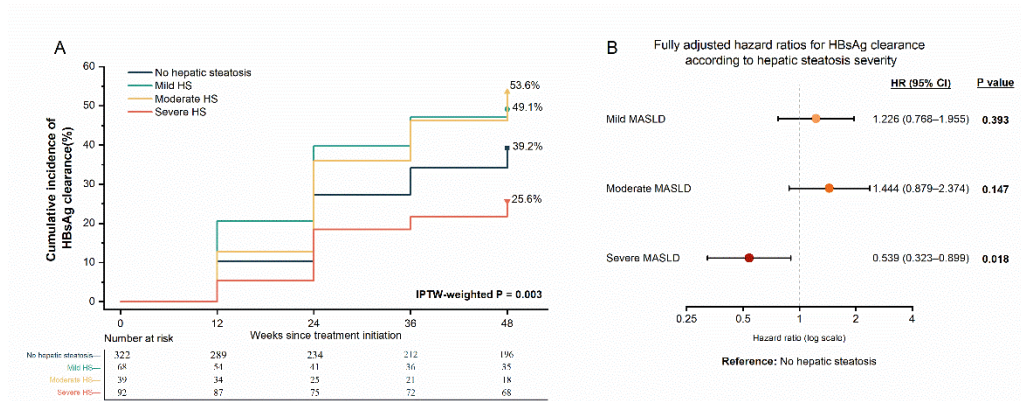


Figure 4. Cumulative probability and adjusted hazard ratios for HBsAg clearance according to hepatic steatosis severity.

(A) IPTW-weighted cumulative probability curves for HBsAg clearance over 48 weeks in patients without hepatic steatosis and those with mild, moderate, or severe MASLD. Numbers below the plot indicate the IPTW-weighted numbers at risk. Between-group differences were assessed using an IPTW-weighted log-rank test. (B) Forest plot of hazard ratios for HBsAg clearance according to hepatic steatosis severity from the fully adjusted IPTW-weighted Cox regression model, with patients without hepatic steatosis as the reference group. Hazard ratios less than 1 indicate a lower hazard of achieving HBsAg clearance.

Abbreviations: HBsAg, hepatitis B surface antigen; MASLD, metabolic dysfunction-associated steatotic liver disease; IPTW, inverse probability of treatment weighting; HR, hazard ratio; CI, confidence interval.

4. Discussion

In this prospective real-world multicenter cohort of 526 HBeAg-negative patients with CHB and low baseline HBsAg levels who completed 48 weeks of PEG-IFN α -based therapy, hepatic steatosis severity was closely associated with serological response. Patients with severe MASLD had the lowest week-48 HBsAg clearance rate, the lowest frequency of early significant HBsAg decline, and the least favorable longitudinal HBsAg kinetics during treatment. After IPTW based on age, sex, and baseline HBsAg level and sequential multivariable adjustment, severe MASLD remained independently associated with a lower likelihood of HBsAg clearance, whereas mild and moderate MASLD were not significantly associated with reduced clearance. These findings suggest a threshold-like pattern in which severe hepatic steatosis, rather than hepatic steatosis per se, may define a metabolically vulnerable phenotype with impaired responsiveness to PEG-IFN α -based functional cure therapy.

HBsAg clearance is a central endpoint in current HBV cure-oriented strategies and is associated with improved long-term clinical outcomes [4-7]. Unlike most studies that evaluated virological suppression during NA therapy, our study focused on PEG-IFN α -induced HBsAg clearance in a population enriched for functional cure potential. Although PEG-IFN α -based regimens can induce durable HBsAg loss in selected patients, treatment response remains heterogeneous even among HBeAg-negative patients with low baseline HBsAg levels [8,9]. Our findings extend existing response-prediction frameworks, which primarily emphasize viral and biochemical factors such as baseline HBsAg level, HBV DNA, ALT activity, HBeAg status, and treatment regimen, by

identifying severe MASLD and cumulative metabolic burden as host metabolic factors associated with a lower probability of functional cure.

Existing evidence on how hepatic steatosis affects antiviral efficacy in CHB remains heterogeneous [16-17]. While some studies suggest that hepatic steatosis impairs the antiviral response [15,20-21], others have found no independent adverse association, particularly in NA-based studies or cohorts with very low baseline HBsAg [9,19,22]. These discrepancies largely stem from substantial heterogeneity in study populations, HBeAg status, baseline HBsAg levels, treatment modalities, and the methods used to define and assess steatosis severity. Therefore, the impact of hepatic steatosis on antiviral outcomes should not be interpreted as uniform across all CHB treatment settings.

The relationship between HBV infection and hepatic steatosis is biologically complex and sometimes paradoxical. Experimental and clinical evidence suggests that hepatic steatosis may be associated with lower HBV replication markers or a higher probability of spontaneous HBsAg seroclearance in some settings [12-13,18]. However, metabolic dysfunction and steatohepatitis may simultaneously promote hepatic inflammation, fibrosis progression, and adverse liver outcomes in patients with CHB [13,23-26]. Our findings help reconcile these apparently divergent observations. The key endpoint in the present study was not baseline viral replication or on-treatment HBV DNA suppression, but treatment-induced HBsAg clearance. Thus, lower viral replication activity or the mere presence of steatosis does not necessarily translate into a higher likelihood of PEG-IFN α -induced functional cure. Instead, severe steatotic burden may reflect a metabolic-inflammatory state that is unfavorable for HBsAg decline and immune-mediated antigen clearance.

An important observation is that the unfavorable association was confined mainly to severe MASLD. Mild and moderate MASLD were not significantly associated with reduced HBsAg clearance after weighting and multivariable adjustment. This pattern suggests that a certain threshold of steatotic burden or accompanying metabolic dysfunction may be required to meaningfully impair interferon responsiveness. Consistent with this interpretation, patients with three or more metabolic risk factors also had a lower likelihood of HBsAg clearance. Accordingly, severe hepatic steatosis may be better understood not as an isolated liver lesion, but as a visible marker of cumulative metabolic dysfunction. Previous CHB studies have shown that metabolic dysfunction and hepatic steatosis are closely associated with liver stiffness, fibrosis burden, and disease progression [24-26]. Together with our dose-response finding based on metabolic risk factor burden, these observations support the concept that systemic metabolic stress, rather than intrahepatic fat accumulation alone, may contribute to impaired PEG-IFN α responsiveness.

Several biological mechanisms may explain the reduced response in patients with severe MASLD. Excessive hepatic lipid accumulation is associated with lipotoxicity, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and chronic low-grade inflammation, all of which may alter the intrahepatic immune microenvironment and innate antiviral signaling [10,23]. More directly, transcriptomic profiling of human liver tissue has shown that comorbid MASH suppresses hepatic interferon-stimulated gene expression and macrophage gene signatures in patients with CHB [27]. In addition, CHB patients with MASLD may show differences in viral variants and immune biomarkers, further supporting altered host-virus-immune interactions in this population [28]. Because PEG-IFN α efficacy depends on intact interferon signaling and immune activation, severe MASLD may represent a host metabolic-immune state that is less permissive to interferon-mediated antiviral activity. Furthermore, severe metabolic dysfunction may influence viral antigen persistence by altering HBV transcriptional regulation, including the activity of cccDNA and integrated HBV DNA, which have been implicated in sustained HBsAg production after functional cure [29,30]. Although these mechanisms remain inferential in our study, they provide biological plausibility for the observed association between severe MASLD and impaired HBsAg kinetics.

These findings have practical implications for patient selection and monitoring in PEG-IFN α -based functional cure strategies. Pretreatment assessment of hepatic steatosis severity and metabolic risk burden may help identify patients with a lower probability of HBsAg clearance before initiating a finite 48-week treatment course. For patients with severe MASLD or high cumulative metabolic burden, clinicians may need to set more cautious expectations, monitor on-treatment HBsAg kinetics closely, and integrate antiviral therapy with metabolic risk management and fibrosis surveillance. This is particularly relevant because steatotic liver disease and metabolic dysfunction are increasingly common in CHB and may contribute to fibrosis burden and liver stiffness progression [24-26]. Our results also support the inclusion of MASLD severity and metabolic risk burden as stratification factors or adjustment variables in future trials evaluating finite or immune-based HBV cure strategies. Whether structured metabolic optimization before or during PEG-IFN α -based therapy can improve HBsAg decline or clearance remains an important question for prospective studies. This study has several strengths. It draws on a multicenter, clinically relevant cohort of HBeAg-negative patients with low baseline HBsAg levels who were favorable candidates for HBsAg clearance; focuses on HBsAg clearance, a high-value endpoint central to the functional cure paradigm; examines both steatosis severity and cumulative metabolic burden rather than a single dichotomous exposure; evaluates both categorical response endpoints and longitudinal HBsAg kinetics; and applies IPTW together with sequentially adjusted Cox

models to reduce confounding bias. The findings were further supported by sensitivity analyses after truncation of stabilized weights.

Several limitations should be acknowledged. First, this was an observational analysis of prospectively collected real-world data; therefore, residual confounding cannot be fully excluded despite IPTW and multivariable adjustment. Patients were derived from two source studies with partly different eligibility criteria, and prior or concurrent NA therapy may have introduced treatment-selection bias. Second, the metabolic burden score was clinically simple but coarse, without waist circumference, direct insulin-resistance measures, or longitudinal metabolic data. Third, steatosis severity was assessed by CAP rather than liver biopsy or magnetic resonance-based fat quantification. Although CAP is validated and practical for grading steatosis, it cannot distinguish simple steatosis from steatohepatitis and may be influenced by hepatic inflammation. Fourth, mechanistic analyses of interferon signaling, cytokine profiles, intrahepatic immune responses, cccDNA activity, and integrated HBV DNA-derived HBsAg production were not available. Finally, the 48-week follow-up precluded assessment of durability, late seroreversion, and long-term clinical outcomes, and subgroup sample sizes limited more granular analyses. Future studies should validate the cumulative metabolic burden framework and integrate metabolic, virological, and immune-phenotypic data to clarify the determinants of PEG-IFN α -induced functional cure.

In conclusion, severe MASLD was associated with unfavorable HBsAg kinetics and a significantly lower likelihood of HBsAg clearance in HBeAg-negative patients with CHB receiving PEG-IFN α -based therapy. These findings suggest that severe hepatic steatosis and metabolic risk burden may compromise interferon-based functional cure responses. Incorporating MASLD assessment into pretreatment evaluation may improve response stratification and guide more individualized HBV functional cure strategies.

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Statements & Declarations

Funding: This study was supported by Capital Clinical Diagnostic Techniques and Translational Application Projects (Z211100002921059), National Key Research and Development Program of Ministry of Science and Technology (2023YFC2308100), High-level Public Health Technical Talents Construction Project of Beijing Municipal Health Commission (Academic Leader-02-14), and Beijing Natural Science Foundation (No. 7222093).

Institutional Review Board Statement: Written informed consent was obtained from all participants prior to data collection. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee under protocol number 2022-067-K.

Acknowledgments: We acknowledge the patients for their participation in the present study. Additionally, we thank the contributions of all study participants for their support in patient recruitment, data collection, and follow-up.

Authors' contributions: Pengxuan Wu designed the study, analyzed the data, and drafted the manuscript. Jianxia Dong, Dacheng Sheng revised the manuscript. Lilin Wang, Xinyue Meng, Jing Zhao were responsible for patient recruitment and data provision. Sujun Zheng and Shan Ren supervised the study, provided critical revisions, and obtained funding. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest related to this study.

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