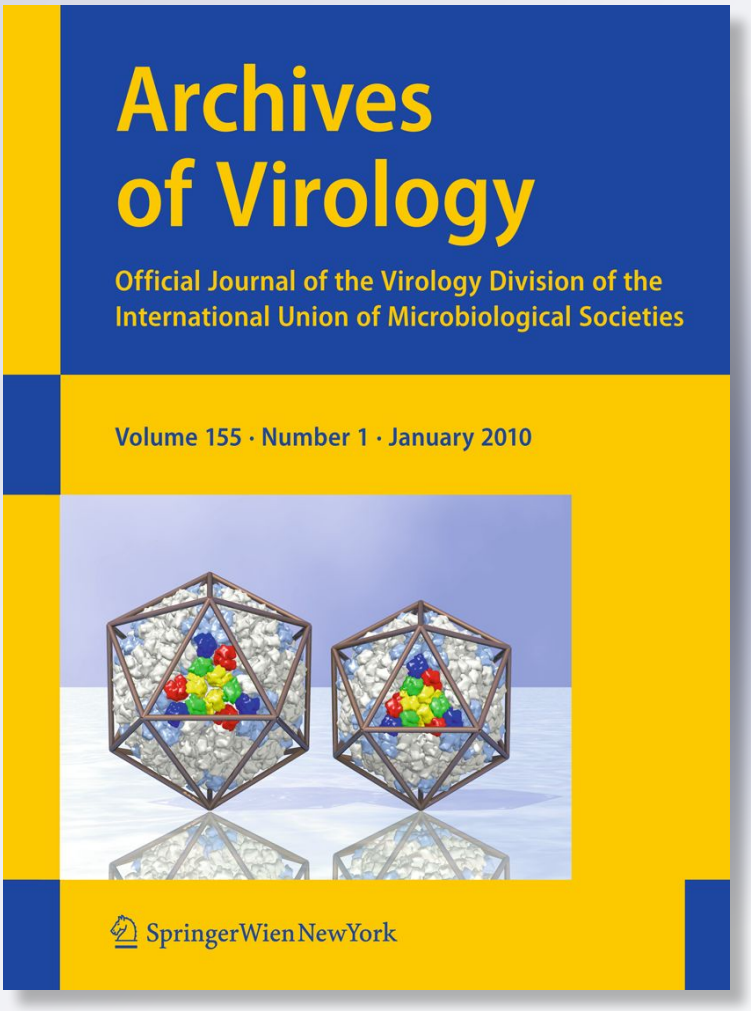
A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients with stringent cessation criteria for adefovir

Minghao Ha, Guotong Zhang, Shu Diao, Mingfang Lin, Liping Sun, Huiyuan She, Chenbao Kuan, Lihui Shen, Chunhong Huang, Wenjuan Shen & Zhongming Huang

Archives of Virology
Official Journal of the Virology Division of the International Union of Microbiological Societies

ISSN 0304-8608

Arch Virol
DOI 10.1007/s00705-011-1163-0



Springer

Arch Virol
DOI 10.1007/s00705-011-1163-0

ORIGINAL ARTICLE

A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients with stringent cessation criteria for adefovir

Minghao Ha · Guotong Zhang · Shu Diao · Mingfang Lin · Liping Sun · Huiyuan She · Chenbao Kuan · Lihui Shen · Chunhong Huang · Wenjuan Shen · Zhongming Huang

Received: 17 August 2011 / Accepted: 22 October 2011

Abstract Adefovir is usually applied for therapy of chronic hepatitis B (CHB), but its effectiveness after cessation is still unknown. This study was to evaluate the effectiveness of adefovir treatment with strict cessation criteria in hepatitis B e antigen (HBeAg)-negative patients and to identify potentially important factors. One hundred forty–five HBeAg-negative CHB patients who had received adefovir treatment for at least 24 months and for whom serum hepatitis B virus (HBV) DNA had remained undetectable for at least 18 months before cessation were included. They were followed up monthly during the first four months and at 3-month or 6-month intervals thereafter. Patients with $\geq 10^4$ copies of HBV DNA per mL were defined as relapsed. In total, 95 patients relapsed within the follow-up time, and more than 93% relapsed within 12 months after adefovir cessation. Cumulative relapse rates at months 6, 12, 24, 36, 48 and 60 were 53.8%, 61.4%, 65.5%, 65.5%, 65.5% and 65.5%, respectively. Age was the only factor associated with relapse, with lower relapse rates in younger patients shown by Cox regression analysis. HBsAg seroconversion occurred in 12 patients, and none of them relapsed during follow-up. The effectiveness of adefovir therapy does not persist in HBeAg-negative CHB patients, even when strict cessation criteria are applied, except for patients aged $\leq$ 25 years. HBsAg seroconversion is the ideal endpoint of adefovir treatment.

M. Ha (✉) · G. Zhang · S. Diao · M. Lin · L. Sun · H. She · C. Kuan · L. Shen · C. Huang · W. Shen · Z. Huang
Department of Infectious Diseases and Hepatology, Shanghai No 7 hospital, Pudong Gaoqiao Datong Road 358, Shanghai 200137, China
e-mail: hmh_1021@sina.com

Introduction

An estimated 400 million people worldwide are chronically infected with hepatitis B virus (HBV). One million die each year from complications of infection, including cirrhosis, hepatocellular carcinoma, or both [1]. Hepatitis B e antigen (HBeAg)-negative chronic hepatitis B occurs during a late phase in the course of HBV infection [2]. Mutations in the precore promoter regions, core promoter regions, or both, which prevent the formation of HBeAg, are selected during or after HBeAg loss and seroconversion to antibody to HBeAg (anti-HBe). The characteristic feature of HBeAg-negative chronic hepatitis B infection is intermittent periods of exacerbation and quiescence. It frequently follows an intense disease course, with low rates of spontaneous recovery [2–4]. Epidemiological data suggest that the median prevalence of HBeAg-negative chronic hepatitis B varies considerably, ranging from 14 percent in the United States and Northern Europe to more than 33 percent in the Mediterranean area, with an increasing prevalence worldwide [3].

Nucleotide analogs (NAs) have been shown to be powerful inhibitors of hepatitis B virus (HBV) *in vitro* and *in vivo* [5, 6]. The advantages of NAs include convenient oral administration and excellent safety profiles. Furthermore, the effectiveness of NAs in interferon-refractory chronic hepatitis B (CHB) patients has been demonstrated [7].

However, the endpoint of treatment with NAs such as adefovir is still uncertain, especially in HBeAg-negative CHB patients. Various cessation criteria have been recommended. The European Association for the Study of the Liver recommends continuation of treatment until hepatitis B surface antigen (HBsAg) is cleared [8]. With the low probability that administration of NAs will lead to HBsAg clearance in CHB patients [9], this would result in life-long

Published online: 15 November 2011

treatment for most patients. The cessation criteria proposed by the Asian Pacific Association for the Study of Liver (APASL) is discontinuation of NA treatment if HBV DNA remaining undetectable on three separate occasions six months apart [10]. However, the effectiveness of NAs treatment after cessation in patients meeting this criterion is still uncertain.

In this prospective study, we included 145 HBeAg-negative CHB patients undergoing adefovir treatment who met cessation criteria that were stricter than those of the APASL. The objective of this study was to evaluate the effectiveness of adefovir treatment with strict cessation criteria and to identify potentially important factors for relapse.

Materials and methods

Study subjects

Adefovir treatment was initiated in 253 patients at our medical center who were seropositive for HBsAg and seronegative for HBeAg more than six months before treatment. Alanine aminotransferase (ALT) levels were no less than two times the upper limit of normal (ULN, 40 IU/L), and serum HBV DNA levels were at least 10^4 copies/mL when initiating adefovir treatment. None of them had signs of decompensated liver damage (jaundice, variceal bleeding, ascites, or encephalopathy).

There were 145 patients who met the cessation criteria (see below). Adefovir treatment in these patients was initiated from June 2002 to June 2007, and ceased from November 2005 to November 2010. No other NA was administered throughout the period of treatment. Other patients were excluded because they failed to achieve a primary response or virologic breakthrough occurred during treatment. The study protocols were approved by the local ethics committee. All patients provided informed written consent.

Cessation criteria were defined as follows: (i) at least 18 months of further treatment after achieving undetectable HBV DNA; (ii) undetectable HBV DNA levels on three separate occasions six months apart by polymerase chain reaction (PCR), also with normal ALT; (iii) total treatment duration of at least 24 months.

Relapse was defined as reappearance of serum HBV DNA ($\geq 10^4$ copies/mL) by PCR.

Methods

All patients were monitored monthly during the first four months after cessation, at months 6, 9 and 12, and at 6-month intervals thereafter. Clinical monitoring, biochemical assessment and serum HBV DNA were evaluated at each visit. Abdominal ultrasonography was considered when necessary.

If serum HBV DNA exceeded 10^4 copies/mL, another sample was evaluated a week later to confirm it. Relapse was defined as elevated HBV DNA titer ($>10^4$ copies/mL) in two consecutive samples at least one week apart.

Hepatitis B virus DNA was quantified by real-time PCR at a resolution of 1×10^3 copies/mL, using a Roche LightCycler (Roche Diagnostics, Basel, Switzerland) and commercial reagents (PG Biotech, Shenzhen, China). Serum HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were detected using an Abbott AxSYM immunoassay system (Abbott Laboratories, Abbott Park, IL, USA) and original reagents. Biochemical parameters of the liver were assessed using a Beckman CX7 Chemistry Analyzer (Beckman Coulter, CA, USA) and original reagents.

Hepatitis B virus genotypes of selected patients were determined by direct sequencing of amplicons from the S region of the HBV genome as described previously [11].

Statistical methods

Results were expressed either as mean ± standard deviation or median and range. Statistical analyses were carried out using Student's t test, Mann-Whitney test, χ^2 test and Fisher's exact test when appropriate. Cumulative rates of relapse were evaluated using the Kaplan-Meier method and were tested by the log-rank test. The Cox proportional hazard model was used to identify factors that are important for relapse, with the variables including age, gender, pretreatment ALT, aspartate aminotransferase (AST) level, pretreatment titer of HBV DNA, combination with interferon, additional treatment duration, and total treatment duration. Differences were considered statistically significant at $P < 0.05$.

Results

Patient characteristics

One hundred forty-five CHB patients (101 males and 44 females) were included in our study. They were HBeAg negative and anti-HBe positive for more than six months before adefovir treatment was initiated. The mean age of the patients was 32.9 ± 16.8 years, and the pretreatment ALT level and serum HBV DNA level were 224 IU/L (80–654 IU/L) and 6.74 $\log_{10}$ copies/mL (4–8.76 $\log_{10}$ copies/mL), respectively. Combination therapy with interferon-α (INF-α) (3 MU or 5 MU, given subcutaneously every other day) was administered to 23 of the 145 patients.

Hepatitis B virus genotypes were determined in 41 patients. All (41/41) were identified as genotype C.

Treatment and follow-up period

A minimum treatment period of 24 months was achieved for each patient, and the median treatment period was 26 months (24–66 months). The median follow-up period was 16 months (1–88 months, mean 23 months); 77.2% (112/145) of the patients were followed up for more than three years, and 47.6% (69/145) were observed for more than five years.

Cumulative relapse rates after adefovir cessation

The cumulative relapse rates after adefovir cessation were analyzed by the Kaplan–Meier method (Fig. 1). The cumulative relapse rates increased steadily after adefovir cessation and reached 65.5% within five years during the follow-up time. Nearly 68.4% of the relapses (65/95) appeared within three months after withdrawal, and over 93% of relapses (89/95) appeared within 12 months. The latest relapse after adefovir cessation was found at month 24.

Hepatitis flares and treatment

Hepatitis flares, defined as an increase in ALT and virologic breakthrough, occurred in 93 of 95 relapsers (97.9%). ALT levels increased from 68 to 798 IU/L with no signs of hepatic decompensation (jaundice, variceal bleeding, ascites, or encephalopathy). Various treatments were introduced according to the patients' medical characteristics, antiviral agents available, and the severity of hepatic inflammation. In brief, 46 patients were retreated with adefovir, 27 with entecavir, and 15 with pegylated INF-α. Seven patients with slightly elevated ALT were followed up without antiviral treatment. Hepatic insufficiency was not found during follow-up in any of the relapsers.

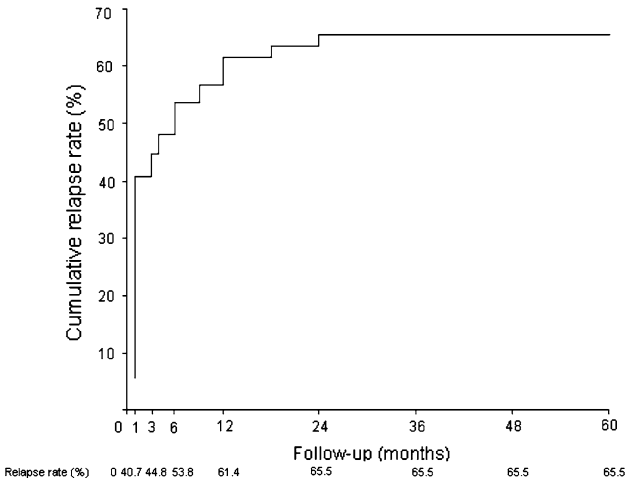


Fig. 1 Cumulative relapse rate after adefovir cessation in 145 hepatitis B e antigen- negative chronic hepatitis B (CHB) patients who met strict cessation criteria. The relapse rate increased from 61.4% after 1 year to 65.5% after 5 years, and nearly one-half of the relapses occurred within six months after adefovir cessation

Factors connectecd with relapse after adefovir cessation

Clinical characteristics and laboratory data, including gender, titer of HBV DNA, pretreatment ALT, total treatment duration, duration of additional treatment, and duration of treatment before HBV DNA seronegativity, were not significantly different between relapsers and non-relapsers (Table 1).

Cox proportional hazard model analysis was used to factors that are important for relapse, and eight variables (age, pretreatment HBV DNA, pretreatment ALT, AST level, gender, combination with interferon, additional treatment duration, and total treatment duration) were included. The results are shown in Table 2.

The analysis showed that age was the factor that correlated with relapse (relative risk [RR] 1.343, confidence interval [CI] 1.013–1.487, P = 0.004), indicating that the older the patient is, the greater the likelihood of relapse. Other variables did not correlate with effectiveness after adefovir cessation.

The data were subsequently stratified according to age, and the cumulative relapsed rates in different age groups were analyzed (Table 3).

The cumulative relapse rate (CRR) in patients aged ≤ 25 years within five years after adefovir cessation was less than a quarter (21.8%), i.e., over three-quarters of patients maintained a sustained effect for five years after cessation. But in patients aged > 25 years, over three-quarters of patients relapsed within the 5-year follow-up time. This indicates that the age of 25 years may be considered a cutoff value for sustained response in HBeAg-negative CHB patients after adefovir cessation (Fig. 2).

The total treatment duration was also stratified to determine its effect on the effectiveness of adefovir cessation. A cutoff value of 30 months was adopted, and the CRRs in patients with different total treatment durations were evaluated. There was no significant difference between the two groups with different total treatment durations (49.3% vs 51.7%, log rank test, P = 0.743).

The data for pretreatment ALT were also evaluated. Patients were stratified into three groups according to their pretreatment ALT levels (80–200 IU/L, 201–400 IU/L, and > 400 IU/L). The CRRs in these groups were not significantly different (56.4%, 54.1%, and 49.7%, respectively, log rank test, P = 0.893).

Table 1 Clinical features and laboratory characteristics of relapsers and non-relapsers

	Relapsers	Non-relapsers	*P* value
Number	95	50	
Age (years)	39.3 ± 18.2	25.1±7.8	0.003
Male/female	63/32	38/12	0.685
Pretreatment ALT (IU/L)	210 (82–653)	243 (80–654)	0.795
HBV DNA (log_{10} copies/mL)	6.96 (4.32–8.76)	6.59 (4.84–8.7)	0.711
Combination with IFN (n)	14	9	0.687
Additional treatment after undetectable HBV DNA (m)	18–48 (24)	18–69 (24)	0.582
Time until undetectable HBV DNA (w)	13 (4–24)	9 (4–24)	0.146
Total treatment (m)	26 (24–47)	26 (24–66)	0.723

ALT alanine aminotransferase, *HBV* hepatitis B virus, *INF* interferon

Table 2 Cox regression analysis of clinical features and relapse after adefovi treatment

Variable	Regression coefficient	Standard error	RR (95% CI)	*P* value
Age (years)	0.072	0.018	1.343 (1.013–1.487)	0.004
Gender	−0.008	0.436	0.891 (0.323–2.764)	0.846
Pretreatment ALT (IU/L)	0.186	0.174	0.981 (0.721–1.392)	0.153
Pretreatment AST (IU/L)	−0.013	0.004	1.148 (0.946–1.568)	0.228
Pretreatment HBVDNA (log_{10} copies/mL)	−0.298	0.134	0.571 (0.414–0.884)	0.171
Combination with IFN	0.264	0.598	1.793 (0.382–2.277)	0.958
Additional treatment after Undetectable HBVDNA (m)	−0.246	0.268	0.452 (0.343–0.756)	0.201
Total treatment (m)	0.098	0.056	1.003 (0.990–1.237)	0.093

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CI* confidence interval, *HBV* hepatitis B virus, *INF* interferon, *RR* relative risk

Table 3 Cumulative relapse rates in different age groups (log-rank test)

Follow-up (months)	Relapse rate (%)				*P* value
	≤25 years	25–35 years	35–45 years	≥45 years	
12	18.3	53.6	67.3	72.6	0.003
24	21.8	61.3	70.2	84.6	0.018
36	21.8	61.3	70.2	84.6	0.018
48	21.8	61.3	70.2	84.6	0.018
60	21.8	61.3	70.2	84.6	0.018

In our study, HBsAg seroconversion occurred in 12 of 145 (8.3%) patients within five years and none of them relapsed during the follow-up time. However, no factors correlating with HBsAg seroconversion were identified.

Discussion

This study is the first to report on hepatitis B relapse after stopping adefovir treatment in Chinese HBeAg-negative CHB patients according to the recommendations of the Asian Pacific Association for the Study of the Liver. The cessation criteria adopted in our study was more stringent than in most prior studies. For patients who met the APASL's criteria, CRRs at 1, 2, 3, 4 and 5 years were 61.4%, 65.5%, 65.5%, 65.5% and 65.5%, respectively. Most of the patients relapsed in the first year after adefovir cessation. Hadziyannis et al. found that when treatment with adefovir was discontinued, the virological, biochemical, and histological benefits that had been gained in the first 48 weeks were lost [12].

The high relapse rate in the study by Hadziyannis et al. might be attributed to the shorter duration of adefovir treatment. However, as described above, the relapse rates were higher than 65% in our study, even though the total treatment duration was 24–66 months. Therefore, our results indicate that the total duration of treatment is not associated with relapse rates. Similarly, Cox regression

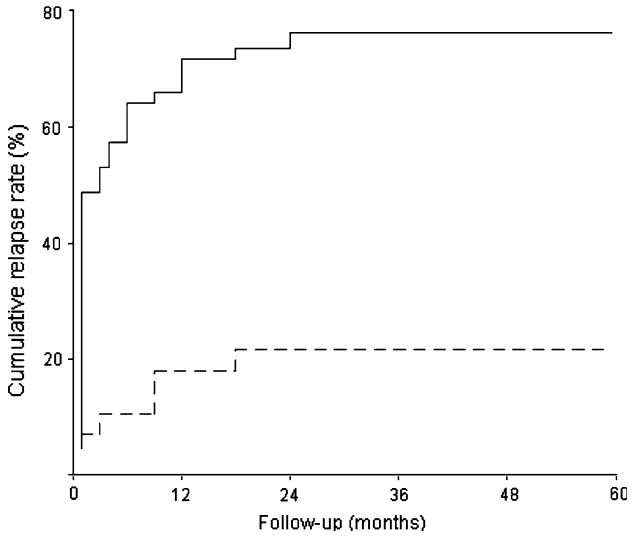


Fig. 2 Cumulative relapse rates (CRR, Kaplan–Meier method) in age subgroups. In patients aged $\leq$ 25 years, the rate of relapse within 5 years of lamivudine withdrawal was only 21.8%, while in patients aged $>$ 25 years, the rate was 75.3% (log rank test, P = 0.132). *Solid line* $>$ 25 years; *Dashed line* $\leq$ 25 years

analysis showed that the duration of additional treatment after HBV DNA became undetectable was not a significant factor for relapse after adefovir cessation. Our findings were quite different from reports of studies on the durability of lamivudine effectiveness in HBeAg-negative patients [13–16], where relapse rates ranged from 30%-56.3% after cessation of lamivudine. The smaller relapse rates reported in those studies may be attributed either to too small sample sizes (31–62 patients) or to the more potent antiviral capability of lamivudine compared with adefovir. Liu et al [17]. and Liang et al. [18] reported that virological relapse in HBeAg-negative CHB patients occurred most often within six months after discontinuation of lamivudine according to the APASL criterion. This was consistent with our study (more than one-half of the relapses occurred within six months after adefovir cessation). Because of the high relapse rates of lamivudine and adefovir, relapse rates after treatment cessation should also be considered when choosing a nucleotide analogue (and not only response and resistance rates).

The most important finding of our study was that age was a significant independent factor for relapse after adefovir cessation in HBeAg-negative CHB patients. This result indicated that the relapse rates increased with the age of the patients, which is consistent with other reports performed in patients undergoing lamivudine cessation [19–21]. Since most chronic HBV infections in endemic areas such as the Asia-Pacific are acquired by perinatal transmission, younger age simply means a shorter period of HBV infection. A shorter infection period of infection made viral clearance easier, resulting in better durability.

Our study demonstrated that age 25 was the cutoff value for relapse rate after adefovir cessation when the data were stratified according to age. For HBeAg-negative CHB patients who meet the strict cessation criteria used in our study and are less than 25 years old, adefovir treatment may be discontinued, but for patients older than 25 years, the best treatment option may be to continue treatment until HBsAg seroconversion.

Our study has some notable limitations. First, adefovir resistance mutations were not analyzed. Second, most of the patients did not undergo a liver biopsy, thus histological data could not be obtained. Third, the HBV genotypes were evaluated only in a small proportion of the patients. The HBV genotype in most of the CHB patients in Shanghai was genotype C (>90% reported in a previous study [22]). Therefore, the results of our study may merely demonstrate the ineffectiveness of adefovir cessation in HBeAg-negative CHB patients infected by HBV genotype C.

In conclusion, the effectiveness of adefovir therapy does not persist in HBeAg-negative CHB patients, even with strict cessation criteria applied, except for patients aged $\leq$ 25 years. HBsAg seroconversion is the ideal endpoint of adefovir treatment.

References

1. Hepatitis B fact sheet no. 204. (2000) Geneva: World Health Organization, October 2000
2. Hadziyannis SJ (1995) Hepatitis B e antigen negative chronic hepatitis B: from clinical recognition to pathogenesis and treatment. Viral Hepat Rev 1:7–36
3. Funk ML, Rosenberg DM, Lok AS (2002) World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 9:52–61
4. Brunetto MR, Stemler M, Schodel E et al (1989) Identification of HBV variants which can not produce precore derived HBeAg and may be responsible for severe hepatitis. Ital J Gastroenterol 21:151–154
5. Proceedings of the European Association for the Study of the Liver (EASL) (2003) International consensus conference on hepatitis B, September 14–16, 2002. J Hepatol 39(Suppl 1):S1–S235
6. Wai CT, Lok AS (2002) Treatment of hepatitis B. J Gastroenterol 37:771–778
7. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C (2000) Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 32:847–851
8. European Association for the Study of the Liver (2009) EASL clinical practice guidelines: management of chronic hepatitis B. J Hepatol 50:227–242
9. Richman DD (2000) The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. Hepatology 32:866–867
10. Liaw YF, Leung N, Kao JH et al (2008) Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2:263–283

11. Lindh M, Andersson AS, Gusdal A (1997) Genotypes, nt1858 variants, and geographic origin of hepatitis B virus—large-scale analysis using a new genotyping method. J Infect Dis 175:1285–1293
12. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ et al (2005) Long-term therapy with Adefovir Dipivoxil for HBeAg-Negative chronic hepatitis B. N Engl J Med 352:2673–2681
13. Fung SK, Wong F, Hussain M et al (2004) Sustained response after a 2-year course of lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. J Viral Hepat 11:432–438
14. Huang YH, Wu JC, Chang TT et al (2003) Analysis of clinical, biochemical and viral factors associated with early relapse after lamivudine treatment for hepatitis B e antigen-negative chronic hepatitis B patients in Taiwan. J Viral Hepat 10:277–284
15. Wang L, Liu F, Liu YD et al (2010) Stringent cessation criterion results in better durability of lamivudine treatment: a prospective clinical study in hepatitis B e antigen-positive chronic hepatitis B patients. J Viral Hepat 17:298–304
16. Song BC, Suh DJ, Lee HC et al (2000) Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 32:803–806
17. Liu F, Wang L, Li XY et al (2011) Poor durability of lamivudine effectiveness despite stringent cessation criteria : A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. J Gastroentol Hepatol 26:456–460
18. Liang Y, Jiang J, Su M et al (2011) Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. Aliment Pharmacol Ther 34:344–352
19. Shin JW, Park NH, Park JH et al (2005) Efficacy of lamivudine re-treatment for relapsed patients after an initial lamivudine therapy in HBeAg-positive chronic hepatitis B. J Viral Hepat 12:393–397
20. Ryu SH, Chung YH, Choi MH et al (2003) Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospectivestudy. J Hepatol 39:614–619
21. Lee HC, Suh DJ, Ryu SH et al (2003) Quantitative polymerase chain reaction assay for serum hepatitis B virus DNA as a predictive factor for post-treatment relapse after lamivudine induced hepatitis B e antigen loss or seroconversion. Gut 52:1779–1783
22. Zeng G, Wang Z, Wen S et al (2005) Geographic distribution, virologic and clinical characteristics of hepatitis B virus genotypes in China. J Viral Hepat 12:609–617