Predictors for early HBeAg loss during lamivudine therapy in HBeAg-positive chronic hepatitis B patients with acute exacerbation

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Abstract

Purpose To examine the rate of early HBeAg loss and predictors of HBeAg loss in HBeAg-positive chronic hepatitis B (CHB) patients with acute exacerbation (AE) treated with lamivudine.

Methods A total of 146 patients diagnosed with CHB and AEs were included in this retrospective study. Patients were divided into two groups: decompensated and compensated.

Results The mean treatment duration for the decompensated and compensated groups was 18.1 and 19.9 months, respectively. Decompensated patients were significantly older and had a higher prevalence of cirrhosis and genotype B infection than compensated patients. Compared to compensated patients, decompensated patients achieved a higher rate of HBeAg loss (25.8 vs. 14.3%; $P = 0.0805$) at 3 months of therapy, a higher rate of serum HBV DNA negativity (53.2 vs. 29.8%; $P = 0.0042$), and a lower rate

of rtM204V/I mutation (3.2 vs. 16.7%; $P = 0.0139$) after 12 months of lamivudine therapy. The rates of HBeAg loss after 6 and 12 months of lamivudine therapy were similar between the two groups. Logistic regression analysis revealed that female gender and baseline ALT level $\geq 1,000$ IU/L, but not decompensations, were significant predictors of HBeAg loss at 3 months; however, only female gender was a significant predictor of HBeAg loss after 6 and 12 months of lamivudine therapy. The early HBeAg losers showed a significantly higher sustained remission rate off lamivudine therapy.

Conclusions Female gender and baseline serum ALT level $\geq 1,000$ IU/L were independent predictors of early HBeAg loss during lamivudine therapy in HBeAg-positive CHB patients with AE.

Keywords Acute exacerbation · Chronic hepatitis B · Decompensation · HBeAg loss · Lamivudine

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Abbreviations

AE	Acute exacerbation
ALT	Alanine aminotransferase
AFP	Alpha-fetoprotein
AST	Aspartate aminotransferase
CHB	Chronic hepatitis B
CI	Confidence interval
HAV	Hepatitis A virus
HBeAg	Hepatitis B e antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
OR	Odds ratio
PT	Prothrombin time
RFLP	Restriction fragment length polymorphism

rtM204V/I	Reverse transcriptase domain 204 methionine-to-valine/isoleucine mutation
SD	Standard deviation
ULN	Upper limit of normal

Introduction

Chronic hepatitis B (CHB) is a common disease with an estimated prevalence of 350 million carriers worldwide. One important landmark in the natural history of CHB is hepatitis B e antigen (HBeAg) seroconversion [1, 2]. The major feature of the disease course preceding HBeAg seroconversion is recurrent serum alanine aminotransferase (ALT) elevations. It is not uncommon to encounter episodes with abrupt ALT elevations to a level over five times the upper limit of normal (ULN), defined as acute exacerbation (AE) in previous studies [2, 3]. The clinical manifestations of AEs vary from absence of symptoms to the typical symptoms of acute hepatitis. Some patients may develop hepatic decompensation or failure [2–4]. Although AE represents the host's immune effort to clear the replicating hepatitis B virus (HBV), how different subsets of HBV-specific T cells, such as CD4+ and CD8+ T cells, and HBV-non-specific inflammatory cells interact to bring about variable degrees of hepatic necroinflammation remains poorly understood [2, 3, 5–7].

Lamivudine is a nucleoside analog with a rapid and potent inhibitory effect on HBV replication [8]. Given its rapid onset of action, lamivudine has been used in the setting of AE, particularly when complicated with decompensation and fulminant hepatic failure [9–14]. Previous clinical trials in HBeAg-positive patients have shown that pretherapy serum ALT level is an important predictor of subsequent HBeAg seroconversion following the institution of lamivudine therapy [15, 16]. Patients with pretherapy ALT levels >5 times the ULN have an HBeAg seroconversion rate of 64% after 1 year of therapy [15]. Only a few studies comprising a small number of patients have reported the long-term therapeutic efficacy of lamivudine in HBeAg-positive patients with AE and decompensation [17–19]. Since hepatic decompensation is often associated with more extensive hepatocyte death and higher ALT levels [4], this clinical setting usually reflects a more vigorous immune response against HBV. However, the early HBeAg loss rates following the institution of lamivudine therapy in HBeAg-positive CHB patients experiencing AE either with or without decompensation have not yet been directly compared in the same study. Furthermore, predictors of early HBeAg loss during the first year following the institution of lamivudine therapy in

HBeAg-positive CHB patients with AE are not clearly defined. Therefore, we conducted this retrospective analysis to examine the early HBeAg loss rate and its predictors during lamivudine therapy in a consecutive cohort of HBeAg-positive CHB patients with AE, including those complicated with clinical decompensation.

Patients and methods

Study design

This retrospective cohort study was performed at the liver unit of the China Medical University Hospital between January 2003 and July 2008. All patients gave written informed consent and the study protocol was approved by the institutional review board of the hospital. Included patients were those who: were >18 years; were HBsAg-positive for >6 months; were HBeAg-positive; presented with episodes of ALT elevations >5 times the ULN (i.e., >200 IU/L); fulfilled the criteria for AE; had not previously received antiviral therapy. Included patients were prescribed lamivudine 100 mg per day. The following patients were excluded: patients who presented with AEs that were associated with anticancer chemotherapy or transarterial chemoembolization for hepatocellular carcinoma; those who showed evidence of superinfection with hepatitis A virus (HAV), hepatitis C virus (HCV) or hepatitis D virus (HDV); those who had evidence of hepatocellular carcinoma and/or reported a past history of habitual alcohol consumption, intravenous drug abuse, homosexual activity or prostitution.

Liver cirrhosis was diagnosed based on at least two of the following three findings: platelet count $<100 \times 10^9/L$, evidence of esophageal varices on endoscopy, and ultrasonographic features consistent with cirrhosis [20]. The therapeutic end point was initially planned to be 6 months after HBeAg seroconversion; however, the actual duration of consolidation therapy was a joint decision made by the attending physician and the patient because the Bureau of National Health Insurance only reimbursed 18 months of lamivudine therapy regardless of the timing of HBeAg loss or seroconversion.

In total, 146 patients were included in the current study. Eligible patients who had received lamivudine for more than 1 year ($N = 136$) or who had already achieved early HBeAg loss despite only receiving lamivudine for less than 1 year ($N = 10$) were consecutively enrolled in this study. Serum albumin, aspartate aminotransferase (AST), ALT, bilirubin, creatinine and prothrombin time (PT) were measured before, during and after treatment with lamivudine. Peak serum alpha-fetoprotein (AFP) levels associated with AE were determined by 3–4 weekly measurements

following the onset of serum ALT elevation. Among the 146 included patients, 62 (42.5%) developed hepatic decompensation with prolongation of PT ≥ 3 s over control and serum total bilirubin level ≥ 2 mg/dL.

Virological study

Virological markers including serum HBsAg, HBeAg, anti-HBe antibody, anti-HCV antibody, IgM anti-HAV, HBV DNA and HBV genotype were measured before administration of lamivudine. Serum HBeAg and anti-HBe were monitored every 3 months and serum HBV DNA was monitored every 6–12 months during and after treatment with lamivudine. The HBsAg, HBeAg, anti-HBe, anti-HCV and IgM anti-HAV were assayed using commercially available enzyme immunoassays (Vitros ECI, Ortho-Clinical Diagnostics, High Wycombe, Buckinghamshire, UK) and the anti-HDV was analyzed by radioimmunoassay (General Biological Cooperation, Hsinchu, Taiwan). All blood samples were processed and stored at -80°C until the time of analysis. Isolation of nucleic acids was performed using the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany). To obtain 40 μL of nucleic acid extract, 250 μL of serum was used. A real-time quantitative PCR assay utilizing a LightCycler (Roche), with a lower limit of detection of 100 copies/mL, was used to measure serum HBV DNA as previously described [21]. For the genotyping of HBV, part of the surface gene was first amplified by PCR, then restriction fragment length polymorphism (RFLP) was performed as described [22]. For the detection of the rtM204V/I mutation, a direct PCR sequencing analysis of amino acids 66–237 of the reverse transcriptase domain was performed. The primers were: 5'-CTCCARTCACTACCAAC-3' and 5'-GGGTTYAAA TGTATACCCA-3'. The PCR product was purified and subjected to direct sequence analysis using the primer 5'-GTATTCATCCCC-3'. The assay can detect the rtM204V/I mutation when the mutant represents at least 20% of the viral population.

Statistical analysis

Continuous data were expressed as mean $\pm$ standard deviation (SD) or median. Categorical data were expressed by frequency and proportion. Baseline characteristics were compared among patients with and without decompensation by Student's *t* test or Mann–Whitney *U* test for continuous variables, and by conventional Chi-square test or Fisher's exact test (if cell count < 5) for categorical variables. Predictors for HBeAg loss on lamivudine therapy were determined by multivariate analysis using logistic regression models. *P* value of < 0.05 was considered statistically significant. All calculations were carried out using

a standard statistical package (SPSS for Windows, version 12, SPSS Inc, Chicago, IL, USA).

Results

Study population

The baseline characteristics of the two groups including gender, serum creatinine level and serum HBV DNA level were not significantly different (Table 1). The decompensated group was composed of significantly older patients (36.1 ± 12.3 vs. 32.2 ± 7.9 ; $P = 0.0282$), more cirrhotic patients (9.7 vs. 1.2%; $P = 0.042$) and more genotype B-infected patients (87.5 vs. 66.3%; $P = 0.0047$). They presented with significantly lower serum albumin level (3.3 ± 0.5 g/dL vs. 4.0 ± 0.3 g/dL; $P < 0.0001$), higher ALT level (1505.6 ± 828.1 IU/L vs. 507.6 ± 303.4 IU/L; $P < 0.0001$), higher PT (7.53 ± 4.95 s prolonged over control vs. 1.95 ± 1.06 s prolonged over control; $P < 0.0001$) and higher total bilirubin level (9.6 ± 6.9 mg/dL vs. 1.3 ± 1.2 mg/dL; $P < 0.0001$). In those patients whose peak serum AFP levels following AE were adequately determined, the decompensated patients had a significantly higher median AFP level (237.5 ng/mL, range 7.72–2810 vs. 15.2 ng/mL, range 1.57–192; $P < 0.0001$).

Therapeutic outcome

The mean duration of lamivudine therapy for the decompensated group and the compensated group was 18.1 ± 7.4 and 19.9 ± 7.1 months, respectively ($P = 0.1466$). Nine patients in the decompensated group and one patient in the compensated group received less than 1 year of lamivudine therapy, but achieved HBeAg loss (14.5 vs. 1.2%; $P = 0.002$). Out of the ten patients who received lamivudine for less than 1 year, six received a mean total duration of therapy for 10 ± 2.1 months because they all achieved HBeAg seroconversion within 3 months of therapy (mean duration of consolidation therapy: 7.0 ± 1.5 months). The other four patients achieved HBeAg loss or seroconversion after 6.5 ± 4.2 months of lamivudine therapy and were still remaining on therapy at the end of the study period (July 2008). Twenty-five patients (40.3%) in the decompensated group discontinued lamivudine therapy. Of them, 8, 11 and 6 patients achieved HBeAg loss, seroconversion and remained HBeAg-positive at the end of therapy, respectively. After a median post-treatment follow-up duration of 4.8 months (range 1–26), five and seven patients with HBeAg loss and seroconversion (12/19 = 63.2%), respectively, relapsed with abnormal serum ALT levels. A total of 42 patients (50%) in the compensated

Table 1 Baseline characteristics of 146 HBeAg-positive chronic hepatitis B patients experiencing acute exacerbation with or without decompensation

	Total (<i>N</i> = 146)	Decompensated (<i>N</i> = 62)	Compensated (<i>N</i> = 84)	<i>P</i> value
Male/female	109/37	48/14	61/23	0.5098 ^a
Age (years old)	33.8 ± 10.2	36.1 ± 12.3	32.2 ± 7.9	0.0282 ^b
<40	107 (73.3%)	41 (66.1%)	66 (78.6%)	
≥40	39 (26.7%)	21 (33.9%)	18 (21.4%)	0.0930 ^a
Liver cirrhosis	7 (4.8%)	6 (9.7%)	1 (1.2%)	0.0420 ^d
Albumin	3.5 ± 0.6	3.3 ± 0.5	4.0 ± 0.3	<0.0001 ^b
Baseline ALT level (IU/L)	931.4 ± 765.6	1,505.6 ± 828.1	507.6 ± 303.4	<0.0001 ^b
200–400	40 (27.4%)	3 (4.8%)	37 (44%)	
400–1,000	57 (39%)	15 (24.2%)	42 (50%)	
≥1,000	49 (33.6%)	44 (71%)	5 (6%)	<0.0001 ^d
Baseline PT: seconds prolonged over control	4.40 ± 4.13	7.53 ± 4.95	1.95 ± 1.06	<0.0001 ^b
<3	88 (60.3%)	4 (6.5%)	84 (100%)	
3–5	18 (12.3%)	18 (29%)	(0%)	
5–10	25 (17.1%)	25 (40.3%)	(0%)	
≥10	15 (10.3%)	15 (24.2%)	(0%)	<0.0001 ^a
Baseline total bilirubin level (mg/dL)	5.9 ± 6.6	9.6 ± 6.9	1.3 ± 1.2	<0.0001 ^b
0.2–2	76 (52%)	1 (1.6%)	75 (89.3%)	
2–5	26 (17.8%)	18 (29%)	8 (9.5%)	
5–10	22 (15.1%)	21 (33.9%)	1 (1.2%)	
≥10	22 (15.1%)	22 (35.5%)	0 (0%)	<0.0001 ^a
Creatinine (mg/dL)	0.90 ± 0.18	0.90 ± 0.21	0.90 ± 0.16	0.9578 ^b
Peak AFP level (ng/mL)	70.1 (1.57–2,810)	237.5 (7.72–2,810)	15.2 (1.57–192)	<0.0001 ^c
	<i>N</i> = 90	<i>N</i> = 50	<i>N</i> = 40	
<20	27 (30%)	2 (4%)	25 (62.5%)	
20–100	27 (30%)	14 (28%)	13 (32.5%)	
≥100	36 (40%)	34 (68%)	2 (5%)	<0.0001 ^a
Log HBV DNA (copies/mL)	7.10 (3.62–9.88)	7.10 (3.62–9.88)	7.14 (4.09–9.76)	0.5466 ^c
	<i>N</i> = 129	<i>N</i> = 55	<i>N</i> = 74	
<6	30 (23.3%)	14 (25.5%)	16 (21.6%)	
6–8	68 (52.7%)	28 (50.9%)	40 (54.1%)	
≥8	31 (24%)	13 (23.6%)	18 (24.3%)	0.8760 ^a
Genotype (B/C)	104/35 (74.8%/25.2%)	49/7 (87.5%/12.5%)	55/28 (66.3%/33.7%)	0.0047 ^a
	<i>N</i> = 139	<i>N</i> = 56	<i>N</i> = 83	

Data are expressed as mean ± SD, median or *N* (%)

ALT alanine aminotransferase, PT prothrombin time, AFP alpha-fetoprotein

^a Chi-square test (for association)^b Student's *t* test (for means)^c Mann–Whitney *U* test (for medians)^d Fisher's exact test (for association)

group discontinued lamivudine therapy, of whom 13, 21 and 8 patients achieved HBeAg loss, seroconversion and remained HBeAg-positive at the end of therapy, respectively. After a median post-treatment follow-up duration of 6.0 months (range 1.5–38), eight and nine patients

with HBeAg loss and seroconversion (17/34 = 50%), respectively, relapsed with abnormal serum ALT levels. Nine (14.5%) and seven (11.3%) patients in the decompensated group developed ascites and hepatic encephalopathy during treatment, respectively. Of them, five

patients (8%) developed both complications. One patient achieved HBeAg loss after 1 month of lamivudine therapy, but underwent orthotopic liver transplantation due to hepatic failure after 2 months of lamivudine therapy. None of them died during the study period. None in the compensated group developed such complications.

The ALT normalization rates in the two groups were not significantly different (91.9 vs. 95.2%; $P = 0.4954$) after 12 months of therapy. The decompensated group achieved a marginally higher HBeAg loss rate than the compensated group at 3 months of therapy (25.8 vs. 14.3%; $P = 0.0805$). However, the HBeAg loss rates were not statistically different after 6 and 12 months of therapy (Table 2). The HBeAg seroconversion rates were not significantly different after 3, 6 and 12 months of therapy (Table 2). The proportion of patients with undetectable serum HBV DNA levels after 12 months of therapy was significantly higher in the decompensated group (53.2 vs. 29.8%; $P = 0.0042$). The incidence of the rtM204V/I mutation after 12 months of therapy was significantly lower in the decompensated group (3.2 vs. 16.7%; $P = 0.0139$).

Baseline factors associated with early HBeAg loss on lamivudine therapy

We next analyzed the baseline factors associated with HBeAg loss after 3, 6 and 12 months of lamivudine treatment in the whole cohort by univariate analysis, including gender, age, liver cirrhosis, decompensation, baseline ALT level, baseline PT, baseline total bilirubin level, peak AFP level, baseline HBV DNA level and HBV genotype. At 3 months of therapy, female gender (odds ratio (OR) 2.79; 95% confidence interval (CI): 1.18–6.67; $P = 0.0178$) and baseline ALT level $\geq 1,000$ IU/L (OR 5.44; 95% CI: 1.58–18.74; $P = 0.0080$) were significantly associated with HBeAg loss, but decompensation (OR 2.09; 95% CI: 0.91–4.78; $P = 0.0805$) was only marginally associated with HBeAg loss (Table 3). None of the other factors were significantly associated with HBeAg loss. At 6 and 12 months of therapy, only female gender was significantly associated with HBeAg loss (OR 3.03; 95% CI: 1.37–6.68; $P = 0.0051$ and OR 2.44; 95% CI: 1.15–5.26; $P = 0.0190$, respectively). None of the other factors were significantly associated with HBeAg loss (Tables 4, 5).

Table 2 Therapeutic outcome of decompensated and compensated HBeAg-positive chronic hepatitis B patients with acute exacerbation on lamivudine therapy

	Total ($N = 146$)	Decompensated ($N = 62$)	Compensated ($N = 84$)	P value
Duration of therapy (months)	19.1 $\pm$ 7.3	18.1 $\pm$ 7.4	19.9 $\pm$ 7.1	0.1466
Treatment with lamivudine for less than 1 year	10 (6.8%)	9 (14.5%)	1 (1.2%)	0.0020*
Patients discontinuing lamivudine treatment after 1 year	67 (45.9%)	25 (40.3%)	42 (50%)	0.2461
Post-treatment follow-up duration (months)	5.5 (1–38)	4.8 (1–26)	6.0 (1.5–38)	0.1714
	$N = 67$	$N = 25$	$N = 42$	
Relapse after discontinuing treatment	43/67 (64.2%)	18/25 (72%)	25/42 (59.5%)	0.3030
Relapse among HBeAg losers	29/53 (54.7%)	12/19 (63.2%)	17/34 (50%)	0.3651
Ascites	9 (6.2%)	9 (14.5%)	0	0.0003
Encephalopathy	7 (4.8%)	7 (11.3%)	0	0.0020
ALT normalization (12 M)	137 (93.8%)	57 (91.9%)	80 (95.2%)	0.4954*
HBeAg loss				
3 M	28 (19.2%)	16 (25.8%)	12 (14.3%)	0.0805
6 M	41 (28.1%)	21 (33.9%)	20 (23.8%)	0.1812
12 M	59 (40.4%)	27 (43.5%)	32 (38.1%)	0.5069
HBeAg seroconversion				
3 M	16 (11%)	7 (11.3%)	9 (10.7%)	0.9123
6 M	27 (18.5%)	12 (19.4%)	15 (17.9%)	0.8178
12 M	34 (23.3%)	14 (22.6%)	20 (23.8%)	0.8621
HBV DNA				
<100 copies/mL (12 M)	58 (39.7%)	33 (53.2%)	25 (29.8%)	0.0042
rtM204V/I mutation (12 M)	16 (11%)	2 (3.2%)	14 (16.7%)	0.0139

Data are expressed as mean $\pm$ SD, median or N (%)

ALT alanine aminotransferase, M month

* Fisher's exact test

Table 3 Baseline factors associated with HBeAg loss after 3 months of lamivudine therapy

Response rates by factor	Responders, N/subtotal (%)	Odds ratio	95% CI	P value
Gender				
Male	16/109 (14.7%)	1	–	0.0178
Female	12/37 (32.4%)	2.79	(1.18, 6.67)	
Age (years old)				
<30	9/56 (16.1%)	1	–	
30–40	10/51 (19.6%)	1.27	(0.47, 3.45)	0.6326
≥40	9/39 (23.1%)	1.19	(0.56, 4.40)	0.3914
Liver cirrhosis				
Present	2/7 (28.6%)	1.74	(0.32, 9.34)	0.6195*
Absent	26/139 (18.7%)	1	–	
Decompensation				
Present	16/62 (25.8%)	2.09	(0.91, 4.78)	0.0805
Absent	12/84 (14.3%)	1	–	
Baseline ALT level (IU/L)				
200–400	3/40 (7.5%)	1	–	
400–1,000	10/57 (17.5%)	2.62	(0.69, 9.92)	0.1529*
≥1,000	15/49 (30.6%)	5.44	(1.58, 18.74)	0.0080
Baseline PT (seconds prolonged over control)				
<3	14/88 (15.9%)	1	–	
3–5	5/18 (27.8%)	2.03	(0.63, 6.54)	0.2316*
5–10	5/25 (20%)	1.32	(0.42, 4.12)	0.2838*
≥10	4/15 (26.7%)	1.92	(0.54, 6.84)	0.3105*
Baseline total bilirubin level (mg/dL)				
0.2–2	11/76 (14.5%)	1	–	
2–5	6/26 (23.1%)	1.77	(0.58, 5.38)	0.3096
5–10	5/22 (22.7%)	1.74	(0.53, 5.66)	0.3563*
≥10	6/22 (27.3%)	2.22	(0.72, 6.81)	0.1627
Peak AFP level (ng/mL)				
<20	3/27 (11.1%)	1	–	
20–100	7/27 (25.9%)	2.80	(0.65, 11.98)	0.2935
≥100	7/36 (19.4%)	1.93	(0.45, 8.24)	0.4944
Log HBV DNA (copies/mL)				
<6	8/30 (26.7%)	1	–	
6–8	13/68 (19.1%)	0.65	(0.24, 1.79)	0.4013
≥8	5/31 (16.1%)	0.53	(0.15, 1.85)	0.3627
Genotype				
B	20/104 (19.2%)	1.15	(0.42, 3.15)	0.7841
C	6/35 (17.1%)	1	–	

Data are expressed as N (%)

ALT alanine aminotransferase, PT prothrombin time, AFP alpha-fetoprotein, CI confidence interval

* Fisher's exact test

Baseline predictors of early HBeAg loss on lamivudine therapy

The independent predictive value of gender, age, decompensation, baseline ALT level, peak AFP level and baseline HBV DNA level for early HBeAg loss following the institution of lamivudine therapy was determined by using stepwise logistic regression analysis. Female gender was an

independent predictor of HBeAg loss at 3, 6 and 12 months of therapy (OR 3.74; 95% CI: 1.44–9.73; $P = 0.0069$; OR 3.56; 95% CI: 1.55–8.18; $P = 0.0028$; OR 2.70; 95% CI: 1.23–5.92; $P = 0.0133$, respectively, Table 6). Baseline ALT level $\geq 1,000$ IU/L was a significant predictor of HBeAg loss at 3 months (OR 9.30; 95% CI: 1.52–56.82; $P = 0.0157$), but not at 6 months (OR 2.60; 95% CI: 0.65–10.32; $P = 0.1755$) or 12 months (OR 1.18; 95% CI:

Table 4 Baseline factors associated with HBeAg loss after 6 months of lamivudine therapy

Response rates by factor	Responders, <i>N</i> /subtotal (%)	Odds ratio	95% CI	<i>P</i> value
Gender				
Male	24/109 (22%)	1	–	0.0051
Female	17/37 (45.9%)	3.03	(1.37, 6.68)	
Age (years old)				
<30	15/56 (26.8%)	1	–	
30–40	13/51 (25.5%)	0.94	(0.39, 2.23)	0.8790
≥40	13/39 (33.3%)	1.37	(0.56, 3.34)	0.4911
Liver cirrhosis				
Present	3/7 (42.9%)	1.99	(0.44, 9.13)	0.4013*
Absent	38/139 (27.3%)	1	–	
Decompensation				
Present	21/62 (33.9%)	1.64	(0.79, 3.39)	0.1812
Absent	20/84 (23.8%)	1	–	
Baseline ALT level (IU/L)				
200–400	10/40 (25%)	1	–	
400–1,000	12/57 (21.1%)	0.80	(0.31, 2.09)	0.6477
≥1,000	19/49 (38.8%)	1.90	(0.76, 4.75)	0.1678
Baseline PT (seconds prolonged over control)				
<3	22/88 (25%)	1	–	
3–5	7/18 (38.9%)	1.91	(0.66, 5.50)	0.2285
5–10	8/25 (32%)	1.41	(0.54, 3.73)	0.4843
≥10	4/15 (26.7%)	1.09	(0.31, 3.80)	0.8908*
Baseline total bilirubin level (mg/dL)				
0.2–2	19/76 (25%)	1	–	
2–5	10/26 (38.5%)	1.88	(0.73, 4.81)	0.1890
5–10	6/22 (27.3%)	1.13	(0.38, 3.30)	0.8295
≥10	6/22 (27.3%)	1.13	(0.38, 3.30)	0.8295
Peak AFP level (ng/mL)				
<20	5/27 (18.5%)	1	–	
20–100	7/27 (25.9%)	1.54	(0.42, 5.68)	0.5127
≥100	11/36 (30.6%)	1.94	(0.58, 6.44)	0.2774
Log HBV DNA (copies/mL)				
<6	9/30 (30%)	1	–	
6–8	20/68 (29.4%)	0.97	(0.38, 2.50)	0.9531
≥8	8/31 (25.8%)	0.81	(0.26, 2.51)	0.7150
Genotype				
B	29/104 (27.9%)	1.12	(0.47, 2.68)	0.8032
C	9/35 (25.7%)	1	–	

Data are expressed as *N* (%)

ALT alanine aminotransferase, PT prothrombin time, AFP alpha-fetoprotein, CI confidence interval

* Fisher's exact test

0.35–3.99; *P* = 0.7957) of therapy (Table 6). No other baseline factor was identified as an independent predictor of early HBeAg loss on lamivudine therapy.

Long-term outcomes of the early HBeAg losers during lamivudine therapy

We addressed whether the 59 patients who lost HBeAg during their first year of lamivudine therapy had better long-term outcomes than the 87 patients who did not.

During a mean follow-up duration of 48.0 ± 19.6 and 46.4 ± 20.5 months, respectively (*P* = 0.3542), the early HBeAg losers showed a significantly higher rate of sustained remission (HBeAg seroconversion and HBV DNA less than 10,000 copies/mL) off lamivudine therapy (30.5 vs. 14.9%; *P* = 0.024) than those who did not lose HBeAg during the first year despite with a similar duration of consolidation therapy after HBeAg seroconversion (8.4 ± 2.1 vs. 8.5 ± 2.6 months; *P* = 0.7431). However, the development of liver cirrhosis (0 vs. 1.2%; *P* = 0.41) and

Table 5 Baseline factors associated with HBeAg loss after 12 months of lamivudine therapy

Response rates by factor	Responders, <i>N</i> /subtotal (%)	Odds ratio	95% CI	<i>P</i> value
Gender				
Male	38/109 (34.9%)	1	–	0.0190
Female	21/37 (56.8%)	2.44	(1.15, 5.26)	
Age (years old)				
<30	22/56 (39.3%)	1	–	
30–40	20/51 (39.2%)	1.00	(0.46, 2.18)	0.9941
≥40	17/39 (43.6%)	1.19	(0.52, 2.70)	0.6748
Liver cirrhosis				
Present	3/7 (42.9%)	1.11	(0.24, 5.16)	1.0000*
Absent	56/139 (40.3%)	1	–	
Decompensation				
Present	27/62 (43.5%)	1.25	(0.64, 2.44)	0.5069
Absent	32/84 (38.1%)	1	–	
Baseline ALT level (IU/L)				
200–400	18/40 (45%)	1	–	
400–1,000	18/57 (31.6%)	0.56	(0.24, 1.30)	0.1780
≥1,000	23/49 (46.9%)	1.08	(0.47, 2.50)	0.8552
Baseline PT (seconds prolonged over control)				
<3	34/88 (38.6%)	1	–	
3–5	7/18 (38.9%)	1.01	(0.36, 2.86)	0.9840
5–10	13/25 (52%)	1.72	(0.70, 4.17)	0.2316
≥10	5/15 (33.3%)	0.79	(0.25, 2.50)	0.6955
Baseline total bilirubin level (mg/dL)				
0.2–2	31/76 (40.8%)	1	–	
2–5	12/26 (46.2%)	1.24	(0.51, 3.03)	0.6326
5–10	7/22 (31.8%)	0.68	(0.25, 1.85)	0.4469
≥10	9/22 (40.9%)	1.00	(0.38, 2.63)	0.9920
Peak AFP level (ng/mL)				
<20	9/27 (33.3%)	1	–	
20–100	10/27 (37%)	1.18	(0.38, 3.57)	0.7757
≥100	15/36 (41.7%)	1.43	(0.51, 4.00)	0.5003
Log HBV DNA (copies/mL)				
<6	12/30 (40%)	1	–	
6–8	26/68 (38.2%)	0.93	(0.39, 2.22)	0.8687
≥8	14/31 (45.2%)	1.23	(0.45, 3.45)	0.6836
Genotype				
B	42/104 (40.4%)	1.02	(0.47, 2.22)	0.9680
C	14/35 (40%)	1	–	

Data are expressed as *N* (%)

ALT alanine aminotransferase, PT prothrombin time, AFP alpha-fetoprotein, CI confidence interval

* Fisher's exact test

hepatocellular carcinoma (3.3 vs. 2.3%; $P = 0.692$) in the two groups was similar.

Discussion

With the advent of oral antiviral agents, initiation of oral antiviral therapy has become the standard of care in HBeAg-positive patients with AE and decompensation as soon as a clinical diagnosis has been achieved. Lamivudine

is the first available agent and has been commonly used in this setting. Whether HBeAg-positive CHB patients with AE and decompensation achieve earlier and higher HBeAg loss rates than patients with AE alone following the institution of lamivudine therapy remains unclear. The results of this study demonstrate that CHB patients with AE and decompensation tend to achieve a higher HBeAg loss rate after 3 months of lamivudine therapy than patients without decompensation (25.8 vs. 14.3%; $P = 0.0805$), but that HBeAg loss rates in patients with or without

Table 6 Multivariate logistic regression analysis of baseline predictors of early HBeAg loss during lamivudine therapy

Factors	Time points (M)	Univariate OR (95% CI)	<i>P</i> value	Multivariate OR (95% CI)	<i>P</i> value
Gender	3	2.79 (1.17, 6.65)	0.00178	3.74 (1.44, 9.73)	0.0069
Female	6	3.01 (1.37, 6.63)	0.0051	3.56 (1.55, 8.18)	0.0028
Male	12	2.45 (1.15, 5.25)	0.0190	2.70 (1.23, 5.92)	0.0133
Age (years)	3	1.39 (0.57, 3.40)	0.4700	2.02 (0.72, 5.67)	0.1824
≥40	6	1.41 (0.64, 3.12)	0.3940	1.75 (0.72, 4.24)	0.2161
<40	12	1.20 (0.57, 2.51)	0.6365	1.27 (0.57, 2.85)	0.5608
Decompensation	3	2.08 (0.91, 4.81)	0.0805	1.22 (0.28, 5.29)	0.7876
Present	6	1.64 (0.79, 3.39)	0.1812	1.24 (0.33, 4.59)	0.7518
Absent	12	1.25 (0.64, 2.44)	0.5069	1.01 (0.31, 3.28)	0.9834
Baseline ALT level (IU/L)					
200–400		1		–	–
400–1,000	3	2.62 (0.67, 10.23)	0.1645	3.42 (0.81, 14.54)	0.0952
≥1,000		5.44 (1.45, 20.45)	0.0122	9.30 (1.52, 56.82)	0.0157
Baseline ALT level (IU/L)					
200–400		1		–	–
400–1,000	6	0.80 (0.31, 2.09)	0.6480	0.89 (0.32, 2.50)	0.8259
≥1,000		1.90 (0.76, 4.76)	0.1705	2.60 (0.65, 10.32)	0.1755
Baseline ALT level (IU/L)					
200–400		1		–	–
400–1,000	12	0.56 (0.24, 1.30)	0.1799	0.59 (0.24, 1.44)	0.2425
≥1,000		1.08 (0.47, 2.50)	0.8552	1.18 (0.35, 3.99)	0.7957
Peak AFP level (ng/mL)	3	1.23 (0.49, 3.08)	0.6622	0.80 (0.25, 2.53)	0.7003
≥100	6	1.32 (0.59, 2.97)	0.4956	1.12 (0.39, 3.18)	0.8321
<100	12	1.17 (0.55, 2.49)	0.6845	1.05 (0.40, 2.77)	0.9246
Log HBV DNA (copies/mL)	3	0.81 (0.28, 2.34)	0.6961	1.06 (0.34, 3.30)	0.9197
≥7	6	0.91 (0.37, 2.26)	0.8465	1.00 (0.38, 2.61)	0.9989
<7	12	1.38 (0.62, 3.10)	0.4334	1.45 (0.62, 3.37)	0.3896

ALT alanine aminotransferase, AFP alpha-fetoprotein, CI confidence interval, M month, OR odds ratio

decompensation become similar after a longer treatment period (i.e., 6 and 12 months of lamivudine therapy). Decompensation by itself was not an independent predictor of HBeAg loss, even early after initiation of lamivudine therapy, whereas a higher baseline serum ALT level was an important predictor of early HBeAg loss. Specifically, patients with baseline serum ALT levels $\geq 1,000$ IU/L and between 400 IU/L and 1,000 IU/L showed incremental rates of HBeAg loss after 3 months of lamivudine therapy compared with patients with baseline ALT levels between 200 IU/L and 400 IU/L. The effects of baseline serum ALT levels on HBeAg loss became less pronounced after 6 and 12 months of lamivudine therapy. This finding is consistent with a recent observation by Tseng et al. [23] that HBeAg-positive CHB patients with pretherapy ALT levels >400 IU/L achieve a higher HBeAg seroconversion rate than patients with pretherapy ALT levels between 200 IU/L and 400 IU/L at 3 and 6 months, but not after 1 year of lamivudine therapy.

While it is not clear whether higher serum ALT levels result from a quantitative or qualitative difference in the host's immune response against HBV, higher serum ALT levels appear to reflect a strong immune activity and confer more effective control over the virus over a period of 3–6 months. Serum ALT level serves as a better marker for the magnitude of host immune response against HBV than clinical decompensation. The effect of higher serum ALT levels on HBeAg loss appears to wane after 3–6 months and patients with different categories of baseline serum ALT levels ultimately attained similar rates of HBeAg loss during the study period. The early HBeAg loss observed in some of the patients included in this study presumably results from the host immune response against HBV rather than the therapeutic effect of lamivudine. This was supported by our long-term observation that patients who lost HBeAg during their first year of lamivudine therapy showed a significantly higher rate of sustained remission off lamivudine therapy than those who did not lose HBeAg

during the first year of therapy. Further, it is unclear whether the achievement of similar rates of HBeAg loss over time occurred naturally or as a result of lamivudine therapy. The immunological mechanisms underlying this phenomenon remain to be investigated.

In this study, female gender was an independent predictor of HBeAg loss during the first year of lamivudine therapy. Specifically, female patients with AE achieved earlier and higher rates of HBeAg loss following the initiation of lamivudine therapy than their male counterparts. The majority of HBV infections are acquired during the neonatal period or early infancy in Taiwan [24, 25]. Earlier studies on the natural history of CHB showed that female patients had a lower rate of abnormal ALT levels and HBeAg seroclearance during the HBeAg-positive phase [26, 27]. The maximal serum ALT levels during the HBeAg-positive immune clearance phase were significantly lower in women [28] suggesting that there might be a gender difference in the magnitude of immune activity required to achieve HBeAg clearance. It is possible that female CHB patients require lower immune activity to clear HBeAg. Accordingly, given a similar severity of AE, female patients could be anticipated to achieve a higher rate of HBeAg loss than male patients once lamivudine therapy has been initiated.

This study showed that patients with AE and decompensation achieved a higher rate of serum HBV DNA negativity (53.2 vs. 29.8%; $P = 0.0042$) and had a lower rate of rtM204V/I mutation (3.2 vs. 16.7%; $P = 0.0139$) than patients with AE alone after 1 year of lamivudine therapy. This finding is consistent with previous studies demonstrating that severe AEs might reduce the risk of rtM204V/I mutation and virological breakthrough during lamivudine therapy in HBeAg-positive CHB patients [17–19]. Since serum HBV DNA levels were not measured at early time points during lamivudine therapy in the majority of included patients, it is not possible to compare the early viral kinetics between the two groups of patients during lamivudine therapy.

There are several limitations of our study that are worthy of mentioning. First, this was a retrospective study and only enrolled previously antiviral treatment-naïve patients who received lamivudine therapy. Therefore, the patient cohort could represent a special group of patients due to selection bias. Secondly, the baseline serum ALT levels before the patients started lamivudine therapy might have been affected by the timing of their presentation to our medical care after the onset of AE. The fact that 29% (18/62) of the decompensated patients had baseline serum ALT <1,000 IU/L suggests that some of the patients might have missed laboratory detection of peak serum ALT levels due to late presentation. Thirdly, owing to our national reimbursement policy, the average treatment duration was only

19.1 months. Therefore, this cohort of patients cannot provide information regarding long-term therapeutic efficacy and resistance profile of lamivudine in HBeAg-positive CHB patients with AE. Despite these limitations, to the best of our knowledge, this is the first study comparing early HBeAg loss rates in HBeAg-positive CHB patients experiencing AE with and without decompensation, examining the independent predictors of early HBeAg loss following the institution of lamivudine therapy, and examining the clinical significance of early HBeAg loss during lamivudine therapy.

In conclusion, genotype B infection was significantly associated with AE and decompensation in HBeAg-positive patients with CHB. Decompensation during AE tended to be associated with earlier HBeAg loss and was significantly associated with increased serum HBV DNA negativity and reduced rtM204V/I mutation in patients receiving lamivudine therapy. Decompensation was not an independent predictor of early HBeAg loss. Instead, female gender and baseline serum ALT level $\geq 1,000$ IU/L were significant independent predictors of early HBeAg loss on lamivudine therapy in HBeAg-positive patients with AE. The early HBeAg losers had a higher sustained remission rate off lamivudine therapy. The long-term prognosis of the early HBeAg losers and the potential roles of these two predictors of early HBeAg loss during therapy with other oral antiviral agents in a similar clinical setting await further study.

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References

1. Fattovich G. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003;23:47–58
2. Chu CM, Liaw YF. Chronic hepatitis B virus infection acquired in childhood: special emphasis on prognostic and therapeutic implication of delayed HBeAg seroconversion. *J Viral Hepat* 2007;14:147–152
3. Liaw YF. Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. *J Gastroenterol Hepatol* 2003;18:246–252
4. Sheen IS, Liaw YF, Tai DI, et al. Hepatic decompensation associated with hepatitis B e antigen clearance in chronic type B hepatitis. *Gastroenterology* 1985;89:732–735
5. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005;5: 215–229
6. Tsai SL, Chen PJ, Lai MY, et al. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. *J Clin Invest* 1992;89:87–96

7. Shimada N, Yamamoto K, Kuroda MJ, et al. HBcAg-specific CD8 T cells play an important role in virus suppression, and acute flare-up is associated with the expansion of activated memory T cells. *J Clin Immunol* 2003;23:223–232
8. Nicoll A, Locarnini S. Review: present and future directions in the treatment of chronic hepatitis B infection. *J Gastroenterol Hepatol* 1997;12:843–854
9. Tsang SWC, Chan HLY, Leung NWY, et al. Lamivudine treatment for fulminant hepatic failure due to acute exacerbation of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2001;15:1737–1744
10. Chan HL, Tsang SW, Hui Y, et al. The role of lamivudine and predictors of mortality in severe flare-up of chronic hepatitis B with jaundice. *J Viral Hepat* 2002;9:424–428
11. Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. *J Hepatol* 2003;38:322–327
12. Yuen MF, Sablon E, Hui CK, et al. Prognostic factors in severe exacerbation of chronic hepatitis B. *Clin Infect Dis* 2003;36:979–984
13. Tsubota A, Arase Y, Suzuki Y, et al. Lamivudine monotherapy for spontaneous severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2005;20:426–432
14. Dai CY, Yu ML, Hsieh MY, et al. Early response to lamivudine therapy in clinically non-cirrhotic chronic hepatitis B patients with decompensation. *Liver Int* 2007;27:1364–1370
15. Chien RN, Liaw YF, Atkins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. *Hepatology* 1999;30:770–774
16. Perrillo RP, Lai CL, Liaw YF, et al. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 2002;36:186–194
17. Akuta N, Tsubota A, Suzuki F, et al. Long-term prognosis by lamivudine monotherapy for severe acute exacerbation in chronic hepatitis B infection: emergence of YMDD motif mutant and risk of breakthrough hepatitis—an open-cohort study. *J Hepatol* 2003;38:91–97
18. Tsubota A, Arase Y, Suzuki F, et al. Severe acute exacerbation of liver disease may reduce or delay emergence of YMDD motif mutants in long-term lamivudine therapy for hepatitis B e antigen-positive chronic hepatitis B. *J Med Virol* 2004;73:7–12
19. Wong VW, Wong GL, Tsang SW, et al. Long-term follow-up of lamivudine treatment in patients with severe acute exacerbation of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B. *Antivir Ther* 2008;13:571–579
20. Lin DY, Sheen IS, Chiu CT, et al. Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. *J Clin Ultrasound* 1993;21:303–308
21. Dai MS, Wu PF, Shyu RY, et al. Hepatitis B virus reactivation in breast cancer patients undergoing cytotoxic chemotherapy and the role of preemptive lamivudine administration. *Liver Int* 2004;24:540–546
22. Mizokami M, Nakano T, Orito E, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 1999;450:66–71
23. Tseng TC, Liu CJ, Wang CC, et al. A higher alanine aminotransferase level correlates with earlier hepatitis B e antigen seroconversion in lamivudine-treated chronic hepatitis B patients. *Liver Int* 2008;28:1034–1041
24. Stevens CE, Beasley RP, Tsui J, et al. Vertical transmission of hepatitis B antigen in Taiwan. *N Engl J Med* 1975;292:771–774
25. Hsu HY, Chang MH, Chen DS, et al. Baseline seroepidemiology of hepatitis B virus infection in children in Taipei, 1984: a study just before mass hepatitis B vaccination program in Taiwan. *J Med Virol* 1986;18:301–307
26. Liaw YF, Chu CM, Huang MJ, et al. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. *Liver* 1984;4:301–306
27. Chu CM, Sheen IS, Lin SM, et al. Sex difference in chronic hepatitis B virus infection: studies of serum HBeAg and alanine aminotransferase levels in 10,431 asymptomatic Chinese HBsAg carriers. *Clin Infect Dis* 1993;16:709–713
28. Chu CM, Hung SJ, Lin J, et al. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004;116:829–834