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Pretreatment Hepatitis B Viral Load Predicts Long-Term Hepatitis B Response After Anti-Hepatitis C Therapy in Hepatitis B/C Dual-Infected Patients

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Background. We aimed to investigate the long-term outcomes in hepatitis B (HBV)/hepatitis C virus (HCV) dual-infected patients after anti-HCV therapy.

Methods. A total of 192 HBV/HCV dual-infected patients who had received pegylated interferon treatment were recruited. The investigation outcomes included HBV DNA ≥2000 IU/mL, with or without alanine aminotransferase (ALT) ≥2-fold the upper limit of normal, and hepatitis B surface antigen (HBsAg) seroclearance.

Results. Four (2.1%) patients developed early HBV reactivation before the end of treatment. Fifty (26.6%) of the remaining patients had an episode of HBV DNA ≥2000 IU/mL in a mean follow-up of 68.8 months. The risk was 4.6 per 100 person years. Only 19 (10.1%) patients developed concomitant ALT flare with oral HBV antiviral therapy; the risk was 1.7 per 100 person years. Despite HBV flare, 67 (34.9%) patients had a favorable outcome of HBsAg seroclearance. The probability was 5.7 per 100 person years. A pretreatment HBV DNA level of 300 IU/mL served as an independent predictor for all the outcomes. The combined pretreatment HBV DNA level and HCV response further enhanced the prediction of HBV flare and HBsAg seroclearance.

Conclusions. A pretreatment HBV DNA level of 300 IU/mL predicts HBV flare and HBsAg seroclearance after anti-HCV therapy.

Keywords. hepatitis B; hepatitis C; dual infection; flare; hepatitis B surface antigen; seroclearance.

A higher risk of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) has been identified in patients with hepatitis B virus (HBV) and hepatitis C virus (HCV) dual infection [1, 2]. Due to aggressive liver disease, management of HBV and HCV dual infection has become especially important. Antiviral therapy with pegylated interferon (peg-IFN) and ribavirin has shown efficacy for HCV eradication in both HBV/HCV dual-infected and HCV monoinfected patients [3]. The new HCV antiviral therapy, direct acting antivirals (DAA), demonstrates a high efficacy and low risk compared to IFN-based treatment [4, 5]. However, a previous study that showed compatible efficacy of DAA therapy in HBV/HCV dual-infected patients and HCV monoinfected patients had only a small sample of patients [6].

In patients with HBV/HCV dual infection, HBV was frequently thought to be suppressed by HCV. The interaction between these two viruses is particularly interesting. Studies of IFN-based therapy have identified diverse HBV responses. In a multicenter trial from Taiwan, a subset of the patients who were hepatitis B e antigen (HBeAg)-negative and HBV/HCV dual-infected had detectable HBV DNA before treatment that became undetectable after IFN therapy. However, there was also a high percentage (36.4%) of patients who had HBV reappearance after treatment [3]. A following study demonstrated similar results with 19.1% of patients having HBV reappearance at 6 months after treatment [7]. Alternatively, a small group of patients (9%–11%) may have the ultimate HBV response, which consists of hepatitis B surface antigen (HBsAg) loss after IFN therapy. The HBsAg loss may even reach 30% of patients after 5 years [8].

DAA did not result in the favorable responses of HBV suppression and HBsAg loss. Instead, episodes of HBV reactivation and even related hepatic failure were the most reported events after DAA therapy in patients with HBV and current or past chronic HCV infection [9–11]. Four (57.1%) and 1 (14.3%) of 7 HBV/HCV dual-infected patients had HBV virological and clinical reactivation, respectively, after DAA therapy [12]. Another report, using stricter criteria, still demonstrated as high as

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16.7% HBV virological reactivation cases [13]. Although a variety of risks for HBV reactivation were reported, none reported a favorable HBV response after DAA therapy.

Although short-term HBV responses have been widely reported, the long-term HBV responses, especially HBV reactivation after IFN or DAA therapy, remain uncertain. Herein, we aimed to investigate the long-term responses of HBV after IFN therapy, including viral reactivation and HBsAg loss, in patients with HBV/HCV dual infection. We also aimed to investigate the factors associated with the long-term responses.

METHODS

From October 1995 to December 2013, more than 4005 chronic hepatitis C patients were treated with IFN-based therapy in our hospital, a tertiary hospital in southern Taiwan. Of these, 249 patients who were dually infected with HBV and HCV were evaluated. After excluding 57 patients who lacked posttreatment follow-up data, a total of 192 HBV/HCV dual-infected patients were finally recruited. The patient allocation flowchart is shown in Figure 1. The inclusion criteria were: (1) aged ≥ 20 years; (2) seropositive for HBsAg, HCV antibodies, and HCV RNA for more than 6 months; (3) completion of IFN-based therapy; and (4) naive for HBV nucleot(s)ide analogue (NUC) therapy before treatment. The exclusion criteria were: (1) human immunodeficiency virus infection; (2) another liver disease apart from HBV and HCV infection, such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson disease; and (3) any other known disease that was not suitable for IFN therapy. All the patients received Peg-IFN α-2a 180 μg or Peg-IFN α-2b 1.5 μg/kg weekly and combination therapy with ribavirin 1000–1200 mg/day. For patients who received treatment before 2008, the treatment duration was 48 weeks for HCV genotype 1 and 24 weeks for genotype 2. After 2008, treatment duration was 24 weeks for genotype 1 and 16 weeks for genotype 2 with rapid virological response (RVR, defined as HCV RNA <50 IU/mL at the fourth week of therapy), and 48 weeks for genotype 1 and 24 weeks for genotype 2 without RVR. The study was conducted according to the Declaration of Helsinki guidelines and Good Clinical Practice principles and was approved by the local ethics committees. Written informed consent was obtained from all patients.

The HBsAg and HBeAg levels were tested by commercially available enzyme-linked immunosorbent assay kits (Abbott Laboratories, North Chicago, IL). Serum HBV DNA level and HBsAg were measured every year, as long as no associated HBV virological reactivation or liver enzyme elevation beyond the upper limit of normal (ULN) was present. HBV DNA level was measured once the aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels were elevated beyond the ULN. The AST/ALT level were measured every 3 months after completion of therapy. The quantification of HBV DNA was by the CobasAmpliPrep/CobasTaqMan HBV assay, with a dynamic range of 20–1.7 × 10^8 IU/mL (CAP/CTM Version 2.0, Roche Diagnostics, Indianapolis, IN). Anti-HCV was determined by a third-generation enzyme immunoassay (Abbott Laboratories). HCV RNA was measured by a qualitative polymerase chain reaction assay, with a detection limit of 50 IU/mL (CobasAmplicor Hepatitis C Virus Test, Version 2.0; Roche Diagnostics, Branchburg, NJ). HCV genotypes were determined by the method described by Okamoto et al [14].

Liver cirrhosis was diagnosed by either histology or ultrasound diagnosis combined with evidence of portal hypertension,

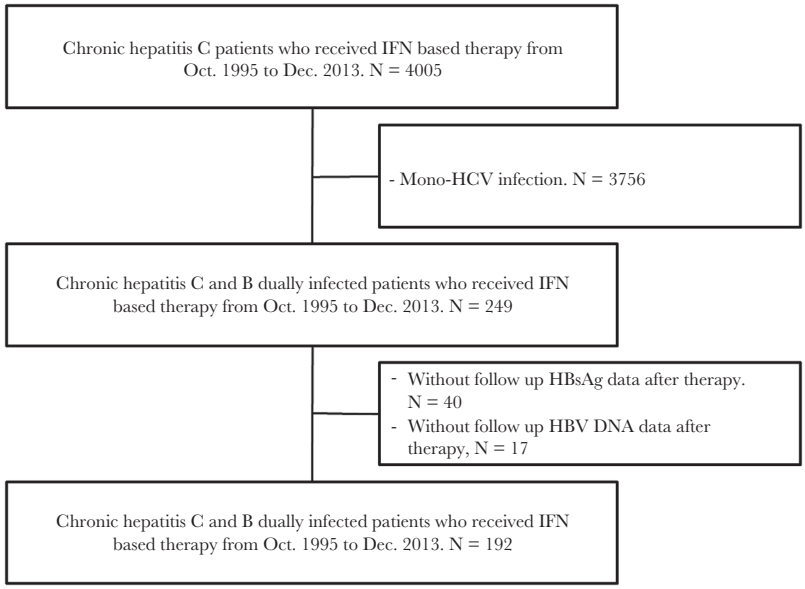

Figure 1. Patient allocation flowchart.

such as splenomegaly, or esophageal or gastric varices. The HCC diagnosis was made by 2 radiological imaging modalities showing the typical pictures of HCC, 1 radiological imaging modality showing the typical pictures of HCC combined with a serum alpha-fetoprotein level ≥ 400 ng/mL, or cytological/histological diagnosis of HCC.

The primary endpoint of the study was the risk of HBV DNA ≥2000 IU/mL, with or without ALT level ≥2-fold ULN after IFN therapy. The secondary endpoints included the rates of HBsAg seroclearance/seroconversion, relapse of HCV after achieving HCV sustained virological response (SVR_{24}), and endpoint-associated factors.

Statistics

Continuous variables are expressed as the means ± standard deviation or medians (interquartile ranges), and categorical variables are expressed as numbers (percentages). Cox proportional hazards regression models were used to identify factors that might relate to HBV virological/clinical flare and HBsAg loss. The cumulative probability of HBV flare and HBsAg loss were analyzed by the Kaplan-Meier actuarial curve method with the log-rank test. All tests were 2-sided, and a P value < .05 was considered to be statistically significant. All analyses were performed by the SPSS 19.0 statistical package (SPSS, Inc., Chicago, IL).

RESULTS

The demographics of all patients are shown in Table 1. Of all the patients, 125 (65.1%) patients were male, with a mean age of 50.5 ± 11.3 years. Thirty-three (17.2%) and 28 (14.6%) of the patients had LC and HCC before therapy, respectively. The mean platelet count was 159.7 ± 55.3 × 10^3/mm^3. The median AST and ALT levels were 65.0 and 88.0 U/L, respectively. More than half (57.3%) of the patients were HCV genotype 1, with a mean viral load of 5.3 ± 1.1 $\log_{10}$ IU/mL. Seven (3.6%) of the patients had a positive HBeAg before therapy. Pretreatment HBV DNA levels were obtained in 163 patients. The median pretreatment HBV DNA level was 2.0 $\log_{10}$ IU/mL. The pretreatment HBV DNA was undetectable in 63.8% of patients. HBV DNA levels <2000 and ≥2000 IU/mL were found in 17.2% and 19.0% of the patients, respectively.

Table 1. Demographics of All IFN-Treated HBV/HCV Dual-Infected Patients

Parameter	Value (N = 192)
Age, y, mean ± SD	50.5 ± 11.3
Male gender, No. (%)	125 (65.1)
Liver cirrhosis, No. (%)	33 (17.2)
HCC, No. (%)	28 (14.6)
Platelets, 10^3/mm^3, mean ± SD	159.7 ± 55.3
AST, U/L, median (IQR)	65.0 (49.3)
ALT, U/L, median (IQR)	88.0 (88.0)
HCV genotype 1, No. (%)	110 (57.3)
HCV RNA, $\log_{10}$ IU/mL, mean ± SD	5.3 ± 1.1
Positive HBeAg, No. (%)	7 (3.6)
Pre-tx HBV DNA, $\log_{10}$ IU/mL, median (IQR)	2.0 (0.8)
Pre-tx HBV DNA, <LLOQ, No. (%)	104/163 (63.8)
Pre-tx HBV DNA, <2000 IU/mL, No. (%)	28/163 (17.2)
Pre-tx HBV DNA, ≥2000 IU/mL, No. (%)	31/163 (19.0)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; IQR, interquartile range; LLOQ, lower limit of quantification; Pre-tx, pretreatment; SD, standard deviation.

Relapse of HCV After HCV SVR_{24}

Overall, 148 (77.1%) of the 192 patients achieved HCV SVR_{24}. For HCV genotype 1 and non-1, the HCV SVR_{24} rates were 70.0% and 86.6%, respectively. During the follow-up period, only 1 (0.7%) patient with HCV SVR_{24} had a relapse of HCV at the 12-month follow-up period. This patient was noncirrhotic and non-HCC, with a genotype 1 HCV infection before therapy. The HBV DNA level was undetectable before treatment and there was also no HBV reactivation episode during the follow-up period.

Early HBV Reactivation Before the End of Treatment

Before the end of treatment, there were a total of 4 patients who developed HBV reactivation (defined as an HBV DNA increase of more than 1 $\log_{10}$ IU/mL from pretreatment and greater than 2000 IU/mL). Three of the 4 patients were HBeAg positive. HBV NUC therapy was prescribed in these 3 patients because of the accompanied clinical hepatitis flare: one at the same time as an HBV flare, 1 at 5 months after stopping IFN therapy, and 1 at 3 years after finishing treatment. The HBV DNA level fluctuated between 2000 and 10 000 IU/mL, but without a clinical hepatitis flare in the remaining patient with a negative HBeAg.

Long-Term Risk of HBV DNA ≥2000 IU/mL and Associated Factors

Eventually, 50 (26.6%) of the 188 patients (excluding the 4 patients with early reactivation) had episodes of HBV DNA ≥2000 IU/mL in a mean follow-up period of 68.8 months. The risk was 4.6 per 100 person years. The 1-, 3-, 5-, 10-, and 15-year cumulative probabilities were 9.3%, 15.5%, 23.6%, 31.9%, and 39.9%, respectively (Figure 2A). Of the 50 patients, only 28 (56.0%) consistently had HBV DNA levels ≥2000 IU/mL.

To identify factors associated with HBV virological flare (≥2000 IU/mL) after the end of treatment (EOT), we perform a Cox regression hazard analysis. The crude analysis showed that positive HBeAg (hazard ratio [HR] 9.8; 95% confidence interval [CI], 3.38–28.72; P < .001), pretreatment HBV DNA level per 1 log increase (HR 1.4; 95% CI, 1.24–1.63; P < .001), and EOT HBV DNA level per 1 log increase (HR 1.5; 95% CI, 1.16–1.95; P = .002) were possible associated factors. The cutoff pretreatment and EOT HBV DNA levels were set as 300 IU/mL and a detectable level. Pretreatment HBV DNA >300 IU/mL remained significant in the univariate analysis. We further adjusted for factors including HCV SVR_{24}, positive HBeAg, and pretreatment

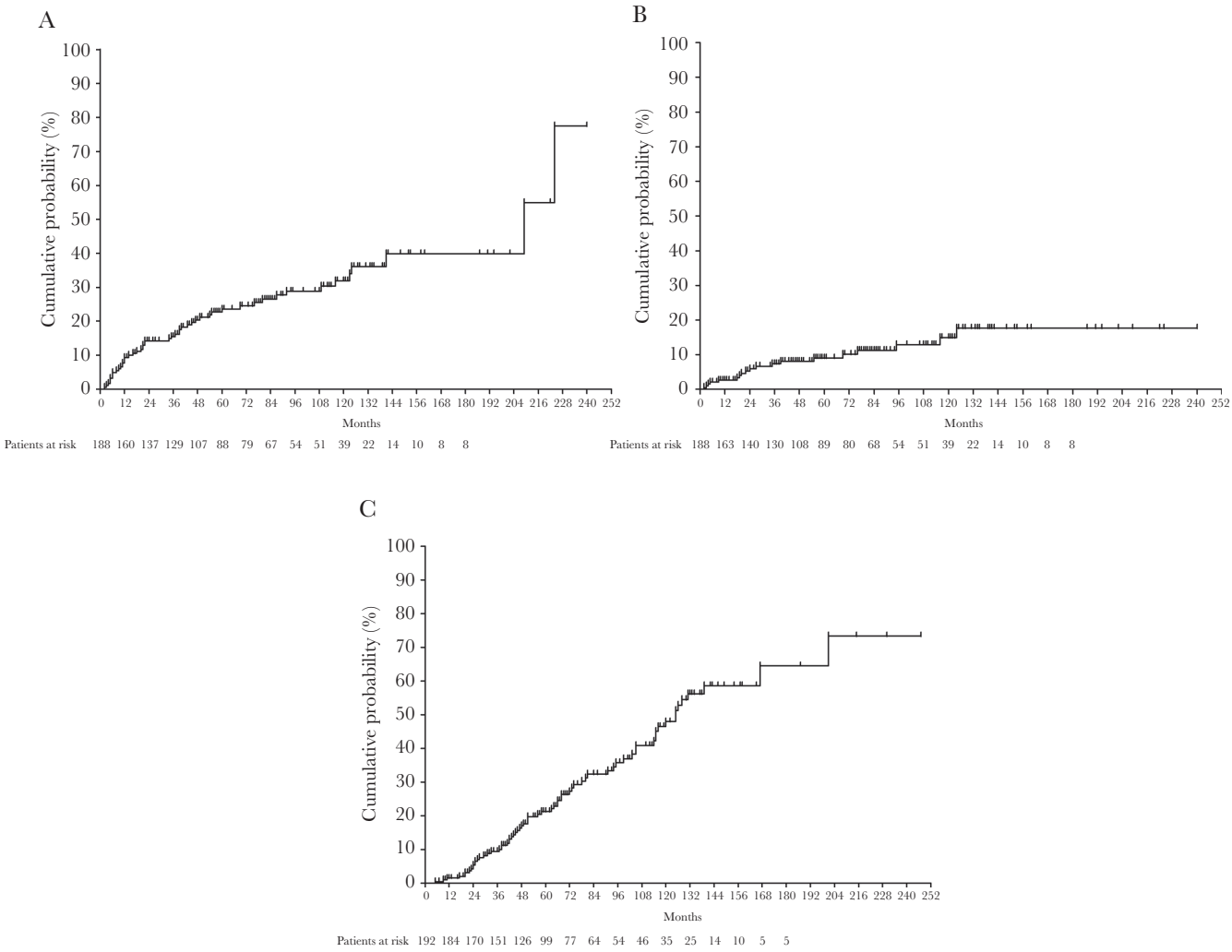

Figure 2. Cumulative probabilities of HBV DNA ≥2000 IU/mL (*A*), HBV DNA ≥2000 IU/mL and ALT ≥2 × ULN after the end of treatment (*B*), and HBsAg seroclearance (*C*) in all patients. Abbreviations: ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ULN, upper limit of normal.

HBV DNA >300 IU/mL. The results identified a positive HBeAg (HR 4.3; 95% CI, 1.38–3.48; P = .012) and pretreatment HBV DNA >300 IU/mL (HR 2.7; 95% CI, 1.41–5.16; P = .003) as the independent factors associated with HBV virological flare (Table 2). The risk of HBV virological flare with a pretreatment HBV DNA >300 IU/mL or ≤300 IU/mL is shown in Figure 3A.

We further analyzed the HBV virological flare in patients with pretreatment HBV DNA ≤300 IU/mL. There was a total of 22 (19.3%) of the 114 patients who had an HBV virological flare after EOT in the mean follow-up period of 76.7 months. The risk was 3.0 per 100 person years. The 1-, 3-, 5-, 10-, and 15-year cumulative probabilities were 4.5%, 10.5%, 14.3%, 21.6%, and 27.7%, respectively (Supplementary Figure 1). Most of the 22 patients (63.6%) had an HBV DNA elevation only transiently. The further Cox regression analysis revealed that there was no factor significantly associated with HBV virological flare, but a trend of EOT HBV DNA level was found (data not shown). Due to small case numbers, there was no significant difference in HBV virological flare between patients with EOT HBV DNA >300 IU/mL or ≤300 IU/mL. However, there was no episode of HBV DNA >2000 IU/mL during the follow-up period in the 5 patients with an EOT HBV DNA ≤300 IU/mL (Supplementary Figure 2).

Long-Term Risk of HBV DNA ≥2000 IU/mL Combined With ALT ≥2-Fold ULN

Of the 188 patients, only 19 (10.1%) patients had an HBV clinical flare (ALT level ≥2-fold UNL twice in 3 months or cirrhosis development following HBV DNA ≥2000 IU/mL) after EOT in a mean follow-up period of 69.5 months. All patients with an HBV clinical flare received NUC therapy. Notably, severe hepatitis flares (ALT level ≥10-fold ULN) occurred in 10 (52.6%) of the 19 patients, including accompanied hepatic decompensation in 6 of these patients. The risk was 1.7 per 100 person years. The 1-, 3-, 5-, 10-, and 15-year cumulative probabilities were 2.7%, 7.4%, 9.1%, 14.9%, and 17.7%, respectively (Figure 2B).

Further analysis of the pretreatment factors associated with HBV clinical flare demonstrated that liver cirrhosis

Table 2. Cox Regression Hazard Analysis of Factors Associated With HBV DNA ≥2000 IU/mL After Treatment

	Crude HR		Adjusted HR	
	(95% CI)	*P*	(95% CI)	*P*
Age, per 1-y increase	1.0 (0.98–1.03)	.893		
Male gender	0.8 (0.46–1.43)	.466		
Liver cirrhosis	1.1 (0.52–2.38)	.781		
HCC	1.5 (0.69–3.15)	.317		
PLT, per 10 × 10^3/mm^3 increase	1.0 (0.98–1.08)	.209		
AST, per 10 U/L increase	1.0 (0.92–1.04)	.503		
ALT, per 10 U/L increase	1.0 (0.96–1.03)	.797		
HCV genotype1	0.7 (0.39–1.20)	.188		
HCV RNA, per 1 log_{10} increase	0.9 (0.67–1.13)	.295		
HCV SVR_{24}	1.8 (0.85–3.86)	.125	1.8 (0.74–4.25)	.199
Positive HBeAg	9.8 (3.38–28.72)	<.001	4.3 (1.38–13.48)	.012
Pre-tx HBV DNA, per 1 log increase	1.4 (1.24–1.63)	<.001		
Pre-tx HBV DNA, >300 IU/mL	3.2 (1.75–5.99)	<.001	2.7 (1.41–5.16)	.003
EOT PLT, per 10 × 10^3/mm^3 increase	1.0 (0.98–1.08)	.331		
EOT ALT, per 10 U/L increase	1.0 (0.95–1.05)	.999		
EOT HBV DNA, per 1 log increase	1.5 (1.16–1.95)	.002		
EOT HBV DNA, detectable	1.7 (0.74–3.76)	.223		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; EOT, end of treatment; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon; PLT, platelet; Pre-tx, pretreatment; SVR_{24}, sustained virological response.

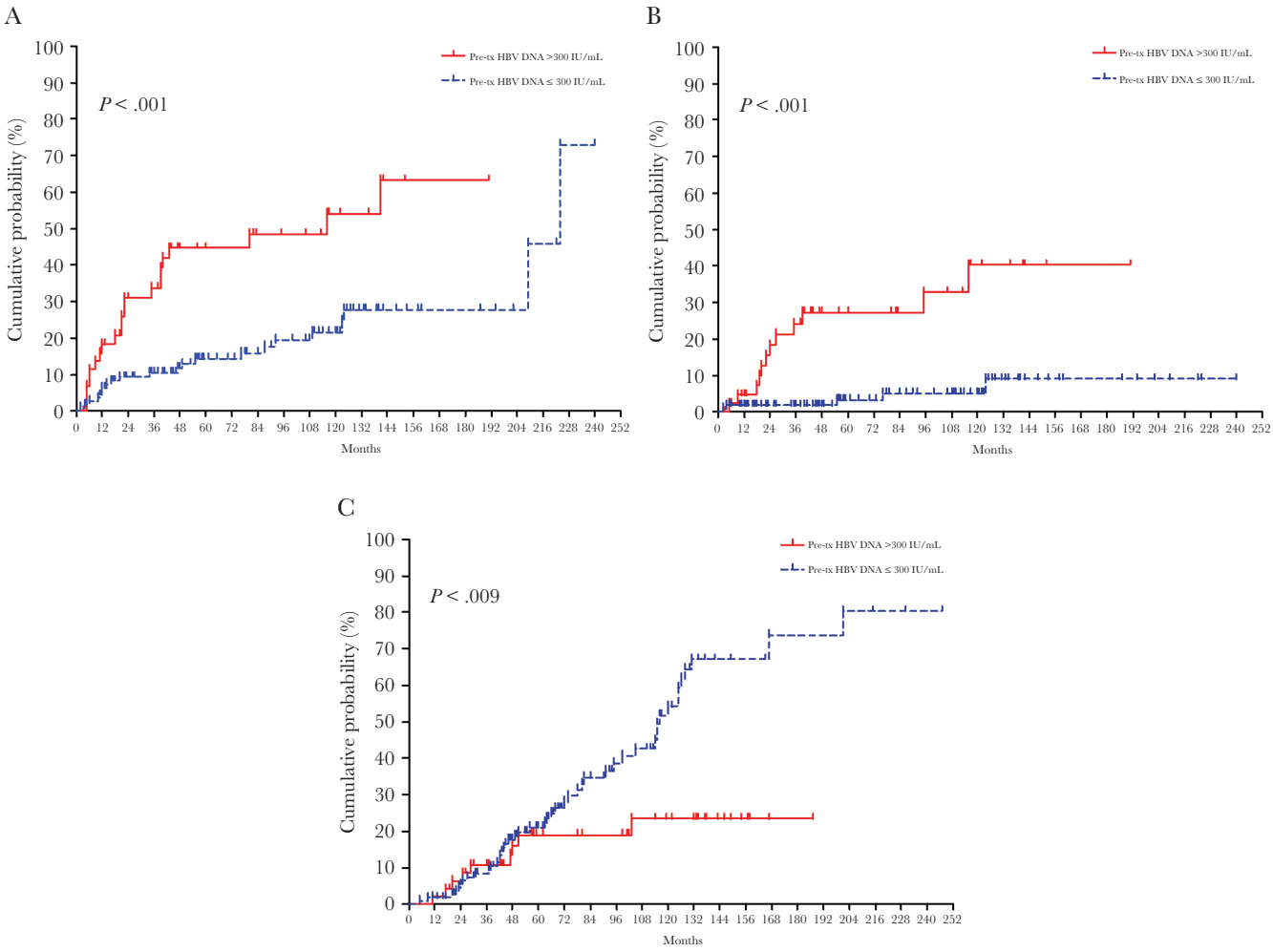

Figure 3. Comparison of HBV DNA ≥2000 IU/mL (*A*), HBV DNA ≥2000 IU/mL and ALT ≥2 × ULN after the end of treatment (*B*), and HBsAg seroclearance (*C*) between patients with pretreatment HBV DNA >300 IU/mL or ≤300 IU/mL. Abbreviations: ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; Pre-tx, pretreatment; ULN, upper limit of normal.

(HR 3.2; 95% CI, 1.25–8.06; P = .015), HCC (HR 4.3; 95% CI, 1.67–10.85; P = .002), positive HBeAg (HR 17.2; 95% CI, 5.51–53.36; P < .001), pretreatment HBV DNA >300 IU/mL (HR 7.1; 95% CI, 2.50–20.25; P < .001), and EOT detectable HBV DNA (HR 4.3; 95% CI, 1.45–12.90; P = .009) were all possible associated factors. After adjusting for the above factors, only positive HBeAg (HR 10.2; 95% CI, 2.41–43.17; P = .002) and pretreatment HBV DNA >300 IU/mL (HR 4.8; 95% CI, 1.07–21.19; P = .040) were found to be independent factors (Supplementary Table). The risk of HBV clinical flare with pretreatment HBV DNA >300 IU/mL or ≤300 IU/mL is shown in Figure 3B.

We further analyzed the HBV clinical flares in patients with pretreatment HBV DNA ≤300 IU/mL. Only 5 (4.4%) of the 114 patients had an HBV clinical flare after EOT. The risk was 0.7 per 100 person years. The 1-, 3-, 5-, 10-, and 15-year cumulative probabilities were 1.8%, 1.8%, 3.2%, 5.1%, and 9.0%, respectively.

Long-Term Probability of HBsAg Loss/Seroconversion and Associated Factors

Although a proportion of the patients suffered the unfavorable outcome of HBV reactivation, we also found as high as 67 (34.9%) of the 192 patients had a favorable outcome of HBsAg loss during a mean follow-up of 73.3 months. The probability of HBsAg loss was 5.7 per 100 person years. The 1-, 3-, 5-, 10-, and 15-year cumulative probabilities were 1.6%, 8.9%, 21.3%, 46.5%, and 64.6%, respectively (Figure 2C). Of the 67 patients, 50 were further examined for hepatitis B surface antibody (HBsAb), and 19 (38.0%) of these patients eventually developed HBsAg seroconversion (negative HBsAg and positive HBsAb). In 41 of the 67 patients with HBsAg loss, HBV DNA level was determined at the time point of HBsAg loss and all had undetectable HBV DNA. In the 86 patients without HBsAg loss, the HBsAg level in the last follow-up was <10 IU/mL in 17 (19.8%), 11–100 IU/mL in 21 (24.4%), 101–1000 IU/mL in 19 (22.1%), and >1000 IU/mL in 29 (33.7%).

We further analyzed the factors associated with HBsAg loss. The crude analysis revealed age >40 years (HR 3.4; 95% CI, 1.38–8.59; P = .008) and pretreatment HBV DNA ≤300 IU/mL (HR 0.4; 95% CI, 0.19–0.82; P = .012) as possible associated factors. Further analysis adjusted for the associated factors revealed both age >40 years (HR 3.2; 95% CI, 1.14–8.84; P = .027) and pretreatment HBV DNA ≤300 IU/mL (HR 0.5; 95% CI, 0.22–0.94; P = .032) as independent factors associated with HBsAg loss (Table 3). The probability of HBsAg loss with pretreatment HBV DNA >300 IU/mL or ≤300 IU/mL is shown in Figure 3C.

Association Between HBV Responses and HCV SVR_{24}

Combining the pretreatment HBV DNA 300 IU/mL and HCV SVR_{24}, we divided patients into 4 groups: (1) pretreatment HBV DNA >300 IU/mL and HCV SVR_{24}; (2) pretreatment HBV DNA >300 IU/mL and HCV non-SVR_{24}; (3) pretreatment HBV DNA ≤300 IU/mL and HCV SVR_{24}; and (4) pretreatment HBV DNA ≤300 IU/mL and HCV non-SVR_{24}. We found that the patients in group 1 were at a higher risk of HBV virological and clinical flare compared to the other 3 groups. Additionally, we identified the most unfavorable group for achieving HBsAg loss as group 2 patients, with none of these patients having HBsAg loss during follow-up (Figure 4).

Table 3. Cox Regression Hazard Analysis of Factors Associated With Hepatitis B Surface Antigen Loss

	Crude HR		Adjusted HR	
	(95% CI)	P	(95% CI)	P
Age, per 1-y increase	1.0 (1.00–1.05)	.028		
Age, >40 y	3.4 (1.38–8.59)	.008	3.2 (1.14–8.84)	.027
Male gender	0.8 (0.49–1.35)	.429		
Liver cirrhosis	1.0 (0.51–1.96)	.993		
HCC	0.8 (0.34–1.82)	.568		
PLT, per 10 × 10^3/mm^3 increase	1.0 (0.94–1.02)	.318		
AST, per 10 U/L increase	1.0 (0.99–1.08)	.098		
ALT, per 10 U/L increase	1.0 (0.99–1.03)	.461		
HCV genotype1	1.1 (0.65–1.72)	.819		
HCV RNA, per 1 log_{10} increase	1.0 (0.78–1.21)	.780		
HCV SVR_{24}	1.1 (0.59–1.94)	.821		
Positive HBeAg	.0 (0.00–2.08)	.111		
Pre-tx HBV DNA, per 1 log increase	0.7 (0.51–0.93)	.015		
Pre-tx HBV DNA, >300 IU/mL	0.4 (0.19–0.82)	.012	0.5 (0.22–0.94)	.032
EOT PLT, per 10 × 10^3/mm^3 increase	1.0 (0.94–1.03)	.486		
EOT ALT, per 10 U/L increase	1.0 (0.99–1.08)	.128		
EOT HBV DNA, per 1 log increase	0.8 (0.60–1.14)	.235		
EOT HBV DNA, detectable	0.6 (0.26–1.29)	.182		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; EOT, end of treatment; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon; PLT, platelet; Pre-tx, pretreatment; SVR_{24}, sustained virological response.

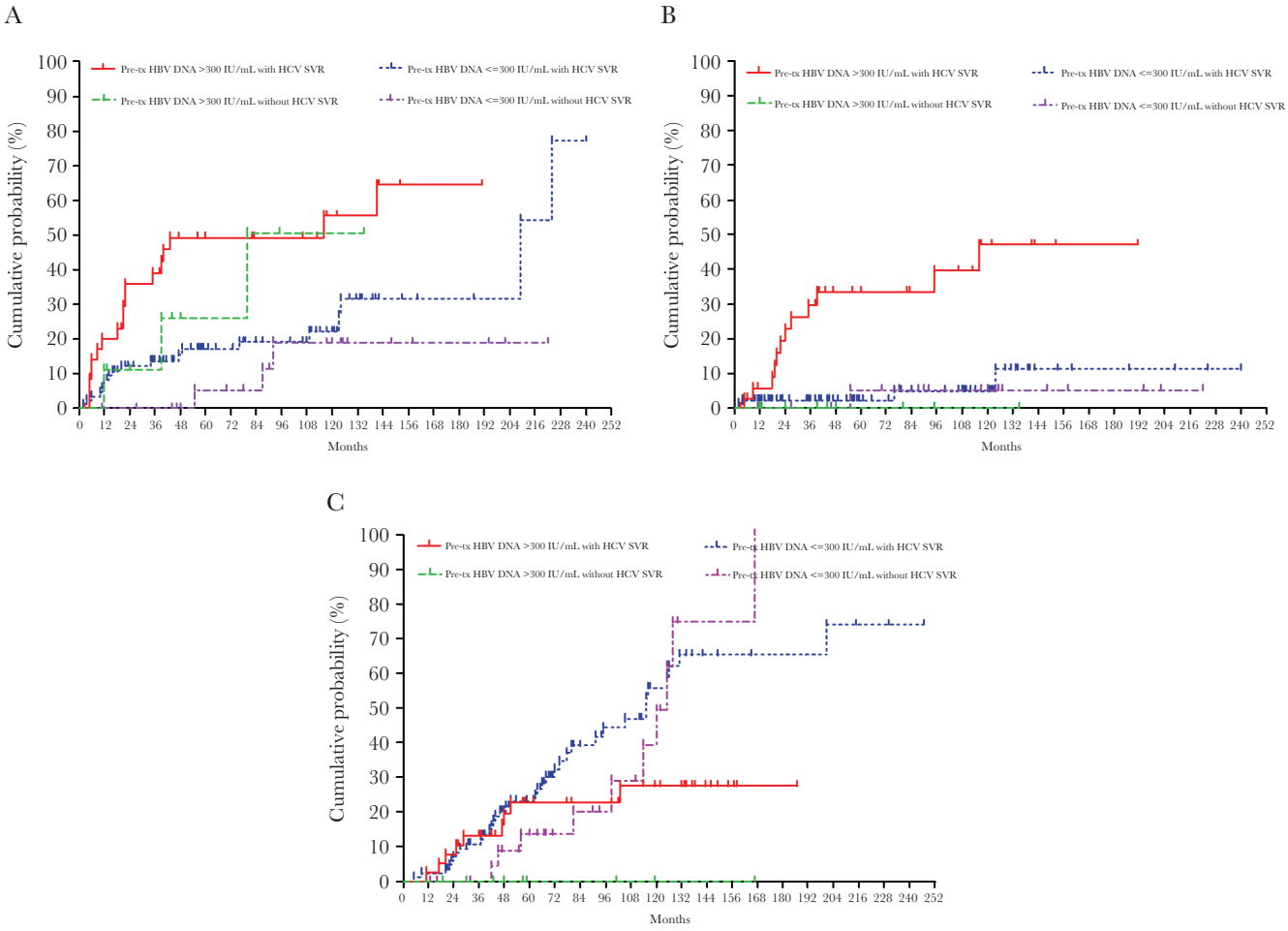

Figure 4. Comparison of HBV DNA ≥2000 IU/mL (*A*), HBV DNA ≥2000 IU/mL and ALT ≥2 × ULN after the end of treatment (*B*), and HBsAg seroclearance (*C*) according to pretreatment HBV DNA of 300 IU/mL and HCV SVR_{24}. Abbreviations: ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; Pre-tx, pretreatment; $SVR_{24,}$ sustained virological response; ULN, upper limit of normal.

DISCUSSION

The significant results in the present study include the finding that only 1 in 10 patients with HBV/HCV dual infection experienced clinical hepatitis flare related to HBV, even though more than 1 in 4 patients had a virological flare after antiviral treatment. However, one-third of patients had a favorable outcome of HBsAg loss eventually. We also identified the outcomes risk for different groups of patients, which will help in the clinical practice of follow-up after therapy.

HBV reactivation after IFN therapy has been reported. In an earlier multicenter trial, posttreatment HBV reappearance was found in 28 (36.4%) of 77 HBV/HCV dual-infected patients with pretreatment undetectable HBV DNA [3]. Our report also demonstrated that 19.1% of patients with pretreatment undetectable HBV DNA exhibited detectable levels 6 months after treatment [7]. However, only short-term results have been reported to date. Regarding the long-term risk of HBV reactivation, there remains uncertainty. To our knowledge, the present study is the first to report the long-term risk of virological and clinical flares related to HBV, and to identify groups at high risk of flare.

HBV reactivation during DAA therapy remains a critical issue [15–17]. In our previous publication, 4 and 1 of the 7 HBV/HCV dual-infected patients had virological and clinical reactivation during DAA therapy, respectively [12]. A recent meta-analysis by Vermehren et al, including 242 HBV/HCV dual-infected patients treated with DAA, further demonstrated a pooled virological reactivation risk of 24% (95% CI, 19%–30%) and clinical reactivation risk of 9% (95% CI, 5%–16%) [18]. The first phase 3b multicenter study, which enrolled 111 Taiwanese patients with HBV/HCV dual infection, also reported as high as 84% of patients with HBV DNA below 20 IU/mL and 53% of patients above 20 IU/mL had increases of HBV DNA greater than 1 $\log_{10}$ IU/mL during and through posttreatment week 12 [19].

Until now, the group of patients at high-risk of reactivation has remained unclear. HBV DNA level was considered a predictor. In the meta-analysis, a significantly lower risk of reactivation was found in patients with pretreatment HBV DNA level

below the lower limit of quantification [18]. However, pretreatment ALT instead of HBV DNA level was reported as the only factor associated with HBV reactivation in the phase 3b multicenter study [19]. In the present study, one of the significant findings was the predictive value of pretreatment HBV DNA level. Patients at high risk of reactivation and low probability of HBsAg seroclearance could be identified with a HBV DNA cutoff level of 300 IU/mL.

The mechanism of HBV reactivation remains unclear. In vitro studies demonstrated suppression of HBV replication by the HCV "core" protein [20–22]. Clinical studies also showed the dominant role of HCV (high HCV and low HBV load) in the majority of studies [23, 24]. In the present study, we found an association between HCV SVR and HBV flare, especially in patients with pretreatment HBV DNA >300 IU/mL. Thus there was a significantly higher rate of HBV clinical flares in this group compared to the other 3 groups of patients (Figure 4). These results correspond with previous studies and demonstrate the necessity of monitoring after HCV viral eradication.

HBsAg seroclearance remains the ultimate goal of HBV antiviral therapy [25–27]. In HBV monoinfected patients, HBsAg seroclearance is difficult to achieve with current antiviral therapy [28–30]. However, it is more frequently achieved in HBV/HCV dual-infected patients. The earlier report using conventional IFN therapy demonstrated 18.5% of patients achieved HBsAg seroclearance during a mean 3.4 years of follow-up [31]. The following study of a multicenter trial from Taiwan demonstrated as high as 30% of patients achieved HBsAg seroclearance in 5 years of posttreatment follow-up after peg-IFN/ribavirin therapy [8]. In the present study, we further demonstrated the persistently increased chance of HBsAg seroclearance after 5 years of follow-up. Even for those who did not achieve HBsAg seroclearance, more than 40% of patients had a relatively low level of HBsAg titer (<1000 IU/mL), which was previously reported to be a safe value for HCC development [32]. Furthermore, we also identified a pretreatment HBV DNA level of 300 IU/mL as a predictor of HBsAg seroclearance, which has not been reported previously. HBsAg level has also been demonstrated as the predictor of HBsAg seroclearance in the earlier report [8]. However, HBsAg level was not analyzed in the present study owing to the lack of stored serum for HBsAg quantification. Whether HBV DNA or HBsAg level would be a better predictor for the long-term outcomes remains uncertain and requires further studies to clarify.

There were several limitations in the present study. First, none of the patients received DAA, which replaced pegylated IFN as the first-line therapy. All-oral DAA therapy was first available to HCV patients in 2015 in Taiwan, and reimbursed by health insurance only for fibrosis stage 3 and 4 patients since 2017. Until now, only a small proportion of HBV/HCV dual-infected patients have received DAA therapy and the posttreatment follow-up duration was also short. Therefore we did not compare the long-term outcomes between the 2 therapies in the present study. However, results of the present study are relevant to outcome prediction and posttreatment monitoring for patients with DAA therapy. Second, several parameters, such as pretreatment HBsAg titer, were not included in the present study because the HBsAg quantification methods were not available for some patients when the treatment started. There were also no stored serum samples available. Although the previous study reported pretreatment HBsAg level as a predictor of HBsAg loss [8], further investigation regarding the role of HBsAg level is still needed. Third, the data of the present study were obtained from a single center without external validation.

In conclusion, patients with HBV/HCV dual infection remain at a high risk of HBV flare during post-IFN treatment follow-up. However, a percentage of patients do achieve favorable outcomes of HBsAg seroclearance and lower levels of HBsAg titer. A pretreatment HBV DNA level of 300 IU/mL is a possible predictor for HBV reactivation.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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