

Long-Term Effect of Interferon Plus Ribavirin on Hepatitis B Surface Antigen Seroclearance in Patients Dually Infected with Hepatitis B and C Viruses

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Abstract

Background: Interferon- α /ribavirin combination therapy might promote hepatitis B surface antigen (HBsAg) seroclearance in patients dually infected with hepatitis B and C viruses (HBV/HCV), but the long-term effect remains unclear. We aimed to investigate the rate of and the factors associated with HBsAg seroclearance during long-term follow-up after interferon- α /ribavirin combination therapy in HBV/HCV dually-infected patients.

Methodology/Principal Findings: Eighty-one patients who received interferon- α /ribavirin combination therapy for 24 weeks with a follow-up period of >24 weeks were enrolled. HBV serological markers and HBV DNA were determined every 6 months. Early and late HBsAg seroclearance were defined as HBsAg loss in less or more than 6 months after end-of-treatment, respectively. Fifteen (18.5%) patients had HBsAg seroclearance during a mean follow-up period of 3.4 (0.5–5.1) years. The 5-year cumulative incidence was 25.6%. Baseline cirrhosis and HBV DNA negativity 1 year after end-of-treatment were independently predictive of HBsAg seroclearance with an odds ratio (OR), 95% confidence intervals (CI) of 16.6, 1.8–153 and 9.2, 1.4–62.1, respectively, by Cox regression hazard analysis. Four patients developed early and 11 developed late HBsAg seroclearance, respectively. Cox regression hazard analysis showed no factor was associated with early HBsAg seroclearance, whilst HBV DNA negativity 1 year after end-of-treatment was the only significant factor predicting late HBsAg loss (OR, 43.0; CI, 2.5–745). Five patients had HBsAg seroconversion with a 5-year cumulative incidence of 8.3%. HBV DNA negativity at baseline and one year after EOT had a trend for HBsAg seroconversion. HCV response did not correlate to HBsAg loss.

Conclusions: We demonstrated that interferon- α /ribavirin had long-term effect on HBsAg seroclearance in dually HBV/HCV-infected patients. Baseline cirrhosis and seroclearance of HBV DNA 1 year after end-of-treatment were significant factors associated with HBsAg seroclearance.

Citation: Yeh M-L, Hung C-H, Huang J-F, Liu C, Lee C-M, et al. (2011) Long-Term Effect of Interferon Plus Ribavirin on Hepatitis B Surface Antigen Seroclearance in Patients Dually Infected with Hepatitis B and C Viruses. PLoS ONE 6(6): e20752. doi:10.1371/journal.pone.0020752

Editor: Man-Fung Yuen, The University of Hong Kong, Hong Kong

Received: December 27, 2010; **Accepted:** May 11, 2011; **Published:** June 13, 2011

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Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the two leading causes of chronic liver disease, liver cirrhosis (LC), and hepatocellular carcinoma (HCC) [1,2]. Since both viruses share the same transmission routes, dual HBV/HCV infection is not uncommon, accounting for 10–40% of patients with chronic hepatitis in Taiwan and Europe [3,4]. Previous studies also indicated that HBV/HCV dually infected patients had a much higher risk of developing LC or HCC than those with HBV or HCV infection alone [5,6]

Seroclearance of hepatitis B surface antigen (HBsAg) is a rare event in chronic hepatitis B (CHB) patients [7,8]. The estimated

annual incidence of HBsAg seroclearance is 0.1–2.26% [9,10]. A recent study demonstrated the annual HBsAg seroclearance rate has quite differed in Hepatitis B e antigen (HBeAg) positive and negative populations (0.39 versus 2.61 per 100 person-years, $P < 0.001$) [10]. HBsAg seroclearance usually confers a favorable outcome and is the optimal treatment goal. However, HBsAg seroclearance is very rarely observed in HBV infected patients receiving current antiviral agents, with an annual rate of 2.4–3.2% with interferon (IFN) or pegylated IFN therapy and only <1% with nucleoside/nucleotide analogues [11,12]

IFN is an antiviral agent approved for the treatment of CHB and chronic hepatitis C. Although IFN monotherapy has been

Table 1. Baseline and follow-up data.

Baseline data	
Male, n (%)	56 (69.1)
Mean age (years), mean \pm SD	46.4 \pm 11.8
Post-treatment follow-up period, year, mean \pm SD (range)	3.7 \pm 1.2 (0.5–4.9)
Baseline ALT level (U/L), mean \pm SD	112.9 \pm 77.8
Liver cirrhosis, n (%)	7 (8.6)
Positive HBeAg, n (%)	4 (4.9)
Baseline HBV DNA negativity, n (%)	34 (42)
Baseline HCV RNA level (log IU/ml), mean \pm SD	5.5 \pm 1.4
Follow-up data	
Achievement of an HCV SVR, n (%)	59 (72.8)
HBV DNA negativity at EOT, n (%)	47 (58)
HBV DNA negativity 6 months after EOT, n (%)	36 (44.4)
HBV DNA negativity 1 year after EOT, n/N (%)	26/65 (40)

SD, standard deviation; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; SVR, sustained virologic response; EOT, end of treatment.

doi:10.1371/journal.pone.0020752.t001

ineffective in treating HBV/HCV dually infected patients [13], the introduction of ribavirin in combination with IFN- α or pegylated IFN- α for HBV/HCV dually infected patients has been shown to be as effective for patients with HCV mono-infection in terms of sustained eradication of HCV viremia [14,15]. Interestingly, the combination therapy could also promote HBsAg seroclearance in HBV/HCV dually infected patients, which can reach 11.2% 6 months after end of treatment [16,17,18,19,20]. Recently, an increased rate of HBsAg clearance to 8.7% was observed in patients with HBeAg-negative chronic hepatitis B after 3 years of peginterferon alpha-2a as a first-line treatment [21]. Whether the rate of HBsAg loss among HBV/HCV dually infected patient increases overtime after IFN plus ribavirin combination therapy has not been well established. We conducted a long-term follow-up study to explore the cumulative rate of and predictive factors associated with HBsAg seroclearance in HBV/HCV dually infected patients after IFN plus ribavirin combination therapy.

Materials and Methods

Patients

The present study is based on three previous studies investigating the efficacy of IFN- α /ribavirin combination therapy in HBV/HCV dually infected patients [16,17,18]. From April 1999 to April 2002, 102 patients with dual HBV/HCV infections were enrolled into the three studies. All the patients were seropositive for HCV antibody (anti-HCV) and HBsAg for more than 6 months. Except three patients, all the others were positive for serum HCV RNA (detect by a standardized qualitative polymerase chain reaction (PCR) assay (Cobas Amplicor HCV Monitor Version 2.0; Roche Diagnostics, Branchburg, NJ, USA; detection limit 50 IU/ml)). Patients with human immunodeficiency virus or hepatitis delta virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, α_1 -antitrypsin deficiency, decompensated cirrhosis, overt hepatic failure, a current or past history of alcohol

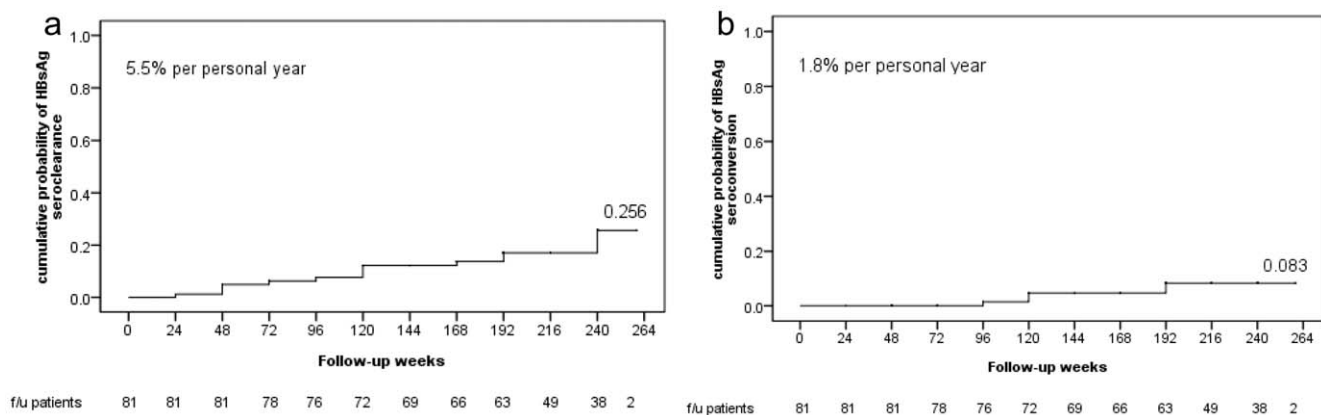


Figure 1. 5-year cumulative probability of HBsAg seroclearance (a) and seroconversion (b) in HBV/HCV dually infected patients. Fifteen patients had HBsAg seroclearance during the follow-up period with a 5-year cumulative incidence of 25.6% (a). HBsAg seroconversion was found in 5 of the 81 patients. The 5-year cumulative incidence was 8.3% (b). HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus.

doi:10.1371/journal.pone.0020752.g001

Table 2. Comparison of baseline and follow-up factors in HBV/HCV dually infected patients who did versus did not achieve HBsAg seroclearance.

	HBsAg seroclearance		P value
	Yes	No	
Number of patients	15	66	
Male, n (%)	10 (66.7)	46 (69.7)	1.000
Age (years)	51.5±9.2	45.2±12.1	0.064
Posttreatment follow-up period, year, Mean±SD (range)	3.3±1.4 (0.5–4.5)	3.7±1.1 (0.5–4.9)	0.274
Baseline ALT level (U/L)	136.6±78.3	107.5±77.3	0.193
Liver cirrhosis, n (%)	3 (20)	4 (6.1)	0.114
Positive HBeAg, n (%)	0 (0)	4 (6.1)	1.000
Baseline HBV DNA negativity, n (%)	10 (66.7)	24 (36.4)	0.043
Baseline HCV RNA level (log IU/ml)	5.7±1.1	5.4±1.5	0.502
Achievement of an HCV SVR, n (%)	10 (66.7)	49 (74.2)	0.537
HBV DNA negativity EOT, n (%)	11 (73.3)	36 (54.5)	0.250
HBV DNA negativity 6 months after EOT, n (%)	9 (60)	27 (40.9)	0.251
HBV DNA negativity 1 year after EOT, n/N (%)	10/12 (83.3)	16/53 (30.2)	0.002

HBsAg, hepatitis B s antigen; SD, standard deviation; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; SVR, sustained virologic response; EOT, end of treatment.

doi:10.1371/journal.pone.0020752.t002

abuse (≥ 80 gm ethanol per day), a psychiatric condition, or evidence of HCC were excluded.

All patients received a regimen of 3–6 MU IFN- α thrice weekly plus ribavirin 800–1200 mg per-day for 24 weeks. Of the 102 patients, 81 who were followed up more than 24 weeks after end of treatment (EOT) were enrolled into the present long-term follow-up study. During the follow-up period, liver function tests, HBV serological markers and HBV DNA were tested at an interval of 6 months. HBsAg, antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), and anti-HCV were detected with commercially available enzyme-linked immunosorbent assay kits (Abbott Laboratories, North Chicago, IL, USA) in each local laboratory unit. Serum HBV DNA was determined by a standardized automated quantitative PCR assay (Cobas Amplicor HBV Monitor; Roche Diagnostics; detection limit 200 copies/ml). Serum HCV RNA levels were measured using the branched DNA assay (VERSANT HCV-RNA 3.0. Assay, Bayer Diagnostics, Emeryville, CA, USA; quantification limit 615 IU/ml). The study was conducted according to the guidelines of the Declaration of Helsinki, with the principles of good clinical practice, and was approved by local ethics committees for patient's chart review and data analysis without informed consent.

Study endpoint

The primary aim of the study was the loss of serum HBsAg in HBV/HCV dually infected patients after IFN- α /ribavirin combination therapy. The secondary aim was to evaluate the factors related to HBsAg loss.

Definitions

HCV sustained virological response (SVR) was defined as undetectable HCV RNA at 24 weeks after EOT. We defined early HBsAg seroclearance as HBsAg loss within 6 months after EOT and late HBsAg seroclearance as HBsAg loss beyond 6 months after EOT. HBsAg seroconversion was defined as HBsAg loss and seropositive anti-HBs.

Statistical analysis

Means and standard deviations were used to describe the distribution of continuous variables. Independent *t*-tests were used for comparing continuous variables. Chi-square and Fisher's exact test were used for comparing categorical variables. Cox proportional hazards regression models were used to identify the independent factors that might influence HBsAg seroclearance. The cumulative probability of HBsAg seroclearance was analyzed by the Kaplan-Meier actuarial curve method with the log-rank test. All tests were two-sided, and a *P* value of <0.05 was considered statistically significant. All analyses were performed using the SPSS 16.0 statistical package (SPSS, Inc., Chicago, IL, USA).

Results

Patient demographics

Table 1 shows baseline and follow-up data of the 81 patients. The mean post-treatment follow-up period was 3.4 ± 1.1 (0.5–5.1) years. Four (4.9%) patients were seropositive for HBeAg and 72 (88.9%) were seropositive for anti-HBe at baseline. Thirty-four (42%) patients had undetectable HBV DNA at baseline. Baseline HCV RNA level was 5.5 ± 1.4 log IU/ml. Fifty-nine (72.8%) patients achieved an HCV SVR after combination therapy. During the follow-up period, 47 (58%), 36 (44.4%), and 26 (40%) patients had undetectable HBV DNA at EOT and 6 and 12 months after EOT, respectively.

Cumulative probability and predictive factors of HBsAg seroclearance

Fifteen patients had HBsAg seroclearance during the follow-up period with a 5-year cumulative incidence of 25.6% (Figure 1a). The annual incidence was 5.5% per person year. We analyzed baseline and follow-up factors associated with HBsAg seroclearance (Table 2). Baseline HBV DNA negativity ($P=0.043$) and HBV DNA negativity 1 year after EOT ($P=0.002$) were significantly positive factors associated with HBsAg seroclearance

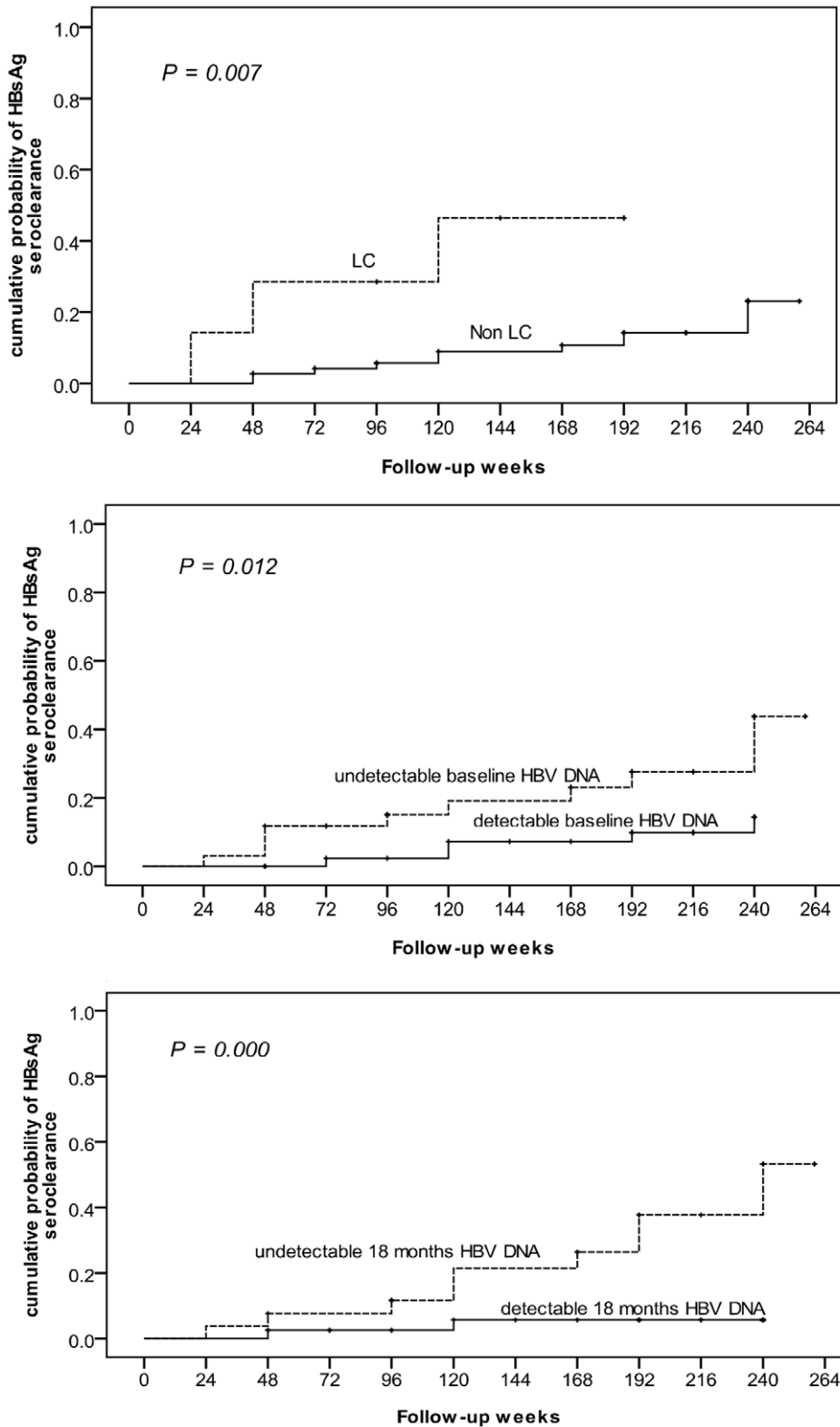


Figure 2. 5-year cumulative probability of HBsAg seroclearance was significantly associated with LC and HBV DNA. Using the Kaplan-Meier method, the 5-year cumulative probability of HBsAg seroclearance was significantly associated with LC ($P = 0.007$), baseline HBV DNA negativity ($P = 0.012$) and HBV DNA negativity 1 year after EOT ($P = 0.000$). HBsAg, hepatitis B surface antigen; LC, liver cirrhosis; HBV, hepatitis B virus; EOT, end of treatment.
doi:10.1371/journal.pone.0020752.g002

Table 3. Comparison of baseline and follow-up factors of HBV/HCV dually infected patients who did versus did not achieve early/late HBsAg seroclearance.

	Early HBsAg seroclearance		P value	Late HBsAg seroclearance		P value
	Yes	No		Yes	No	
Number of patients	4	77		11	66	
Male, n (%)	2 (50)	54 (70.1)	0.583	8 (72.7)	46 (69.7)	1.000
Age (years)	56.0±7.3	45.9±11.8	0.095	49.8±9.6	45.2±12.1	0.234
Post-treatment follow-up period, year, Mean±SD (range)	3.9±0.9 (2.5–4.5)	3.7±1.2 (0.5–4.9)	0.711	3.1±1.6 (1.0–4.5)	3.7±1.1 (0.5–4.9)	0.209
Baseline ALT level (U/L)	168.5±64.2	110.0±77.7	0.144	125.0±82.4	107.5±77.3	0.494
Liver cirrhosis, n (%)	2 (50)	5 (6.5)	0.036	1 (9.1)	4 (6.1)	0.548
Positive HBeAg, n (%)	0 (0)	4 (5.2)	1.000	0 (0)	4 (6.1)	1.000
Baseline HBV DNA negativity, n (%)	4 (100)	30 (39)	0.028	6 (54.5)	24 (36.4)	0.322
Baseline HCV RNA level (log IU/ml)	6.4±0.7	5.4±1.4	0.197	5.5±1.1	5.4±1.5	0.949
Achievement of an HCV SVR, n (%)	3 (75)	56 (72.7)	1.000	7 (63.6)	49 (74.2)	0.479
HBV DNA negativity EOT, n (%)	3 (75)	44 (57.1)	0.635	8 (72.7)	36 (54.5)	0.335
HBV DNA negativity 6 months after EOT, n (%)	4 (100)	32 (41.6)	0.035	5 (45.5)	27 (40.9)	1.000
HBV DNA negativity 1 year after EOT, n/N (%)				8/9 (88.9%)	16/53 (30.2)	0.001

HBsAg, hepatitis B s antigen; SD, standard deviation; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; SVR, sustained virologic response; EOT, end of treatment.
doi:10.1371/journal.pone.0020752.t003

in univariate analysis. HBsAg seroclearance developed among 29.4% (10/34) of patients with baseline HBV DNA negativity and 38.5% (10/26) of patients with HBV DNA negativity 1 year after EOT, which was significantly higher than the 10.6% (5/47) among patients with baseline HBV DNA positivity ($p = 0.043$) and the 5.1% (2/39) among patients with HBV DNA positivity 1 year after EOT ($P = 0.002$), respectively. Baseline HCV RNA level and HCV SVR achievement were not associated with HBsAg seroclearance. Using the Kaplan-Meier method, we observed that the cumulative probability of HBsAg seroclearance was significantly associated with LC ($P = 0.007$), baseline HBV DNA negativity ($P = 0.012$) and HBV DNA negativity 1 year after EOT ($P = 0.000$, Figure 2). In multivariate analysis by using Cox regression hazard analysis, LC ($P = 0.013$; odds ratio = 16.6; 95% CI = 1.8–153.0) and HBV DNA negativity 1 year after EOT ($P = 0.023$; odds ratio = 9.2; 95% CI = 1.4–62.1) were the independent factors associated with HBsAg seroclearance.

We also compare the long-term HBsAg seroclearance rate in our conventional interferon group to our another cohort using Pegylated-Interferon (Peg-IFN) in HBV/HCV co-infected patients. During 3.5 years post-treat follow up period, 12 of 69 patients had HBsAg seroclearance in our cohort and 12 of 36 patients had HBsAg seroclearance in Peg-IFN cohort. There is no significant difference between the two groups (Chi-Square test, P value = 0.087).

Early and late HBsAg seroclearance

Of the 15 patients who achieved HBsAg seroclearance, 4 showed seroclearance in less than 6 months after EOT. In univariate analysis, LC, baseline HBV DNA negativity and HBV DNA negativity 6 months after EOT were significantly associated with early HBsAg seroclearance ($P = 0.036$, 0.028, 0.035, respectively, Table 3). By using Kaplan-Meier method, the cumulative probability of early HBsAg seroclearance remained significantly associated with LC, HBV DNA negativity at baseline and 6 months after EOT ($P = 0.001$, 0.016, 0.023, respectively, Figure 3).

Nevertheless, in multivariate analysis by using Cox regression hazard analysis, no factor was independently associated with the development of early HBsAg seroclearance.

Eleven of the remaining 77 patients achieved HBsAg seroclearance beyond 6 months after EOT. In univariate analysis, HBV DNA negativity 1 year after EOT was the only significant factor related to late HBsAg seroclearance ($P = 0.001$, Table 3). By using Kaplan-Meier method, the cumulative probability of late HBsAg seroclearance was significantly associated with HBV DNA negativity 1 year after EOT ($P = 0.001$, Figure 4). Cox regression hazard analysis showed HBV DNA negativity 1 year after EOT was the only significant factor related to late HBsAg seroclearance ($P = 0.010$; odds ratio = 43.0; 95% CI = 2.5–745).

Comparing the three cohort groups of early, late and non-HBsAg loss, we found a trend of LC and baseline HBV DNA negativity for the overall three groups ($P = 0.012$, 0.011).

Cumulative probability and predictive factors of HBsAg seroconversion

HBsAg seroconversion was found in 5 of the 81 patients. One patient achieved seroconversion by 18 months, two by 24 months, and two by 42 months after EOT. The 5-year cumulative incidence was 8.3% (Figure 1b). The annual incidence was 1.8% per person year. All five patients were HBeAg seronegative and anti-HBe seropositive at baseline. Comparing the three groups of HBsAg seroconversion, HBsAg seroclearance without seroconversion and non-HBsAg seroclearance, there was a trend of baseline HBV DNA negativity and HBV DNA negativity one year after EOT ($P = 0.032$, 0.004) (Table 4).

Discussion

Loss of HBsAg is the ultimate endpoint of antiviral therapy for chronic HBV infection. It is accompanied by disease remission in terms of ALT normalization and by a significantly decreased risk of LC, hepatic failure, and HCC [22].

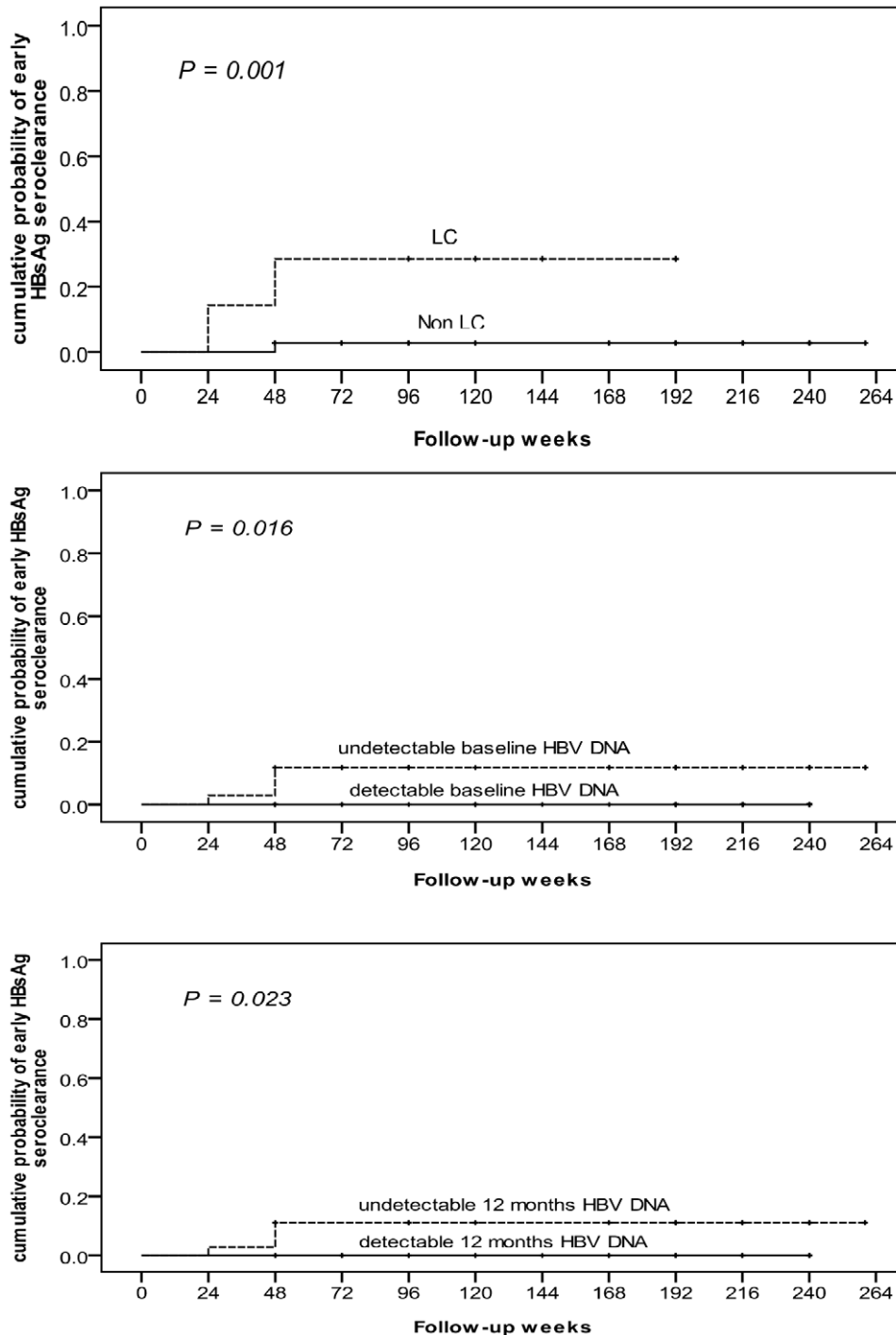


Figure 3. Cumulative probability of early HBsAg seroclearance was significantly associated with LC and HBV DNA. Using Kaplan-Meier method, the cumulative probability of early HBsAg seroclearance remained significantly associated with LC, HBV DNA negativity at baseline and 6 months after EOT ($P=0.001$, 0.016 , 0.023). HBsAg, hepatitis B surface antigen; LC, liver cirrhosis; HBV, hepatitis B virus; EOT, end of treatment. doi:10.1371/journal.pone.0020752.g003

Spontaneous seroclearance of HBsAg is a rare event in CHB, with an estimated annual incidence of 0.1% to 0.8% [9]. HBsAg seroclearance is also observed in patients receiving treatment for CHB including nucleoside/nucleotide analogues and IFN. Several studies of lamivudine treatment reported absence or less than 2% HBsAg seroclearance [23,24,25]. One-year treatment with adefovir dipivoxil resulted in 1.6% HBsAg

seroclearance, and a recent study using tenofovir disoproxil fumarate for 48 weeks resulted in 3% HBsAg seroclearance [26]. Another study reported a 5% HBsAg seroclearance rate after standard IFN- α treatment for 32 weeks [27]. A higher HBsAg seroclearance rate of 7–11% was reported after 1-year pegylated IFN treatment in patients with CHB mono-infection [21,28].

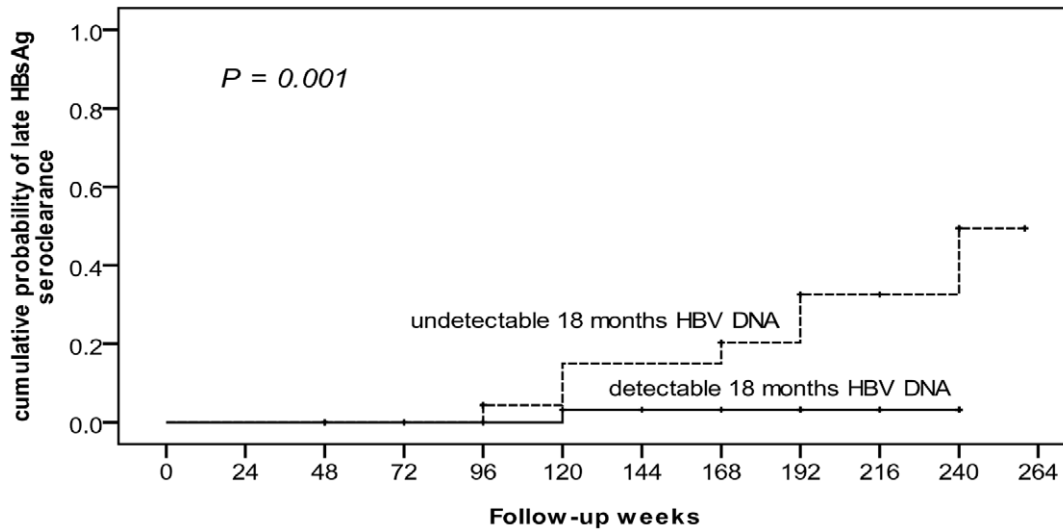


Figure 4. Cumulative probability of late HBsAg seroclearance was significantly associated with HBV DNA. Using Kaplan-Meier method, the cumulative probability of late HBsAg seroclearance was significantly associated with HBV DNA negativity 1 year after EOT ($P=0.001$). HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; EOT, end of treatment. doi:10.1371/journal.pone.0020752.g004

Clinical and laboratory studies have shown that the HBV and HCV interact with each other and affect immune responses. Most dually infected cases have detectable levels of HCV viremia and very low values of serum HBV DNA [29,30]. In cases of HCV superinfection with HBV, HBsAg seroclearance has been reported [31]. A large-scale prospective trial designed to evaluate the efficacy of pegylated IFN- α -2a plus ribavirin combination in HBV/HCV dually infected patients reported 11.2% HBsAg seroclearance [19]. The long-term data reported here show that 25.6% of HBV/HCV dually infected patients treated with IFN- α /ribavirin combination therapy achieved HBsAg seroclearance during the 5-year follow-up period. This

higher rate of HBsAg seroclearance suggests that the viral interaction between HBV and HCV may influence the rate of HBsAg seroclearance.

Many factors are related to HBsAg seroclearance, including HBV genotype. Yuen et al. reported that HBsAg carriers with genotype B infection were more likely to clear HBsAg than those with genotype C infection [22]. Others reported higher HBsAg seroclearance in genotype A as compared to genotypes B, C, or D after pegylated IFN- α treatment in HBeAg-positive patients [21,28,32]. In HBe-negative patients, Marcellin et al. reported that HBsAg seroclearance did not appear to be genotype related [21]. In the present study, most of our patients were HBeAg-

Table 4. Comparison of baseline and follow-up factors in HBV/HCV dually infected patients who did achieve HBsAg seroconversion versus HBsAg seroclearance without seroconversion and non-HBsAg seroclearance.

	HBsAg seroclearance with seroconversion		Non-HBsAg seroclearance	P value
	Yes	No		
Number of patients	5	10	66	
Male, n (%)	2 (40)	8 (80)	46 (69.7)	0.801
Age (years)	49.4 \pm 10.4	52.5 \pm 9.0	45.2 \pm 12.1	0.162
Posttreatment follow-up period, year, Mean \pm SD (range)	2.7 \pm 1.3 (1.5–4.5)	4.0 \pm 0.7 (2.5–4.5)	3.7 \pm 1.1 (0.5–4.9)	0.071
Baseline ALT level (U/L)	81.4 \pm 24.9	164.2 \pm 82.0	107.5 \pm 77.3	0.063
Liver cirrhosis, n (%)	1 (20)	2 (20)	4 (6.1)	0.100
Positive HBeAg, n (%)	0 (0)	0 (0)	4 (6.1)	0.354
Baseline HBV DNA negativity, n (%)	3 (60)	7 (70)	24 (36.4)	0.032
Baseline HCV RNA level (log IU/ml)	5.5 \pm 1.1	5.8 \pm 1.1	5.4 \pm 1.5	0.748
Achievement of an HCV SVR, n (%)	3 (60)	7 (70)	49 (74.2)	0.658
HBV DNA negativity EOT, n (%)	3 (60)	8 (80)	36 (54.5)	0.138
HBV DNA negativity 6 months after EOT, n (%)	4 (80)	5 (50)	27 (40.9)	0.344
HBV DNA negativity 1 year after EOT, n/N (%)	5/5 (100)	5/7 (71.4)	16/53(30.2)	0.004

HBsAg, hepatitis B s antigen; SD, standard deviation; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; SVR, sustained virologic response; EOT, end of treatment.

doi:10.1371/journal.pone.0020752.t004

negative. However, the influence of HBV genotype was not evaluated due to limited serum sample availability.

HBV DNA is another factor associated with HBsAg seroclearance. Several studies reported that serum HBV DNA becomes undetectable 1 to 2 years after HBsAg seroclearance [22,33]. In the present study, HBV DNA negativity 1 year after EOT was the most significant predictive factor of HBsAg seroclearance. We also found a 53.3% 5-year cumulative incidence of HBsAg seroclearance in patients with HBV DNA negativity 1 year after EOT. The findings implied that HBsAg seroclearance might be promoted by IFN/RBV in HBV/HCV dually-infected patients through the achievement of HBV DNA seroclearance.

Interestingly, we found 35.5% of patients who had HBV DNA negativity 6 months after EOT had reappearance of HBV DNA 1 year after EOT. The positive predictive value of HBsAg seroclearance in patients with HBV DNA negativity 6 months after EOT was only 25%. Using the corresponding SVR definition of HCV is, therefore, not suitable for HBV response, and cannot predict subsequent HBsAg seroclearance. Three of six (50%) patients who had detectable HBV DNA 6 months after EOT and became undetectable 1 year after EOT had HBsAg seroclearance in the long-term follow-up period. Based on this result, HBV DNA 1 year after EOT, instead of 6 months after EOT, might be recommended for predicting HBsAg seroclearance in clinical settings.

We also observed that liver cirrhosis was another predictive factor for HBsAg seroclearance. Increasing age has been associated with HBsAg seroclearance [34,35]. In the current study, HBsAg seroclearance rate in older patients tended to be higher than that of their counterparts. A longer time period of infection, an older age and a lower HBV DNA level are at risk of cirrhosis and may largely contribute to this observation.

More recently, the low baseline levels of HBsAg was demonstrated to be significantly associated with HBsAg clearance 6 months after antiviral therapy in patients dually infected with HBV/HCV [20]. However, we did not quantify the levels of HBsAg due to no serum sample available.

References

- Chuang WL, Chang WY, Lu SN, Su WP, Lin ZY, et al. (1992) The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. A case-control study. *Cancer* 69: 2052–2054.
- Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, et al. (2004) Impact of acute hepatitis C virus seroconversion in patients with chronic hepatitis B virus infection. *Gastroenterology* 126: 1024–1029.
- Kaklamani E, Trichopoulos D, Tzonou A, Zavitsanos X, Koumantaki Y, et al. (1991) Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. *JAMA* 265: 1974–1976.
- Yang JF, Lin CI, Huang JF, Dai CY, Lin WY, et al. (2010) Viral Hepatitis Infections in Southern Taiwan: A Multicenter Community-based Study. *Kaohsiung J Med Sci* 26: 461–469.
- Donato F, Boffetta P, Puoti M (1998) A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer* 75: 347–354.
- Yang HI, Lu SN, Liaw YF, You SL, Sun CA, et al. (2002) Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 347: 168–174.
- Hsu HY, Chang MH, Lee CY, Chen JS, Hsu HC, et al. (1992) Spontaneous loss of HBsAg in children with chronic hepatitis B virus infection. *Hepatology* 15: 382–386.
- Villeneuve JP, Desrochers M, Infante-Rivard C, Willems B, Raymond G, et al. (1994) A long-term follow-up study of asymptomatic hepatitis B surface antigen-positive carriers in Montreal. *Gastroenterology* 106: 1000–1005.
- Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC (1991) Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. *Hepatology* 13: 627–631.
- Liu J, Yang HI, Lee MH, Lu SN, Jen CL, et al. (2010) Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. *Gastroenterology* 139: 474–482.
- Andersson KL, Chung RT (2009) Monitoring during and after antiviral therapy for hepatitis B. *Hepatology* 49: S166–173.
- Liaw YF, Chu CM (2009) Hepatitis B virus infection. *Lancet* 373: 582–592.
- Pontisso P, Gerotto M, Benvegna L, Chemello L, Alberti A (1998) Coinfection by hepatitis B virus and hepatitis C virus. *Antivir Ther* 3: 137–142.
- Liu CJ, Chen PJ, Chen DS (2009) Dual chronic hepatitis B virus and hepatitis C virus infection. *Hepatology* 49: 517–525.
- Pothoff A, Wedemeyer H, Boecher WO, Berg T, Zeuzem S, et al. (2008) The HEP-NET B/C co-infection trial: A prospective multicenter study to investigate the efficacy of pegylated interferon-alpha2b and ribavirin in patients with HBV/HCV co-infection. *J Hepatol* 49: 688–694.
- Chuang WL, Dai CY, Chang WY, Lee LP, Lin ZY, et al. (2005) Viral interaction and responses in chronic hepatitis C and B coinfecting patients with interferon-alpha plus ribavirin combination therapy. *Antivir Ther* 10: 125–133.
- Hung CH, Lee CM, Lu SN, Wang JH, Tung HD, et al. (2005) Combination therapy with interferon-alpha and ribavirin in patients with dual hepatitis B and hepatitis C virus infection. *J Gastroenterol Hepatol* 20: 727–732.
- Liu CJ, Chen PJ, Lai MY, Kao JH, Jeng YM, et al. (2003) Ribavirin and interferon is effective for hepatitis C virus clearance in hepatitis B and C dually infected patients. *Hepatology* 37: 568–576.
- Liu CJ, Chuang WL, Lee CM, Yu ML, Lu SN, et al. (2009) Peginterferon alfa-2a plus ribavirin for the treatment of dual chronic infection with hepatitis B and C viruses. *Gastroenterology* 136: 496–504 e493.
- Yu ML, Lee CM, Chuang WL, Lu SN, Dai CY, et al. (2010) HBsAg profiles in patients receiving peginterferon alfa-2a plus ribavirin for the treatment of dual chronic infection with hepatitis B and C viruses. *J Infect Dis* 202: 86–92.
- Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. (2009) Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. *Gastroenterology* 136: 2169–2179, e2161–2164.
- Yuen MF, Wong DK, Sablon E, Tse E, Ng IO, et al. (2004) HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. *Hepatology* 39: 1694–1701.
- Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hamm HW, et al. (1999) Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 341: 1256–1263.

HBsAg seroconversion is not infrequent in Caucasian patients undergoing IFN therapy [36], but is very rare in the Asian population [37]. A recent study demonstrated a 3.5% HBsAg seroconversion rate at 3 years post-treatment in HBeAg-negative patients treated with pegylated IFN- α -2a [21]. In the present study, the 5-year cumulative incidence of HBsAg seroconversion was 8.3%. The fact that a substantially higher rate of HBsAg seroconversion was found in our study as compared to studies of CHB mono-infection suggests that HCV superinfection may interact with HBV and enhance the appearance of anti-HBs after loss of HBsAg [38,39].

In conclusion, we demonstrated that interferon- α /ribavirin had long-term effects on HBsAg seroclearance in dually HBV/HCV infected patients with a 5-year cumulative incidence of approximately 25%. Baseline cirrhosis and HBV DNA seronegativity 1 year after EOT were significant factors predicting HBsAg seroclearance. Those patients with sustained undetectable HBV DNA after combination therapy had a higher probability of late HBsAg seroclearance. The 5-year cumulative incidence of HBsAg seroconversion was 8.3%. HBV DNA negativity at baseline and one year after EOT had a trend for HBsAg seroconversion.

Acknowledgments

The authors thank secretary and serum processing help from Taiwan Liver Research Foundation (TLRF). The foundation did not influence how the study was conducted or the approval of the manuscript. The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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24. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, et al. (2000) Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology* 32: 828–834.
25. Perrillo RP, Lai CL, Liaw YF, Dienstag JL, Schiff ER, et al. (2002) Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 36: 186–194.
26. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, et al. (2008) Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med* 359: 2442–2455.
27. Janssen HL, Gerken G, Carreno V, Marcellin P, Naoumov NV, et al. (1999) Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 30: 238–243.
28. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, et al. (2008) Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology* 135: 459–467.
29. Chu CM, Yeh CT, Liaw YF (1998) Low-level viremia and intracellular expression of hepatitis B surface antigen (HBsAg) in HBsAg carriers with concurrent hepatitis C virus infection. *J Clin Microbiol* 36: 2084–2086.
30. Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, et al. (2001) Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. *Hepatology* 34: 404–410.
31. Liaw YF (2002) Hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. *J Gastroenterol* 37 Suppl 13: 65–68.
32. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, et al. (2006) Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 101: 297–303.
33. Lioriot MA, Marcellin P, Bismuth E, Martinot-Peignoux M, Boyer N, et al. (1992) Demonstration of hepatitis B virus DNA by polymerase chain reaction in the serum and the liver after spontaneous or therapeutically induced HBeAg to anti-HBe or HBsAg to anti-HBs seroconversion in patients with chronic hepatitis B. *Hepatology* 15: 32–36.
34. Chu CM, Liaw YF (2007) HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. *Hepatology* 45: 1187–1192.
35. Tai DI, Tsay PK, Chen WT, Chu CM, Liaw YF (2010) Relative roles of HBsAg seroclearance and mortality in the decline of HBsAg prevalence with increasing age. *Am J Gastroenterol* 105: 1102–1109.
36. Manns MP (2002) Current state of interferon therapy in the treatment of chronic hepatitis B. *Semin Liver Dis* 22 Suppl 1: 7–13.
37. Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, et al. (2001) Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: The effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology* 34: 139–145.
38. Chien RN (2008) Current therapy for hepatitis C or D or immunodeficiency virus concurrent infection with chronic hepatitis B. *Hepatol Int* 2: 296–303.
39. Bini EJ, Perumalswami PV (2010) Hepatitis B virus infection among American patients with chronic hepatitis C virus infection: prevalence, racial/ethnic differences, and viral interactions. *Hepatology* 51: 759–766.