

Hepatitis B surface antigen seroconversion is associated with favourable long-term clinical outcomes during lamivudine treatment in HBeAg-negative chronic hepatitis B patients

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SUMMARY. The aims of this study were to assess hepatitis B surface antigen (HBsAg) seroconversion and to determine its impact on the natural course of the disease in patients with HBeAg-negative chronic hepatitis B (CHB) during lamivudine (LMV) treatment. A total of 183 consecutive patients with HBeAg-negative CHB who were treated with LMV were included in the study. Data were retrospectively collected from outpatient visit charts. The primary endpoint was HBsAg seroconversion to anti-HBs. The secondary endpoint was to determine the development of cirrhosis. Loss of HBsAg was confirmed in 10 patients and seroconversion to anti-HBs in nine patients during LMV treatment or after its discontinuation. HBsAg seroconversion was achieved on-treatment in four patients after a median treatment duration of 30 months and off-treatment in the remaining five patients in a median 61 months after LMV discontinuation. The cumulative probability of HBsAg seroconversion increased

from 0.6% at 1 year and 1.9% at 5 years to 21.5% at 10 years of LMV during and after LMV treatment. HBsAg clearance was preceded by undetectable serum hepatitis B virus (HBV) DNA. The majority of the patients responding to treatment had undetectable HBV DNA levels at 24 weeks of treatment. The cumulative probability of LMV resistance increased from 2.2% at 1 year to 37.3% at 5 years. No baseline parameter predicting either HBsAg seroconversion or the emergence of LMV resistance was identified. None of the patients with HBsAg seroconversion experienced virological breakthrough or disease progression during the follow-up period. These results indicate that HBsAg seroclearance can occur in patients with HBeAg-negative CHB under LMV therapy and predicts better clinical outcome.

Keywords: chronic hepatitis B, hepatitis B surface antigen quantification, hepatitis B virus, lamivudine.

INTRODUCTION

The primary adverse outcome of chronic hepatitis B virus (HBV) infection is cirrhosis and its complications, including portal hypertension and hepatocellular carcinoma (HCC), which can lead to death [1–7]. This outcome is consistently

Abbreviations: Anti-HBs, hepatitis B surface antibody; CHB, chronic hepatitis B; ETV, entecavir; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LMV, lamivudine; TDF, tenofovir disoproxil fumarate.

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related with serum HBV DNA levels and serum hepatitis B surface antigen (HBsAg) positivity [8–10]. The goals of antiviral therapy in chronic hepatitis B (CHB) infection are sustained suppression of HBV replication and subsequently to prevent disease progression and development of cirrhosis and its complications. Currently, several oral antiviral agents, including lamivudine (LMV), adefovir (ADV), tenofovir disoproxil fumarate (TDF), telbivudine, and entecavir (ETV) and immunomodulators including interferons (IFNs), are approved to treat CHB infection. It has been reported that the response to antiviral therapy in CHB infection is associated with a reduction in the occurrence of liver-related morbidity and mortality [2–7].

The ultimate goal of antiviral therapy is HBsAg loss and seroconversion to hepatitis B surface antibody (anti-HBs). HBsAg seroconversion has been associated with HBV viral suppression, biochemical normalization, histological improvement and better clinical outcome [4–7]. HBsAg seroconversion can be achieved after IFN-based therapy,

whereas it is suboptimal under oral antivirals [10–12]. In fact, spontaneous HBsAg seroconversion has also occurred, at a rate of 0.5–1.7% per year [13,14]. This number is higher in Western HBV populations than in Asian HBV populations [13,14].

Lamivudine, in use since 1995, is the first oral nucleoside analogue to be administered for the treatment of CHB infection. It has been shown to be effective in inhibiting HBV replication and in ameliorating liver disease [15–18]. LMV also decreases the incidence of HCC as well as delays disease progression [19–21]. Unfortunately, the effectiveness of long-term LMV treatment decreases dramatically as a result of the emergence of resistant mutant HBV strains [22,23]. Therefore, the aims of this study were to assess HBsAg seroconversion to anti-HBs in HBeAg-negative CHB patients treated with long-term LMV and to determine its impact on the natural course of the disease in such patients during LMV treatment or after its discontinuation (on-treatment and off-treatment follow-up periods).

MATERIALS AND METHODS

Patients

This was a retrospective single-centre study. Between January 2001 and July 2009, a total of 2737 patients with HBV infection were seen in the Liver Diseases Outpatient Clinic. Among them, a total of 183 consecutive patients with HBeAg-negative CHB (M/F: 120/63, mean age: 45.5 ± 11.0 years) treated with LMV, 100 mg daily, were retrospectively analysed. All patients were originally diagnosed with CHB, and none had cirrhosis. The diagnosis of CHB infection was made on the basis of biochemical, serological and histological data, when available. All patients were followed up for at least 6 months. Thirty-four per cent of the patients had used standard IFN previously (6–9 MU, three times per week, for 6–12 months). Data were collected from outpatient visit charts. Upon admission to our Clinic, 98 patients had already received LMV for at least 1 year and the remaining 85 patients were newly started on LMV treatment. Among them, 56 were still on treatment during the analysis. The 183 patients with CHB had received LMV treatment for varying durations; 21 for 1 year, 21 for 2 years, 33 for 3 years, 35 for 4 years, 24 for 5 years, 23 for 6 years, 18 for 7 years, 4 for 8 years, 2 for 9 years and 2 for 10 years.

HBV DNA quantification

Hepatitis B virus DNA levels were measured using two different methods according to the available technology. At first, HBV DNA levels were tested using a commercial liquid-hybridization assay (Digene, Gaithersburg, MD, USA), with the lower limit of detection of this assay being 5 pg/mL of viral DNA. Then, a more sensitive real-time polymerase

chain reaction (PCR)-based commercial method to quantify HBV DNA viral load was used, with a detection limit of 6 IU/mL as stated by the manufacturer (COBAS TaqMan, Roche Diagnostics, Mannheim, Germany).

Testing for antiviral resistance

For the amplification of the HBV-pol region, an 800 bp PCR fragment covering the whole HBV-pol gene was amplified using the following primers: CLC188 5'-TCC CCA ACC TCC AAT CAC-3' and CLC887 5'-AAA CCC AAA AGA CCC ACA A-3'. The PCR was run for 35 cycles with denaturation at 95 °C for 45 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min. Sequencing of the amplified fragments was carried out with a sequencing PCR of 25 cycles of 10 s at 94 °C, 5 s at 50 °C and 4 min at 60 °C using a Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Definition

The primary outcome measure was HBsAg seroconversion to anti-HBs. The secondary outcome measure was to determine the development of cirrhosis and its complications, including the development of ascites, variceal bleeding, hepatic encephalopathy and HCC. A virological breakthrough was defined as $>1 \log_{10}$ increase in serum HBV DNA level above nadir. When LMV resistance developed, patients received ADV and/or TDF monotherapy or ADV and/or TDF with LMV combination therapy.

Follow-up

Patients were seen at 3-month intervals in the outpatient clinic during the on- or off-treatment follow-up periods. Physical examination and biochemical and serological tests were performed at each visit. Mutation analysis was performed when viral breakthrough was detected. Further investigations included surveillance for HCC with ultrasound and alpha-fetoprotein determinations every 6–12 months as well as clinical assessment for liver decompensation. Possible adverse effects of LMV were also assessed.

Statistical analyses

Nominal variables were assessed by chi-square test or Fisher's exact test, where applicable. Comparison between two groups was evaluated by Student's *t*-test or Mann–Whitney *U*-test, where applicable. To define independent risk factors of an outcome variable (loss of HBsAg, seroconversion to anti-HBs and LMV resistance), logistic regression analysis was used. Odds ratios and their 95% confidence intervals were calculated. A *P* value <0.05 was considered significant. SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Baseline demographic, clinical and laboratory characteristics are shown in Table 1. Among 183 patients, 66% were men, and the median age was 45.5 ± 11.0 years (range: 20–79 years). The mean baseline serum alanine aminotransferase (ALT) and HBV DNA levels were 128.3 ± 128.9 IU/L (range: 13–776 IU/L) and 7.1 ± 1.0 log 10 copies/mL (range: 4.5–9.8 log 10 copies/mL), respectively. The mean disease duration and mean duration of LMV treatment were 74.2 ± 62.1 months (median: 63.0 months) and 34.4 ± 22.4 months (median: 27 months), respectively.

Loss of HBsAg was confirmed in a total of 10 patients (5.5%, 10/183) and seroconversion to anti-HBs in nine patients (4.9%) during the on- and off-LMV treatment follow-up periods. HBsAg seroconversion was achieved on-treatment in four patients in a median interval of 30 months (range: 10–57 months) from the initiation of LMV treatment, and off-treatment in the remaining five patients, in a median interval of 61 months (range: 27–75 months) after the discontinuation of LMV treatment. The former five patients with HBsAg seroconversion had used LMV for a median of 36 months (range: 12–60 months). The cumulative probability of HBsAg seroconversion increased from 0.6% at 1 year and 1.9% at 5 years to 21.5% at 10 years during LMV treatment or after its discontinuation (Fig. 1). One patient with HBsAg loss did not experience seroconversion after 55 months of follow-up, even though this patient was HBV DNA negative by PCR (COBAS Taqman).

Hepatitis B surface antigen seroclearance was preceded by undetectable serum HBV DNA. At the time of HBsAg seroconversion to anti-HBs, all patients had HBV DNA negativity

Table 1 Baseline characteristics of 183 patients with HBeAg-negative chronic hepatitis B (CHB)

Mean $\pm$ SD (median)	$n = 183$
Mean age (years)	45.5 ± 11.0
Gender (M/F)	120/63
Mean disease duration (months)	74.2 ± 62.1 (63)
Mean duration of lamivudine treatment (months)	34.4 ± 22.4 (27)
Previous IFN- α therapy	62 (33.8%)
Baseline serum alanine aminotransferase levels (normal limit: <37 IU/mL)	128.3 ± 128.9 (136)
Baseline serum hepatitis B virus (HBV) DNA levels (log 10 copies/mL)	7.1 ± 1.0 (7.08)
Histological activity index*	7.9 ± 3.6 (7.0)
Presence of fibrosis*	71 (65.1%)

*Available in 109 patients with CHB.

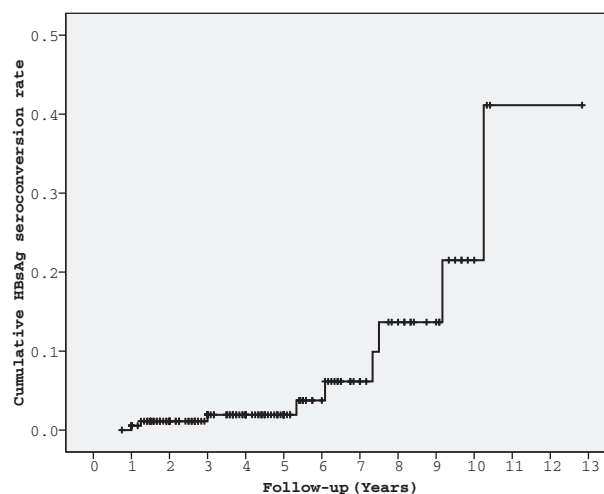


Fig. 1 Cumulative probability of hepatitis B surface antigen seroconversion in patients with HBeAg-negative chronic hepatitis B during and after lamivudine treatment follow-up periods.

and serum ALT normalization. HBsAg seroclearance (achieved either during LMV treatment or after the discontinuation of LMV treatment) was also associated with sustained HBV DNA suppression and ALT normalization. By univariate and multivariate analysis, including patient age, gender, disease duration, duration of LMV treatment, previous history of IFN treatment, baseline serum ALT, HBV DNA levels and the presence of liver fibrosis, no baseline parameter predicting HBsAg seroclearance was identified (Table 2).

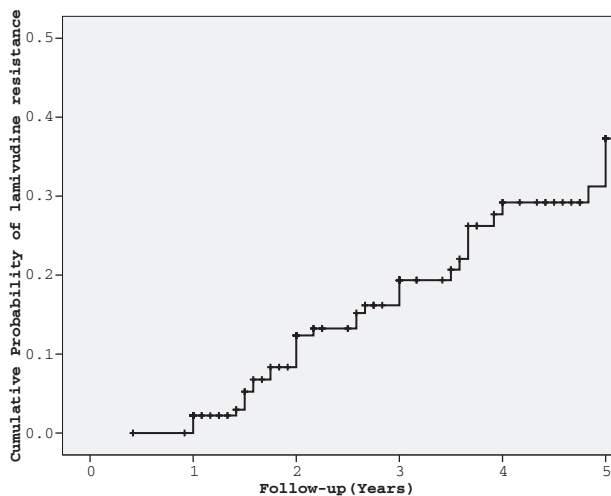
A virological breakthrough occurred in 39 patients on-treatment. LMV-resistant mutations were detected in 24 patients: rtM204V/rtL180M in 12 patients, rtM204I in 11 patients and rtL180M in one patient. The cumulative probability of LMV resistance was 2.2%, 12.4%, 19.4%, 29.2% and 37.3% after 1, 2, 3, 4 and 5 years of the LMV treatment, respectively (Fig. 2). When virological breakthrough occurred, the mean serum ALT and HBV DNA levels were 69.1 ± 77.2 IU/L (median: 52.0 IU/mL) and 5.6 ± 1.7 log 10 copies/mL (median: 5.0 log 10 copies/mL), respectively. When baseline characteristics were compared between patients with/without LMV resistance, no significant difference was observed (Table 3).

Follow-up

Lamivudine treatment was generally well tolerated. None of the patients discontinued LMV therapy because of its adverse effects. Among the 183 patients, two developed cirrhosis and HCC: LMV resistance and HCC developed at 36 and 48 months, and 60 months of LMV treatment, respectively. Additionally, one patient developed HCC without cirrhosis at 45 months of LMV treatment. None of the patients with

Table 2 Predictive factors associated with loss of hepatitis B surface antigen

Variable	Odds ratio	95% Confidence interval	P value
Age	0.971	0.912–1.034	0.356
Gender	1.053	0.254–4.358	0.944
Disease duration	0.999	0.987–1.010	0.819
Treatment duration	0.998	0.968–1.029	0.909
Previous IFN- α therapy used	1.600	0.414–6.184	0.496
Baseline serum alanine aminotransferase levels	1.002	0.996–1.009	0.468
Baseline serum HBV DNA levels	1.033	0.626–1.706	0.898
Presence of fibrosis	1.892	0.115–31.120	0.655

**Fig. 2** Cumulative probability of development of lamivudine (LMV) resistance in patients with HBeAg-negative chronic hepatitis B during LMV treatment.

HBsAg seroclearance experienced virological breakthrough or progressed to cirrhosis and its complications.

Fifty-six patients responded to LMV treatment. Forty-two patients had available serum HBV DNA levels at week 24;

83% ($n = 35$) had undetectable HBV DNA levels, while in the remaining seven patients, HBV DNA declined from 7.2 log 10 copies/mL to 4.7 log 10 copies/mL. Four of the seven showed a decrease in HBV DNA levels by >2 log 10 copies/mL, and only three showed a decrease by <2 log 10 copies/mL as compared to baseline; all three had undetectable HBV DNA levels during the visits that followed. Among the 56 patients who responded to LMV treatment, only one progressed to HCC without cirrhosis.

DISCUSSION

This is the first detailed single-centre study demonstrating HBsAg seroconversion to anti-HBs occurring in patients with HBeAg-negative CHB during or after LMV treatment. Nine patients (5%) experienced HBsAg seroconversion to anti-HBs; four were on LMV treatment and experienced seroconversion in a median interval of 30 months after the initiation of LMV, whereas the remaining five experienced seroconversion in a median interval of 61 months after the discontinuation of LMV treatment. The cumulative HBsAg seroconversion rates increased steadily over time (Fig. 1). This result is consistent with previous studies demonstrating that HBsAg seroconversion occurred in patients with HBeAg-negative CHB during and after treatment with IFN or

Table 3 Comparison between patients with/without lamivudine resistance

Variable	Without resistance ($n = 144$)	With resistance ($n = 39$)	P value
Age (>40 years)	66%	79.5%	0.11
Gender (Male)	65.3%	66.7%	0.871
Disease duration			0.540
Treatment duration (≤ 60 months)	85.4%	89.7%	0.488
Previous IFN- α therapy used	36.1%	25.6%	0.223
Baseline serum alanine aminotransferase levels (≤ 2 UNL)	37.1%	45.5%	0.487
Baseline serum hepatitis B virus DNA levels ($>10^7$ copy/mL)	56.1%	57.9%	0.894
Presence of fibrosis	60.9%	81.8%	0.075

nucleos(t)ide analogues [10,12,24,25]. Manesis *et al.* [24] reported that both IFN and LMV treatment suppressed serum HBsAg levels in treatment-responsive patients with HBeAg-negative CHB; however, HBsAg suppression was more gradual in LMV-treated compared with IFN-treated patients. The investigators suggested that among the baseline characteristics, low baseline HBsAg level was the only significant predictive factor for HBsAg seroconversion by multivariate analysis [24]. These results indicate that LMV treatment induced HBsAg seroconversion in patients with HBeAg-negative CHB.

The currently available inferior and superior oral antiviral agents have generally failed to cause HBsAg seroclearance in patients with HBeAg-negative CHB in a short period of time [12,24,25]. In the present study, none of the 10 patients with HBsAg seroclearance experienced HBsAg seroconversion except 1, at 10 months of the treatment, during the 48 weeks of LMV treatment. This result is consistent with previous studies demonstrating that no HBsAg decline was observed during the 48 weeks of either LMV or ETV treatment [12,24–26]. In addition, Manesis *et al.* [24] reported that the estimated time to HBsAg seroclearance was approximately a median 10.6 years after effective LMV treatment. However, in a previous case series study, HBsAg seroclearance was observed in five patients with HBeAg-negative CHB in a mean of 9.2 ± 5.9 months after starting ETV treatment [27]. This result indicates that a longer treatment duration is necessary for HBsAg seroconversion to anti-HBs in patients with HBeAg-negative CHB during treatment with nucleos(t)ide analogues.

Several investigators have suggested that baseline predictive factors including HBV genotype (infection with genotypes A and D vs genotypes B and C) and the patient's ethnicity (Caucasian vs Asian) were most commonly associated with HBsAg seroclearance [5,28,29]. In Turkey, nearly all of the CHB cases are a result of infection with genotype D [30]. In the present study, no other baseline characteristic was identified to predict HBsAg seroclearance in patients with HBeAg-negative CHB during or after LMV treatment periods.

Several studies previously demonstrated that early and maximal viral suppression is an important point regarding success in the treatment of CHB patients with antiviral agents [31–34]. However, the optimal 'early time' and HBV DNA cut-off level at baseline or during treatment are not well defined. In fact, the measurement of serum HBV DNA level while on treatment predicts those who will achieve an ideal response to antiviral therapy, rather than those who will have treatment failure [34]. In the present study, HBV suppression kinetics of the 56 patients who were on LMV treatment with a demonstrated response were retrospectively analysed. At week 24, 42 of them had available serum HBV DNA levels; 83% of these patients ($n = 35$) had undetectable HBV DNA levels, four showed a decrease in HBV DNA levels of $>2 \log_{10}$ copies/mL, and only three showed

a decrease of $<2 \log_{10}$ copies/mL as compared to baseline, and all three of these had undetectable HBV DNA levels during the following visits. This result indicates that the measurement of serum HBV DNA levels during antiviral therapy, especially with inferior antiviral agents, should be performed as early as possible during treatment to predict treatment and clinical outcome.

Hepatitis B surface antigen seroclearance predicts long-lasting HBV suppression, diminished disease progression and improved clinical prognosis as well as decreased risk of cirrhosis and its complications. In the present study, HBsAg seroclearance was preceded by serum HBV viral suppression and ALT normalization. All LMV-treated patients with HBsAg seroclearance also had undetectable serum HBV DNA level and normal serum ALT level, and none experienced disease progression or developed hepatic decompensation and its complications. Liver disease progressed to cirrhosis in only two patients, and both experienced LMV treatment failure. This observation is consistent with previous reports documenting that LMV delays clinical progression in patients with CHB as a result of significantly reducing the incidence of decompensation and the risk of HCC [7,18–21]. These data indicate that LMV-induced HBsAg seroclearance predicts better clinical outcome.

The emergence of HBV-resistant mutants has been reported, leading to treatment failure and progression to liver disease accompanied by decompensation [2,3,35]. The emergence of LMV-resistant mutants increases progressively over the course of treatment [22,23,35]. During the first 5 years of LMV treatment, approximately 14–32% of patients become resistant to LMV each year [22,23,35]. In fact, it is very well known that patients with CHB successfully treated with LMV, who maintained wild-type HBV mutants, had a significantly lower risk of liver disease progression [18]. The most important finding of the present study is that the rate of LMV resistance was low, with a rate of 2.2% at 1 year of treatment. This low resistance rate continued beyond 1 year, and the cumulative probability of LMV resistance was 37% after 5 years of treatment (Fig. 2). This discrepancy may be difficult to explain, but is probably related to the fact that the majority of the patients in this study had achieved viral suppression. During the emergence of LMV resistance, biochemical and virological breakthrough was observed, and the patients received ADV and/or TDF monotherapy or ADV and/or TDF with LMV combination therapy [previously published, Ref. 36]. None of them developed hepatic decompensation. It is likely that LMV is effective in patients with HBeAg-negative CHB when wild-type HBV is maintained during treatment.

This study had several limitations. First, this was a retrospective study, so data were collected from outpatient visit charts. Second, half of the patient population had already been on LMV treatment before admission to our centre. Moreover, LMV was discontinued early in some patients, and those data were not well investigated. Third, several

investigators recently reported HBsAg decrease during antiviral therapy as being predictive of long-term HBsAg seroclearance [10,37–43]. Unfortunately, analysis of HBsAg titres was not included in this study because of lack of availability of the necessary quantitative tests.

In conclusion, LMV can induce HBsAg seroconversion in patients with HBeAg-negative CHB both during and after

LMV treatment. Long-term treatment with nucleos(t)ide analogues is necessary for HBsAg seroconversion in the majority of the patients. HBsAg seroclearance was preceded by serum HBV viral suppression and predicts better clinical outcome. The measurement of serum HBV DNA level as early as possible in the treatment should predict treatment outcome.

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