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Original Article

End-of-treatment HBsAg, HBcrAg and HBV RNA predict the risk of off-treatment ALT flares in chronic hepatitis B patients

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KEYWORDS

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Abstract *Background/Purpose(s):* Since ALT flares after therapy withdrawal are associated with adverse outcomes, risk stratification is of major importance. We aimed to study whether off-treatment flares are related with virological outcomes, and if serum levels of novel biomarkers at end-of-treatment (EOT) can predict flares.

Methods: Chronic hepatitis B patients who participated in three global randomised trials of peginterferon-based therapy were studied (99–01, PARC, ARES). HBV RNA, HBsAg and HBcrAg were quantified at EOT. Associations between EOT biomarker levels and flares were assessed as continuous data and after categorisation. Flares were defined as ALT $\geq 5 \times$ ULN during six months after therapy cessation.

Results: We included 344 patients; 230 HBeAg-positive and 114 HBeAg-negative. Patients were predominantly Caucasian (77.0%) and had genotype A/B/C/D in 23.3/7.3/13.4/52.3%. Flares were observed in 122 patients (35.5%). Flares were associated with lower rates of sustained response (3.5% vs 26.8% among patients with and without a flare; $p < 0.001$). Higher HBsAg (OR 1.586, 95%CI 1.231–2.043), HBV RNA (OR 1.695, 95%CI 1.371–2.094) and HBcrAg (OR

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1.518, 95%CI 1.324–1.740) levels were associated with higher risk of flares ($p < 0.001$). Combinations of biomarkers further improved risk stratification, especially HBsAg + HBV RNA. Findings were consistent in multivariate analysis adjusted for potential predictors including HBeAg-status and EOT-response (HBV DNA <200 IU/mL).

Conclusion: Off-treatment ALT flares were not associated with favourable virological outcomes. Higher EOT serum HBsAg, HBcrAg and HBV RNA were associated with a higher risk of flares after therapy withdrawal. These findings can be used to guide decision-making regarding therapy discontinuation and off-treatment follow-up.

Trial registration: ClinicalTrials.gov: NCT00114361, NCT00146705, NCT00877760.

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Abbreviations

ALT	alanine aminotransferase
cccDNA	covalently closed circular DNA
cDNA	complementary DNA
CHB	chronic hepatitis B
c/mL	copies/millilitre
ETV	entecavir
HBV	hepatitis B virus
HBcrAg	hepatitis B core-related antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
IU/mL	international units/millilitre
LAM	lamivudine
LOD	lower limit of detection
NA(s)	nucleos(t)ide analogue(s)
EOT	end-of-treatment
OR	odds ratio
PCR	polymerase chain reaction
PEG-IFN	peginterferon
RACE	rapid amplification of cDNA ends
RBV	ribavirin
SR	sustained response
ULN	upper limit of normal
U/mL	units/millilitre

Introduction

First-line treatment options for chronic hepatitis B (CHB) patients comprise pegylated-interferon (PEG-IFN) and nucleos(t)ide analogues (NAs). The main goal of antiviral treatment is off-treatment sustained suppression of viral replication or HBsAg loss, thereby limiting hepatic complications, such as progression to liver cirrhosis and hepatocellular carcinoma (HCC).¹ Unfortunately, treatment withdrawal in HBsAg-positive patients may result in viral rebound, which has been associated to the occurrence of ALT flares.^{2,3} Whether such ALT flares are beneficial or harmful for the host is a contentious issue because they have been associated with increased sustained response rates as well as a higher risk of subsequent liver decompensation.^{4,5}

Given the potential risk of adverse outcomes with off-treatment ALT flares, risk stratification is of major

importance. The occurrence of ALT flares has been reported to be associated with both host-related characteristics, such as age and sex, and viral factors, such as HBeAg-status and end-of-treatment HBV DNA levels.^{4,6} The recently identified serum biomarkers hepatitis B virus (HBV) RNA and hepatitis B core-related antigen (HBcrAg), and hepatitis B surface antigen (HBsAg) reflect covalently closed circular DNA (cccDNA) transcriptional activity, and consequently, intrahepatic viral replication.^{7–9} These factors could therefore also be related to the risk of off-treatment flares.

In this study, we therefore aimed to study (1) the relationship between off-treatment ALT flares and virological outcomes and (2) whether EOT levels of HBsAg, HBcrAg and HBV RNA can be used to predict the risk of ALT flares.

Patients and methods

Study population

In this study we included chronic hepatitis B (CHB) patients who participated in three global randomised controlled trials (the 99–01, PARC and ARES studies). Trial design and inclusion criteria have been described in detail elsewhere.^{10–12} In short, in the 99–01 study HBeAg-positive patients ($n = 266$) were randomised to receive either 100 μ g/week PEG-IFN alpha-2b mono-therapy or PEG-IFN plus 100 mg/day lamivudine combination-therapy for 52 weeks.¹⁰ The PARC study enrolled HBeAg-negative patients ($n = 133$), who were randomised for 180 μ g/week PEG-IFN alpha-2a monotherapy or PEG-IFN plus 1000–2000 mg ribavirin combination therapy for 48 weeks.¹¹ In the ARES study, HBeAg-positive patients ($n = 175$) started with 0.5 mg/day entecavir (ETV) monotherapy and were subsequently randomised to either PEG-IFN alpha-2a add-on therapy from week 24 to week 48 ($n = 85$) or continuing ETV ($n = 90$). Responders (defined as HBeAg loss and HBV DNA <200 IU/mL at week 48) continued with ETV consolidation therapy until week 72, after which treatment was ceased.¹² For this study, we included only those patients with PEG-IFN add-on therapy, who received ETV consolidation therapy until week 72 (i.e. those who discontinued therapy). The original study protocols have been approved by the medical ethical committees and are in line with the Declaration of Helsinki of 1975.

For the current analysis, only patients with EOT data on at least one biomarker (HBsAg, HBcrAg and/or HBeAg) were

eligible for enrolment. Since the risk of an off-treatment flare is negligible in patients with HBsAg loss, we excluded patients who were HBsAg negative at EOT (Fig. 1).

Endpoints

An off-treatment flare was defined as an increase of serum ALT five times the ULN within six months after EOT.^{4,13} The time point of a flare was defined at the peak value of the ALT rise. If a patient experienced more than one flare, the first one was used for classification. An early flare was defined as a flare that occurred within 12 weeks after EOT, whereas a late flare was defined as a flare that occurred beyond 12 weeks after EOT. Sustained response (SR) was defined as HBV DNA <2000 IU/mL six months after treatment cessation. HBsAg loss was assessed at end of follow-up and during long term follow-up.^{10,11,14} EOT response was defined as patients who had suppressed HBV DNA (<200 IU/mL) levels at EOT, in line with the original ARES study protocol.¹²

Laboratory measurements

Serum HBV RNA, HBsAg, HBcrAg were measured at EOT and during follow-up. HBV DNA and ALT were quantified at EOT and, in most patients, every four weeks during the six months follow-up period. HBV RNA was quantified using rapid amplification of complementary DNA (cDNA)-ends (RACE)-based real-time polymerase chain reaction (University Hospital Leipzig, Germany). This technique has been described in detail elsewhere.¹⁵ The assays' lower limit of detection (LOD) was 800 copies/millilitre (c/mL).¹⁶ Quantification of HBsAg was performed using Abbott Architect (Abbott, Abbott Park, IL), with a LOD of 0.05 IU/mL. HBcrAg was quantified using Lumipulse® G HBcrAg-assay (Fujirebio Europe) according to the manufacturer's instructions, with a lower limit of quantification (LOQ) of 1000 U/mL (3 log) and lower limit of detection of 2 log.¹⁷ The LOD for HBV DNA was 400 copies/mL (~80 IU/mL, in-house TaqMan PCR assay, Rotterdam, the Netherlands) for 99–01,¹⁰ 35 copies/mL (Taqman, Roche Diagnostics, Basel, Switzerland) for PARC¹¹ and 20 IU/mL (Cobas TaqMan 48, Roche Diagnostics, Basel, Switzerland) for ARES¹² participants. ALT, prothrombin time and bilirubin tests were performed by automated techniques at the participating centres.^{10–12}

Statistical analysis

Descriptive data were described as numbers (with percentages), medians (with interquartile range; IQR) and means (\pm standard deviation; SD). Associations between biomarker levels and ALT flares were assessed using continuous data (with associations assessed using logistic regression and AUROC) and after categorisation (<3 log versus >3 log for HBsAg, undetectable versus detectable for HBV RNA, and for HBcrAg <3 log versus >3 log for HBeAg-negative and <6 log versus >6 log for HBeAg-positive patients; based on mean levels at EOT). HBsAg and HBcrAg were also combined by calculating the previously reported SCALE-B score, calculated as $35 \times \text{HBsAg (log IU/mL)} + 20 \times \text{HBcrAg (log U/mL)} + 2 \times \text{age (year)} + \text{ALT (U/L)}$. This scoring

system has been developed to predict the risk of a clinical relapse in patients with finite NA therapy.¹⁸ For this score HBcrAg levels of 2 log were recoded to 1 log, in compliance with the original report.¹⁸

Associations between novel biomarkers and off-treatment outcomes (flares and SR) were also assessed using multivariate logistic regression, for which each biomarker was entered into a model comprising age, sex, HBV genotype A, EOT response, ALT at EOT, and HBeAg-status at baseline. Differences were considered statistically significant when $p < 0.05$. IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) was used for graphical representation of the results.

Results

Patient characteristics

In total, 344 patients were included (Table 1, Fig. 1); 230 HBeAg-positive and 114 HBeAg-negative. An EOT response was observed in 147 patients (57.1%).

Off-treatment ALT flare characteristics

An off-treatment ALT flare was observed in 122 patients (35.5%); median ALT level at the peak of the flare was $9.4 \times$ the ULN (IQR 6.9–16.4). In 74 patients (60.7%) the peak of the flare occurred ≥ 12 weeks after EOT (i.e. a late flare; median 12 weeks after EOT, IQR 8–20). Among the 122 patients with an off-treatment ALT flare, 38 patients (31.1%) experienced concomitant bilirubin elevation, with a median of $24 \mu\text{mol/mL}$ (range 11–152). A bilirubin level $>50 \mu\text{mol/mL}$ was observed in seven patients (5.7%). Prothrombin time elevation was observed in 20 patients (range $1.01\text{--}10.4 \times$ ULN). None of the patients developed encephalopathy.

Off-treatment ALT flares are associated with lower rates of sustained response and HBsAg loss

Occurrence of an off-treatment flare was associated with a lower probability of SR; 4/113 patients (3.5%) with a flare achieved SR compared to 53/198 (26.8%) patients without a flare (OR 0.100, 95% CI 0.035–0.286, $p < 0.001$). Findings were consistent among the subgroup of patients with an EOT response; 0% of the patients with an off-treatment flare achieved SR compared to 40.0% without a flare ($p < 0.001$). Similarly, occurrence of a flare was not associated with a more pronounced off-treatment HBsAg decline, and none of the patients with an off-treatment ALT flare achieved HBsAg loss during follow-up (Table 1).

Associations between viral biomarkers and off-treatment ALT flares

HBsAg

Higher HBsAg levels at EOT were associated with a higher risk of off-treatment ALT flares (OR 1.586, 95% CI 1.231–2.043,

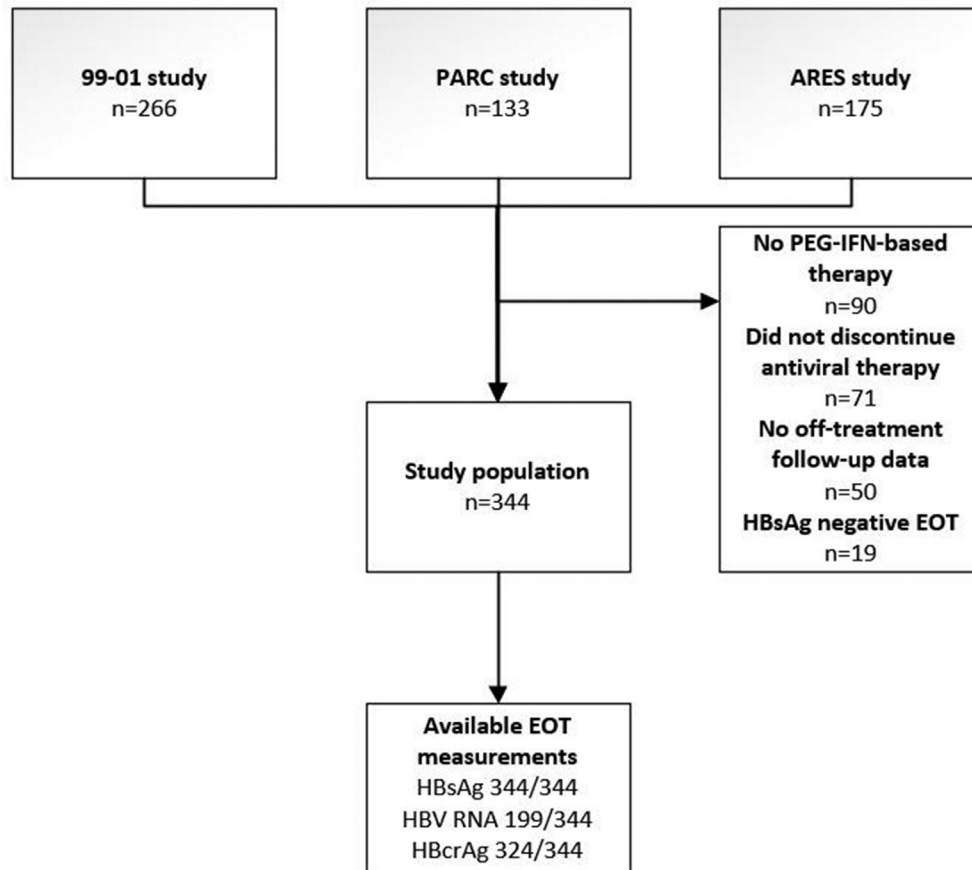


Figure 1. Flowchart. Abbreviations: ALT, alanine aminotransferase; EOT, end-of-treatment; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen; HBeAg, HBsAg, quantitative hepatitis B surface antigen; PEG-IFN, peginterferon.

$p < 0.001$; AUROC 0.608, 95% CI 0.548–0.668, $p = 0.001$). Among the 261 patients with HBsAg levels of >3 log, 107 patients (41.0%) experienced a flare. In contrast, only 15/83 patients (18.1%) with HBsAg levels of <3 log experienced a flare ($p < 0.001$, Fig. 2).

Conversely, higher HBsAg levels at EOT were associated with a lower risk of SR (OR 0.480, 95% CI 0.371–0.621, $p < 0.001$; AUROC 0.237, 95% CI 0.171–0.304, $p < 0.001$). SR was achieved in 25/234 (10.7%) versus 32/77 (41.6%) patients with HBsAg levels of >3 log versus <3 log at EOT ($p < 0.001$, Fig. 3). HBsAg loss was exclusively observed in patients with HBsAg levels of <3 log (7.3% versus 0.0% in patients with HBsAg >3 log, $p < 0.001$).

Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Fig. 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figs. 2–5).

HBV RNA

Higher HBV RNA levels at EOT were associated with an increased risk of off-treatment ALT flares (OR 1.695, 95%CI 1.371–2.094, $p < 0.001$; AUROC 0.726, 95%CI 0.645–0.807, $p < 0.001$). Amongst the 72 patients with detectable HBV RNA levels, 39 patients (54.2%) experienced a flare compared to 18/127 patients (14.2%) with undetectable HBV RNA levels ($p < 0.001$, Fig. 2).

Conversely, higher HBV RNA levels at EOT were associated with a lower risk of SR (OR 0.119, 95%CI 0.017–0.807, $p = 0.029$; AUROC 0.278, 95%CI 0.202–0.354, $p < 0.001$). SR was achieved in 2/68 (2.9%) patients with detectable HBV RNA, versus 35/107 (32.7%) patients with undetectable HBV RNA levels at EOT ($p < 0.001$, Fig. 3). HBsAg loss was exclusively observed in patients with undetectable HBV RNA levels (2.5% versus 0.0% in patients with detectable levels, $p = 0.184$).

Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Fig. 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figs. 2–5).

HBcrAg

Higher HBcrAg levels were associated with off-treatment ALT flares (OR 1.518, 95% CI 1.324–1.740, $p < 0.001$; AUROC 0.720, 95% CI 0.663–0.777, $p < 0.001$). Amongst the 185 patients (57.1%) with high HBcrAg levels (>3 log for HBeAg-negative and >6 log for HBeAg-positive), 87 patients (47.0%) experienced an off-treatment flare. On the contrary, only 26/139 patients (18.7%) with low HBcrAg levels (<3 log for HBeAg-negative and <6 log for HBeAg-positive patients) experienced a flare ($p < 0.001$, Fig. 2).

Conversely, higher HBcrAg levels at EOT were associated with a lower risk of SR (OR 0.658, 95%CI 0.565–0.767,

Table 1 Patient characteristics.

Characteristics	Flare ^g (n = 122)	No flare ^g (n = 222)	p-value
Age at inclusion, years (median, IQR)	35 (28–44)	34 (26–46)	0.930
Male (n, %)	86 (70.5)	173 (77.9)	0.126
Race (n, %)			
Caucasian	92 (75.4)	173 (77.9)	0.588
Asian	22 (18.0)	40 (18.0)	
Other	8 (6.6)	9 (4.1)	
HBV genotype (n, %)			
A	20 (16.4)	60 (27.0)	0.232
B	8 (6.6)	17 (7.7)	
C	18 (14.8)	28 (12.6)	
D	71 (58.2)	109 (49.1)	
Other	5 (4.1)	8 (3.6)	
Study treatment (n, %)			
PEG-IFN mono	61 (50.0)	105 (47.3)	0.108
PEG-IFN + LAM	42 (34.4)	65 (29.3)	
PEG-IFN + RBV	18 (14.8)	39 (17.6)	
PEG-IFN + ETV	1 (0.8)	13 (5.9)	
Laboratory results at end of treatment			
HBeAg status EOT, positive (n, %)	74 (60.7)	67 (30.2)	< 0.001
ALT (median, IQR) ^a	1.7 (1.0–2.7)	1.1 (0.8–1.7)	< 0.001
HBV DNA ^c (mean, ±SD)	5.1 (2.7)	3.1 (2.4)	< 0.001
HBV RNA ^b (mean, ±SD)	4.4 (1.6)	3.2 (1.3)	< 0.001
HBsAg ^e (mean, ±SD)	3.8 (0.8)	3.4 (1.3)	< 0.001
HBcrAg ^d (mean, ±SD)	6.4 (1.7)	4.9 (2.0)	< 0.001
Treatment response			
EOT response ^e	30 (24.6)	117 (52.9)	< 0.001
Sustained response ^f (n, %)	4/113 (3.5)	53/198 (26.8)	< 0.001
HBsAg loss	0 (0)	6/209 (2.9)	0.061

^a times the upper limit of normal (ULN), U/L.

^b Logarithmic scale, c/mL.

^c Logarithmic scale, IU/mL.

^d Logarithmic scale, U/mL.

^e EOT response is defined as HBV DNA <200 IU/mL at end of treatment.

^f Sustained response is defined as HBV DNA <2000 IU/mL six months after end of treatment.

^g Flare is defined as ALT >5× the upper limit of normal after end-of-treatment.

Abbreviations: HBV, hepatitis B virus; PEG-IFN, peginterferon; LAM, lamivudine; RBV, ribavirin; ETV, entecavir; HBcrAg, Hepatitis B core related Antigen; HBeAg, Hepatitis B e Antigen; HBsAg, quantitative hepatitis B surface antigen; EOT, end-of-treatment; IQR, inter-quartile range. † HBV DNA <200 IU/mL at end-of-treatment.

$p < 0.001$; AUROC 0.240, 95%CI 0.181–0.299, $p < 0.001$). SR was achieved in 42/125 (33.6%) with low HBcrAg levels versus 14/179 (7.8%) in patients with high HBcrAg levels ($p < 0.001$, Fig. 3). HBsAg loss was exclusively observed in patients with low HBcrAg levels (4.4% versus 0.0% in patients with high levels, $p = 0.004$).

Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Fig. 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figs. 2–5).

Combinations of biomarkers may further stratify ALT flare risk

HBsAg and HBV RNA

Among the 69 patients with both detectable HBV RNA levels and HBsAg >3 log, 36 patients (52.2%) experienced an off-

treatment ALT flare. In contrast, none of the 42 patients (0.0%) with undetectable HBV RNA levels and HBsAg <3 log experienced a flare ($p < 0.001$, Fig. 2). Findings were consistent among patients with an EOT response; 62.5% of the patients with concomitant elevated levels of HBV RNA and HBsAg experienced a flare, compared to 0.0% of whom both biomarkers were low ($p < 0.001$).

Conversely, higher concomitant HBsAg and HBV RNA levels at EOT were associated with a lower risk of SR. SR was achieved in 23/36 (63.9%) with low levels versus 2/65 (3.1%) in patients with high levels ($p < 0.001$, Fig. 3). HBsAg loss was exclusively observed in patients with low levels of concomitant HBsAg and HBV RNA (7.3% versus 0.0% in patients with high levels, $p = 0.004$).

HBsAg and HBcrAg: SCALE-B

Higher SCALE-B scores were associated with higher risk of an off-treatment flare. Among the 218 patients with a

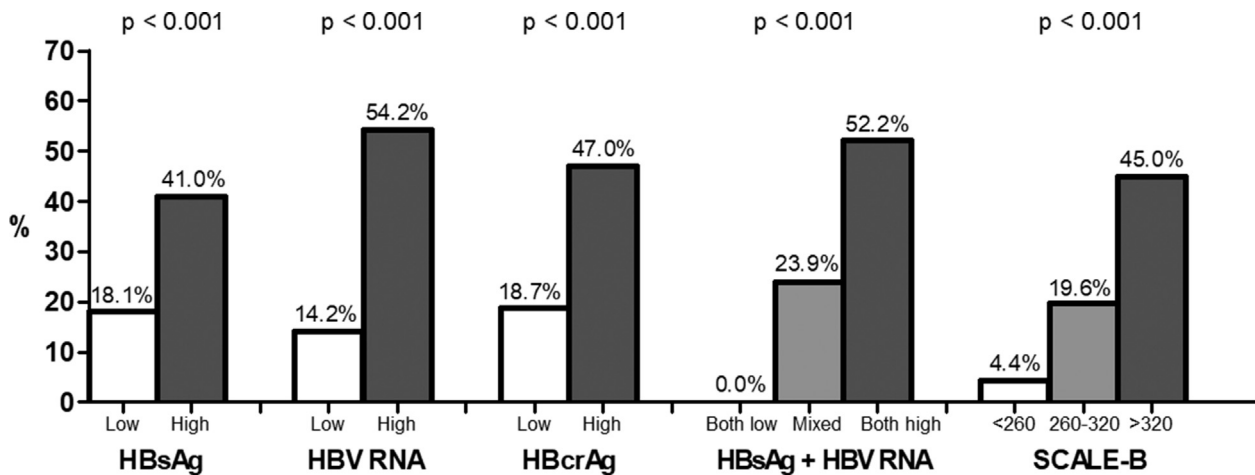


Figure 2. Rates of off-treatment ALT flares ($ALT \geq 5x$ ULN) in the overall cohort, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment. Biomarker levels were categorised as low versus high for HBsAg (<3 log versus >3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (<3 log versus >3 log for HBeAg-negative and <6 log versus >6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg <3 log and undetectable HBV RNA), both high (HBsAg >3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

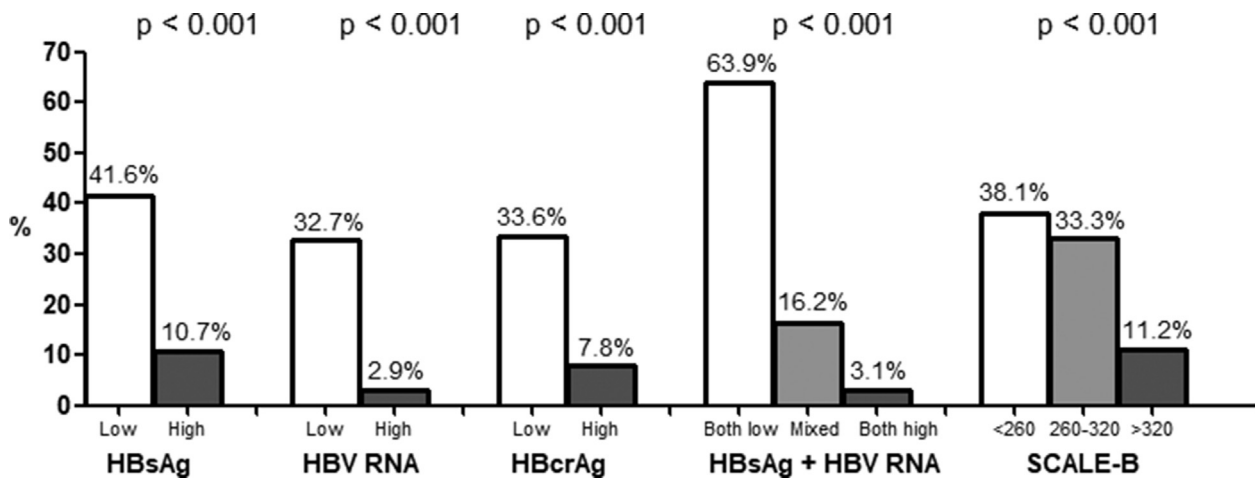


Figure 3. Rates of sustained response (HBV DNA < 2000 IU/mL six months after treatment withdraw) in the overall cohort, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment. Biomarker levels were categorised as low versus high for HBsAg (<3 log versus >3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (<3 log versus >3 log for HBeAg-negative and <6 log versus >6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg <3 log and undetectable HBV RNA), both high (HBsAg >3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

SCALE-B score of ≥ 320 , 98 patients (45.0%) experienced an off-treatment flare. In contrast, a flare was observed in 2/45 patients (4.4%) with a SCALE-B score of <260 ($p < 0.001$, Fig. 2). A similar trend was observed among patients with an EOT response; 20/58 patients (34.5%) with a SCALE-B score of ≥ 320 experienced a flare, compared to 2/39 patients (5.1%) with a SCALE-B score of <260 ($p = 0.001$).

Conversely, higher SCALE-B score at EOT was associated with a lower risk of SR. SR was achieved in 16/42 (38.1%) with a SCALE-B score of <260 versus 23/206 (11.2%) in patients with a SCALE-B score of ≥ 320 ($p < 0.001$, Fig. 3). HBsAg loss was exclusively observed in patients with SCALE-

B score of <320 (6.0% versus 0.0% in patients with levels of ≥ 320 , $p < 0.001$).

Discussion

Off-treatment ALT flares are frequently observed after therapy withdrawal in patients with CHB, with previous studies hinting at a possible beneficial effect.^{2,5} This study, a pooled analysis of three randomised controlled trials, demonstrates that off-treatment flares were not associated with increased rates of HBsAg decline or sustained response. Higher EOT levels of HBsAg, HBcrAg and HBV RNA

Table 2 Multivariate analysis.

	Flare			Sustained response		
	aOR	95%CI	p-value	aOR	95%CI	p-value
HBsAg ^a	1.386	1.041–1.845	0.025	0.562	0.423–0.745	<0.001
HBV RNA ^a	1.494	1.129–1.976	0.005	0.127	0.017–0.919	0.041
HBcrAg ^a	1.517	1.208–1.905	<0.001	0.476	0.332–0.681	<0.001

^a Adjusted for age, sex, genotype A, EOT response (HBV DNA <200 IU/mL), ALT at EOT, and HBeAg-status at baseline. Abbreviations: HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; aOR, adjusted odds ratio.

were associated with higher risk of ALT flares and a lower risk of sustained response or HBsAg loss. These biomarkers could therefore be used to stratify relapse risk in patients being evaluated for therapy discontinuation.

Rapid increases in ALT levels, also known as flares, can occur during the natural course of the chronic HBV infection, or in relationship with (discontinuation of) antiviral therapy. It has been debated whether rise in ALT after antiviral therapy withdrawal could be beneficial. This concept is illustrated by a study by Hadziyannis and colleagues, who observed an off-treatment ALT rise in 76% patients of the patients who discontinued NA treatment, with a high subsequent rate of HBsAg loss (20%) within the first year of follow-up, suggesting a beneficial effect of an ALT flare.⁵ In addition, on-treatment flares during PEG-IFN therapy have been associated with rapid subsequent HBsAg decline and clearance.⁶ However, such a benefit may be restricted to patients with a host-dominant flare (i.e. a flare not preceded by rapid HBV DNA increase),^{6,19} and the benefit of off-treatment flares remains uncertain. The findings from the current study provides no support for the hypothesis that off-treatment ALT flares lead to favourable virological outcomes.

Given the absence of sufficient evidence that off-treatment ALT flares increase the chances of treatment response, it is important to consider that ALT flares may also be harmful.^{4,19–21} Hepatic decompensation, some with fatal outcome, have been described in patients that discontinued NAs and experienced rise in ALT, particularly in patients with more advanced liver disease.^{22,23} Moreover, severe off-treatment ALT flares are not restricted to finite NA therapy, but are also observed in trials with PEG-IFN therapy or novel HBV agents such as nucleic acid polymers (NAPs).^{21,24,25} Thus, ALT flares are commonly observed during or after finite treatment with all different antiviral therapies, making risk stratification essential.⁴ Identification of patients at high risk of flares may trigger intensive post-treatment follow-up and early retreatment, which might help prevent hepatic decompensation or other fatalities.

Since HBV DNA levels are often low or undetectable in patients considered eligible for finite treatment, other biomarkers are required for risk stratification. In the last couple of years, several more serum biomarkers have been identified, including quantitative HBsAg, HBV RNA and HBcrAg. These serum biomarkers might reflect intrahepatic viral replication in various degrees during the different phases of HBV infection, through associations with the cccDNA.^{7–9} Therefore, these biomarkers may serve as marker for intrahepatic transcriptional activity and

consequently predict off-treatment sustained response and the risk of flares. In our study, higher levels of these biomarkers were associated with higher risk of flares and lower risk of sustained response or HBsAg loss. These associations were sustained in multivariate analysis and were consistent across subgroups. Albeit the biomarkers are interrelated, a combination of low biomarker levels identified patients with the highest likelihood of favourable outcomes after therapy cessation. Our findings are consistent with previous smaller studies that only studied viral antigens and/or HBV RNA.²⁶

Our findings may have important clinical implications, as they can be used both to select patients most likely to achieve sustained viral suppression (or HBsAg loss) after therapy withdrawal, and to identify patients in need of careful monitoring. Furthermore, the observation that individual biomarkers are also able to predict off-treatment outcomes suggests that they should be evaluated in studies of novel antivirals that target specific parts of the HBV replication cycle and therefore have profound effects on some, but not all biomarkers (e.g. capsid assembly modulators [CAMs], which have a profound effect on HBV DNA and RNA, but less so on HBcrAg and HBsAg).^{27,28}

Strengths of this study include the large cohort of both HBeAg-positive and–negative patients who participated in three global randomised controlled trials. In addition, since ALT levels were quantified in the majority of patients every four weeks during a follow-up period of six months, we were able to identify a large number of off-treatment ALT flares. Also, since HBcrAg and HBV RNA levels are frequently low or undetectable in HBeAg negative patients, more sensitive assays may further improve predictive performance. This also applies to HBV RNA, which is frequently below the LOD in virally suppressed patients. Finally, our results are based on patients treated with PEG-IFN (+/–NA) therapy. Validation in NA treated patients and/or patients treated with combination regimens containing novel antivirals is warranted. In addition, despite the large number of patients in the overall cohort, stratification resulted in a limited number of patients per subgroup. Nevertheless, subgroup analysis across HBeAg-status, treatment regime and EOT response categories showed homogeneous results (Supplementary Figs. 1–3) with the overall population, supporting the robustness of our findings.

In conclusion, our study demonstrated that off-treatment ALT flares were not associated with favourable virological outcomes and should therefore be considered an undesirable event. Higher levels of HBV RNA, HBcrAg and HBsAg at EOT are associated with higher risk of flares. These findings may be used to select patients most likely to

achieve sustained response or HBsAg loss after therapy discontinuation, and to identify patients eligible for intensive post-treatment follow-up.

Funding statement

This work was supported by the Foundation for Liver and Gastrointestinal Research, Rotterdam, the Netherlands. HBcrAg testkits were provided free of charge by Fujirebio.

Ethics approval statement

The original study protocols have been approved by the medical ethical committees and are in line with the Declaration of Helsinki of 1975.

Patient consent statement

Patients provided written consent for the original study protocols.

Data availability statement

Data not publicly available.

Consent for publication

All authors reviewed and approved the final manuscript.

Author's contributions

MS, SB, RdM and HLAJ conceived the study. SB and MS performed statistical analysis. SB and MS made graphic images, interpretation of data and revision of the manuscript. SB and MS wrote the manuscript, which was revised by all authors. All authors reviewed and approved the final manuscript.

Declaration of competing interest

SB received an unrestricted research grant from Gilead. AB has been received research grants from Roche, Gilead Sciences, Fujirebio, and Janssen. RdK has received honoraria for consulting/speaking from Gilead, Janssen, AbbVie, and Norgine and received research grants from Gilead and Janssen. FvB has received research support and provided consultancy for Roche. TB has received research support, consulting and/or speaking fees from Gilead, Roche, Merck, AbbVie, Bristol-Myers Squibb, and Janssen. BH has received research support and consultancy fees from Intercept, Cymabay, Genfit, Mirum, Albireo, Calliditas and Chemomab. HJ has received research support, consulting and/or speaking fees from Gilead, Roche, Merck, AbbVie, Bristol-Myers Squibb, Arbutus, Janssen and MedImmune. MS has received speaker's fees and research support from Roche, Innogenetics, BMS, Gilead and Fujirebio. The other authors report no disclosures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2022.06.002>.