ORIGINAL RESEARCH

Genotypic Resistance Remains A Concern In Chronic Hepatitis B Patients With High Viral Load After Lamivudine And Adefovir Combination Therapy

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Aims: Previous studies have shown that baseline high viral load is closely related to treatment response in chronic hepatitis B (CHB). This study was designed to evaluate the differences of treatment responses between de novo lamivudine (LAM) plus adefovir (ADV) combination therapy compared with entecavir monotherapy (ETV).

Methods: A total of 185 HBeAg-positive CHB patients with high viral load were enrolled and assigned to the LAM+ADV group (n=90) or ETV group (n=95). Clinical variables are extracted from medical records.

Results: No significant differences in baseline variables were found between the two groups before antiviral treatment. After 104 weeks of antiviral therapy, the mean HBV DNA viral load in the LAM+ADV group decreased from 8.01 ± 0.65 $\log_{10}$ copies/mL to 0.41 ± 1.04 $\log_{10}$ copies/mL, compared with 8.04 ± 0.57 $\log_{10}$ copies/mL to 0.57 ± 1.28 $\log_{10}$ copies/mL in the ETV group (P=0.35). The virological response rate of LAM+ADV group was 82.2% (74/90) at 104 weeks of treatment, and 80.0% (76/95) in the ETV group (P=0.70). For HBeAg serological responses, HBeAg loss occurred in 23.3% (21/90) and 17.9% (17/95) in the LAM+ADV group and the ETV group, respectively (P=0.36). HBeAg seroconversion was observed in 15.6% (14/90) and 15.8% (15/95) in the LAM+ADV group and ETV group, respectively (P=0.96). However, after 104 weeks of treatment, genotypic resistance was confirmed in 8 cases in the LAM+ADV group, a proportion of 8.8% (8/90), compared with an absence of genotypic resistance in the ETV group (P=0.003).

Conclusion: Both de novo combination therapy of LAM+ADV and ETV monotherapy could effectively inhibit HBV replication in patients with high viral load. However, the rate of genotypic resistance in LAM+ADV treatment remains a concern. For CHB patients with high viral load, ETV treatment may be superior.

Keywords: chronic hepatitis B, high viral load, nucleos(t)ide analogs, virologic response

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Introduction

The goal of chronic hepatitis B (CHB) therapy is long-term inhibition of HBV replication and to achieve HBeAg seroconversion in HBeAg-positive patients.[1–3] Studies have shown that baseline HBV DNA load is an important predictor of cirrhosis and hepatocellular carcinoma in CHB patients.[4–7] The higher the baseline viral load, the greater the risk of cirrhosis and hepatocellular carcinoma in patients.[8]

Long-term nucleoside (acid) drugs (NUC) have been shown to effectively inhibit HBV replication, thereby preventing cirrhosis and hepatocellular carcinoma.[9–11]

However, currently limited evidence suggested that virological response is not satisfactory enough with NUC therapy in patients with high viral load CHB at baseline. GLOBE study data suggest that for HBeAg-positive CHB patients, baseline HBV DNA load ≥9 $\log_{10}$ copies/mL is less likely to obtain virological and serological responses.[4] So far, the optimal treatment plan for patients with baseline high viral load CHB is still unclear. Although guidelines recommend that for patients with high viral load, potent NUC with high resistance barrier may be more suitable.[9] The heavy economic burden has made it not widely implemented in low- and middle-income areas.[12] Hence, in People's Republic of China, CHB guideline recommends that combination therapy of NUC with no cross-resistance may be another choice for those CHB patients with high viral load.

Lamivudine (LAM), the first oral agent approved for treatment of CHB, has a well-established safety and efficacy profile.[12–16] However, the clinical benefit is difficult to sustain over a long-term treatment, owing to the selection of HBV mutants to resistance, which occur at rates of 14% to 32% annually.[1] Management of LAM-resistant CHB requires rescue therapy with appropriate complementary drugs without cross-resistance, such as adefovir dipivoxil (ADV). Entecavir (ETV), with potent HBV inhibition and a high barrier to resistance, is initially recommended as a first-line NUC in most guidelines.[8,17–19] However, it is still unclear which treatment is better for CHB patients with high viral load. Therefore, the aims of this study were to investigate the efficacy of de novo combination therapy of LAM with ADV (LAM+ADV) and ETV monotherapy.

Subjects And Methods
Patient Informations
This study is a retrospective study. A total of 185 CHB patients aged 16–61 years were included in the study. The diagnostic criteria for CHB were HBsAg positive for more than 6 months.[20] Patients enrolled were required to meet HBeAg(+) and HBeAb(−). High viral load was defined as baseline serum HBV DNA $\geq10^7$ copies/mL. Patients were excluded if previous history of antiviral therapy with NUCs or interferon; patients are pregnant or have alcohol abuse; clear clinical medical evidence to confirm that

patients have metabolic liver disease, autoimmune liver disease, cirrhosis, primary hepatocellular carcinoma; serum viral markers suggest that patients are co-infected with HCV, HDV or HIV.

The study was approved by the Ethics Committee of Affiliated Hospital of Youjiang Nationalities Medical College. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was obtained from all patients enrolled.

Laboratory Tests
Serum ALT levels were measured by automated techniques. Levels of HBV serological markers were determined using a commercially available radioimmunoassay (ARCHITECT i2000SR, Illinois, USA). Serum HBV DNA levels were measured using real-time PCR quantification.

Efficacy And Safety Endpoints
The primary efficacy endpoint for this study was the proportion of CHB patients with virological response, which defined as HBV DNA below the detection line (100 copies/mL). Secondary efficacy endpoint included the mean reduction in serum HBV DNA viral load; the proportion of CHB patients who received HBeAg serological response; and the proportion of CHB patients who achieved a biochemical response; proportion of CHB patients experienced with viral breakthrough and drug resistance.

Safety indicators included drug side effects or laboratory abnormalities in patients enrolled from LAM+ADV combination therapy or ETV monotherapy, including serum creatinine levels in the LAM+ADV combination group.

Statistical Analysis
Continuous variables were expressed as mean and standard deviation, and categorical variables were expressed as percentages. The HBV DNA levels were expressed in logarithmic units ($\log_{10}$ IU/mL). The χ^2 test and t test were applied when appropriate, to determine whether the results were statistically different. The statistical significance of all tests was set as $P<0.05$ by two-tailed tests. Data analyses and quality control procedures were performed using SPSS for Windows, version 13.0 (SPSS Inc. 233 South Wacker Drive, 11th Floor, Chicago, USA).

Results

Baseline Characteristics Of Patients Enrolled

A total of 185 patients were included, including 121 male patients and 64 female patients, with an average age of 30.8±8.5 years. Among them, 90 cases were in the LAM +ADV group and 95 cases in the ETV group. The baseline characteristics and demographic data of the two groups were balanced and comparable, as shown in Table 1.

Virologic Response

The reduction of viral load in the two groups is shown in Figure 1. Serum HBV DNA load decreased significantly both in the LAM+ADV group and ETV group. No significant difference was observed in the decrease of HBV DNA between the two groups (P=0.35).

Virological response rate of the two groups gradually increased after treatment. The virological response rate is similar with no statistical difference between the two groups at 52 weeks or 104 weeks (P_{52w}=0.79, P_{104w}=0.55), as shown in Figure 2.

HBeAg Serologic Responses

At the 52th week of treatment, HBeAg loss was observed in 17.5% (16/90) and 13.3% (13/95) patients in the LAM +ADV group and the ETV group, respectively (P=0.44). HBeAg serological conversion rate was 12.2% (11/90) and 10.5% (10/95) in the LAM+ADV group and the ETV group, respectively (P=0.72).

At the 104th week of treatment, HBeAg loss occurred in 23.3% (21/90) and 17.9% (17/95) in the LAM+ADV group and the ETV group, respectively (P=0.36). HBeAg seroconversion observed in 15.6% (14/90) and 15.8% (15/95) in LAM+ADV group and ETV group respective (P=0.96), as shown in Figure 3.

Table 1 Baseline Characteristics Of Patients Enrolled

	LAM+ADV group	ETV group	P-value
Sample size	90	95	–
Age, years	31.53±8.81	30.22±8.18	0.48
Sex, M/F	61/29	60/35	0.51
ALT, U/L	213.52±120.99	234.46±160.95	0.50
HBV DNA load, $\log_{10}$IU/mL	8.01±0.65	8.02±0.57	0.90
Genotypes, B/C	78/12	81/14	0.78
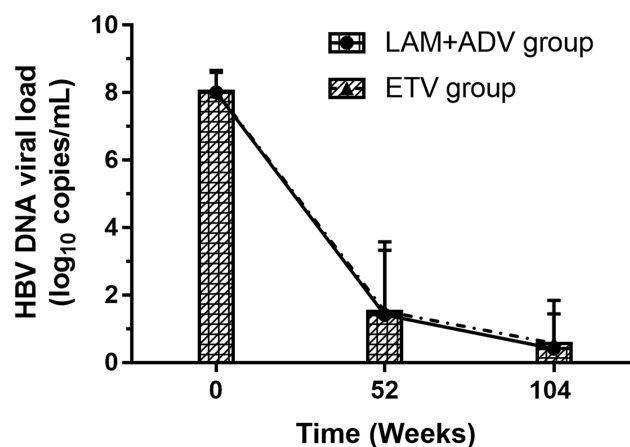
Figure 1 HBV DNA viral load reduction during 104-week treatment. Serum HBV DNA load decreased significantly both in the LAM+ADV group and ETV group. Mean HBV DNA viral load in the LAM+ADV group decreased from 8.01±0.65 $\log_{10}$ copies/mL to 0.41±1.04 $\log_{10}$ copies/mL, compared with 8.04±0.57 $\log_{10}$ copies/mL to 0.57±1.28 log10 copies/mL in the ETV group (P=0.35).
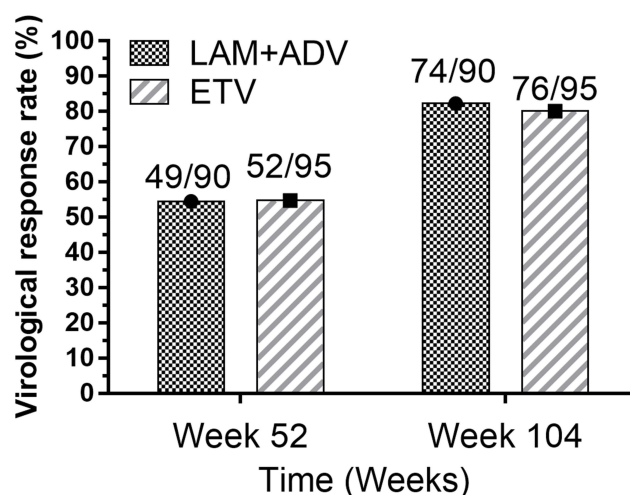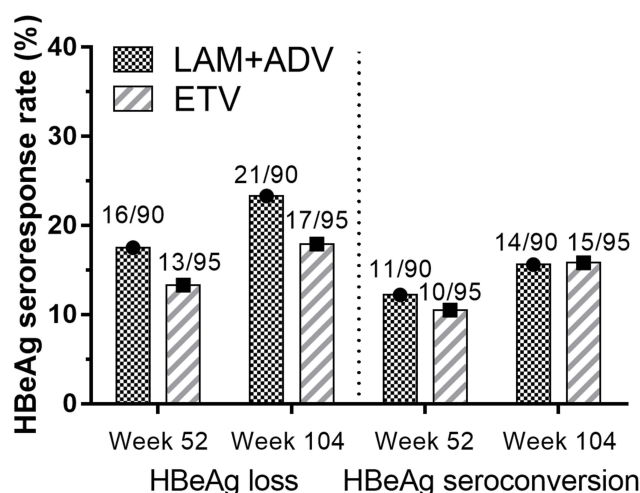

Figure 2 Virological response rate during 104-week treatment. Virological response rate of the two groups gradually increased after treatment. The virological response rate of LAM+ADV group was 82.2% (74/90) in 104 weeks of treatment, and 80.0% (76/95) in ETV group (P=0.70). The virological response rate is similar with no statistical difference.
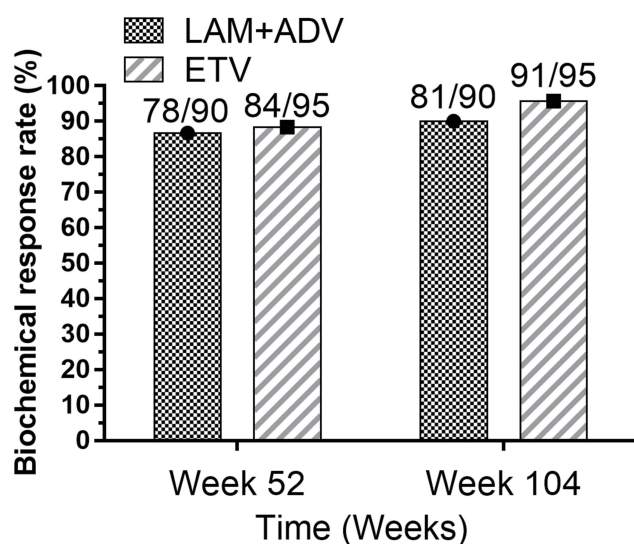
Biochemical Response Rate

The ALT normalization rate in the LAM+ADV group was 86.7% (78/90), compared with 88.4% (84/95) in the ETV group (P=0.72) at week 52. With the prolongation of treatment period, the ALT normalization rate gradually increased. At 104 weeks of treatment, the ALT normalization rate of LAM+ADV group and ETV group were 90.0% (81/90) and 95.7% (91/95), respectively (P=0.12), as shown in Figure 4.

Genotypic Resistance Rate

Among the patients enrolled, there were eight episodes of confirmed genotypic resistance after 104-week treatment. All

Figure 3 HBeAg serologic responses rate during 104-week treatment. HBeAg loss was observed in 17.5% (16/90) and 13.3% (13/95) patients in the LAM+ADV group and the ETV group respective at the 52th week of treatment (P=0.44). At the 104th week of treatment, HBeAg loss occurred in 23.3% (21/90) and 17.9% (17/95) in the LAM+ADV group and the ETV group, respectively (P=0.36). HBeAg serological conversion rate was 12.2% (11/90) and 10.5% (10/95) in the LAM+ADV group and the ETV group at the 52th week of treatment, respectively (P=0.72). While at week 104, 15.6% (14/90) and 15.8% (15/95) in LAM+ADV group and ETV group respective achieve HBeAg seroconversion (P=0.96).

Figure 4 Biochemical response rate during 104-week treatment. ALT normalization rate in the LAM+ADV group was 86.7% (78/90), compared with 88.4% (84/95) in the ETV group (P=0.72) at week 52. At 104 weeks of treatment, the ALT normalization rate of LAM+ADV group and ETV group were 90.0% (81/90) and 95.7% (91/95), respectively (P=0.12).

8 patients were in the LAM+ADV group. After assessing by evaluating genotypic changes using HBV polymerase/ reverse transcriptase assay, the type of resistance was observed (6 cases with rtM204V+rtL180M and 2 cases with rtA181T/V, respectively) in the LAM+ADV group with a corresponding resistance rate of 8.8%, compared with absence of genotypic resistance in the ETV group (P=0.003).

Univariate And Multivariate Analysis For 104-Week Treatment Response

Univariate and multivariate analyses were designed to aid in the prediction of virologic response at week 104 (Table 2). The baseline ALT levels and baseline serum HBV DNA levels were strong predictors for the virologic response at week 104 (P=0.03 and P=0.01, respectively).

Drug Safety

Both LAM+ADV and ETV were well tolerated through the 52 weeks. No serious adverse events were identified after 104-week treatment. No patients experienced an on-treatment hepatic flare or liver failure during treatment periods. No renal relative adverse events occurred that were attributed to the study drug by the clinical investigators. An absence of renal impairment, with blood creatinine concentrations greater than 1.2 mg/dL.

Discussion

Studies have shown that high baseline HBV DNA viral load is an independent risk factor of LAM resistance and the risk of developing HCC.[21–25] It is still necessary to explore antiviral treatment strategy that benefits those CHB patients with high baseline HBV DNA viral load. Although the guidelines recommend that ETV can be used for those population, the heavy economic burden makes ETV not widely available in low- and middle-income areas. The Chinese guidelines recommend that the combined use of LAM and ADV may be another option, but there is still insufficient evidence to support.[26] The results of this study suggest that both ETV monotherapy and LAM+ADV combination therapy can effectively inhibit HBV replication in this population. There was no significant difference in virological response, HBeAg serological response, and biochemical response between the two groups at 104 weeks. However, the LAM+ADV group still had a higher resistance rate compared to the ETV group. Taken together, for CHB patients with high viral load, ETV is still more suitable than LAM+ADV.

Studies have reported that after 3 years of ETV treatment, 90.2% of patients with baseline serum HBV DNA viral load <5.9 $\log_{10}$ copies/mL achieved virological response, while only 66.2% of patients with baseline HBV DNA viral load ≥8 $\log_{10}$ copies/mL achieved.[27] Another study found that baseline HBV DNA viral load <9 $\log_{10}$ copies/mL is a strong predictor of virological response at 104 weeks.[4] Our study confirmed that patients

Table 2 Univariate And Multivariate Analysis For Virologic Response

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P-value	OR	95% CI	P-value
Sex	0.72	0.19–1.76	0.37			
Age	0.89	0.84–1.51	0.81			
ALT level	1.82	1.84–5.18	0.02	1.72	1.51–9.77	0.03
HBV DNA levels	2.67	1.31–8.13	0.01	2.05	1.19–5.34	0.01
Type of treatment	1.74	0.43–4.29	0.64			

with baseline high HBV DNA viral load were unable to achieve the same high level of virological response as normal CHB patients. Our data presented in this study suggest that there is no statistically significant difference in 104-week virological response rates between LAM+ADV de novo combination therapy and ETV monotherapy. This suggests that for such patients, de novo LAM+ADV combination therapy and ETV monotherapy can both effectively inhibit HBV replication, reduce serum ALT levels and liver inflammation, and delay disease progression. However, significantly more cases of genetic resistance occurred in the LAM+ADV group. LAM resistance can lead to subsequent treatment difficulties, especially switch to ETV treatment.[28,29] LAM resistance can also lead to a decrease in the efficacy of ETV. For this part of the population, tenofovir may be a good choice.[14,30] In a meta-analysis included four randomized controlled trials and cohort study, the authors reported that combination therapy with LAM and ADV was more effective than ETV monotherapy.[31] However, in the included studies, none of them enrolled patients with high viral load. In addition, some patients enrolled in those studies were HBeAg-negative. The different clinical characteristics of enrolled patients will affect the results of the study.

According to the study data, baseline high ALT levels are an important predictor of virological response in these patients at 104 weeks. The 2-year GLOBE study data showed that baseline ALT levels ≥2 ULN could predict good virological and serological responses after 2 years of telbivudine in CHB patients.[4] For CHB patients with high viral load and lower baseline ALT level, more attention should be paid to their virological decline and timely intervention to prevent the adverse consequences of persistent viremia.

Lamivudine and adefovir are not the first-line drugs recommended by American Association for the Study of Liver Disease for the treatment of CHB.[8] ETV and tenofovir are the recommended first-line drugs. However, in developing countries, ETV and tenofovir are not fully acceptable to

all patients because of their high prices. Therefore, in People's Republic of China, how to improve the therapeutic effect of drugs and reduce the economic burden is a serious problem. De novo lamivudine plus adefovir is a recommended antiviral treatment to solve the problem. There is no cross-resistance between lamivudine and adefovir, so combination therapy can reduce the rate of resistance. However, our study shows that for CHB patients with high viral load, even patients received treatment with de novo lamivudine plus adefovir, they still have higher resistance rate than ETV monotherapy. Therefore, it needs reconsideration whether we should recommend lamivudine plus adefovir combination therapy in order to reduce the economic burden. Although lamivudine and adefovir are not the first-line antiviral drugs. There are some studies reported previously comparing lamivudine+adefovir and ETV.[12,30] But as far as we know, this study is the first one to demonstrate that CHB patients with high viral load still have a high resistance rate after de novo lamivudine plus adefovir combination therapy. A cost-effectiveness study supporting the use of ETV as first-line therapy for HBeAg seropositive patients.[32] Although the price of the LAM+ADV and ETV is different in different regions, the conclusion is still some value in People's Republic of China. In People's Republic of China, the cost of the ETV (530 yen/month) is greater than the LAM+ADV (393 yen/month), a cost-effectiveness analysis is necessary for those CHB patients with high viral load.

One limitation of this study was the retrospective design. Another limitation was the relatively small sample size enrolled in this study. A large randomized controlled prospective study is still needed to evaluate the long-term antiviral treatment effect in CHB patients with baseline high HBV DNA viral load.

In conclusion, our data suggest that both LAM+ADV de novo combination therapy and ETV therapy can effectively inhibit HBV replication and improve HBeAg seroconversion in CHB patients with high viral load. However, patients treated with LAM combined with ADV still need

to be alert to the possibility of HBV resistance. During the antiviral period, close attention should be paid to virological breakthroughs. And when conditions permit, ETV is still a better choice for CHB patients with high viral load.

Abbreviations

ADV, adefovir dipivoxil; ALT, alanine aminotransferase; CHB, chronic hepatitis B; ETV, entecavir; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LAM, lamivudine; NUC, nucleos(t)ide analog.

Ethics And Consent

The Institutional Review Board of the Affiliated Hospital of Youjiang Nationalities Medical College approved the study. All procedures followed were in accordance with the ethical standards of the Responsible Committee on Human Experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for inclusion in the study.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int*. 2016;10:1–98.
2. Cai SH, Lu SX, Liu LL, Zhang CZ, Yun JP. Increased expression of hepatocyte nuclear factor 4 alpha transcribed by promoter 2 indicates a poor prognosis in hepatocellular carcinoma. *Therap Adv Gastroenterol*. 2017;10:761–771. doi:10.1177/1756283X17725998
3. Ou H, Cai S, Liu Y, Xia M, Peng J. A noninvasive diagnostic model to assess nonalcoholic hepatic steatosis in patients with chronic hepatitis B. *Therap Adv Gastroenterol*. 2017;10:207–217. doi:10.1177/17562 83X16681707
4. Liaw YF, Gane E, Leung N, et al. 2-year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology*. 2009;136:486–495. doi:10.1053/j.gastro.2008.10.026
5. Yu MW, Chang HC, Chen PJ, et al. Increased risk for hepatitis B-related liver cirrhosis in relatives of patients with hepatocellular carcinoma in northern Taiwan. *Int J Epidemiol*. 2002;31:1008–1015. doi:10.1093/ije/31.5.1008
6. Wu X, Cai S, Li Z, et al. Potential effects of telbivudine and entecavir on renal function: a systematic review and meta-analysis. *Virol J*. 2016;13:64. doi:10.1186/s12985-016-0522-6
7. Xue X, Cai S. Comment on "Assessment of liver stiffness in pediatric fontan patients using transient elastography". *Can J Gastroenterol Hepatol*. 2016;2016:9343960. doi:10.1155/2016/9343960
8. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*. 2016;63:261–283. doi:10.1002/hep.28156
9. European Association For The Study Of The Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67:370–398. doi:10.1016/j.jhep.2017.03.021
10. Al AH, Peedikayil MC, Al QM, et al. HBsAg clearance in chronic hepatitis B patients with add-on pegylated interferon alfa-2a to ongoing tenofovir treatment: a randomized controlled study. *Saudi J Gastroenterol*. 2017;23:190–198.
11. Bertuccio P, Turati F, Carioli G, et al. Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol*. 2017;67:302–309. doi:10.1016/j.jhep.2017.03.011
12. Cai S, Yu T, Jiang Y, Zhang Y, Lv F, Peng J. Comparison of entecavir monotherapy and de novo lamivudine and adefovir combination therapy in HBeAg-positive chronic hepatitis B with high viral load: 48-week result. *Clin Exp Med*. 2016;16:429–436. doi:10.1007/s10238-015-0373-2
13. Xue X, Cai S, Ou H, Zheng C, Wu X. Health-related quality of life in patients with chronic hepatitis B during antiviral treatment and off-treatment. *Patient Prefer Adherence*. 2017;11:85–93. doi:10.2147/PPA.S127139
14. Zeng J, Cai S, Liu J, Xue X, Wu X, Zheng C. Dynamic changes in liver stiffness measured by transient elastography predict clinical outcomes among patients with chronic hepatitis B. *J Ultrasound Med*. 2017;36:261–268. doi:10.7863/ultra.15.12054
15. Hu Y, Xu C, Xu B, et al. Safety and efficacy of telbivudine in late pregnancy to prevent mother-to-child transmission of hepatitis B virus: a multicenter prospective cohort study. *J Viral Hepat*. 2018;25:429–437. doi:10.1111/jvh.12834
16. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology*. 2006;130:678–686. doi:10.1053/j.gastro.2005.11.016
17. Kurokawa M, Hiramatsu N, Oze T, et al. Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection. *J Gastroenterol*. 2012;47:577–585. doi:10.1007/s00535-011-0522-7
18. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology*. 2009;50:661–662. doi:10.1002/hep.23190
19. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine*. 2012;30:2212–2219. doi:10.1016/j.vaccine.2011.12.116
20. Cai SH, Lv FF, Zhang YH, Jiang YG, Peng J. Dynamic comparison between Daan real-time PCR and Cobas TaqMan for quantification of HBV DNA levels in patients with CHB. *BMC Infect Dis*. 2014;14:85. doi:10.1186/1471-2334-14-85
21. European Association For The Study Of The Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. *J Hepatol*. 2012;57:167–185. doi:10.1016/j.jhep.2012.02.010
22. Galle PR, Forner A, Llovet JM, et al. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol*. 2018;69:182–236. doi:10.1016/j.jhep.2018.03.019
23. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006;295:65–73. doi:10.1001/jama.295.1.65
24. Chu CM, Liaw YF. Hepatitis B virus-related cirrhosis: natural history and treatment. *Semin Liver Dis*. 2006;26:142–152. doi:10.1055/s-2006-939752
25. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med*. 2011;365:1118–1127. doi:10.1056/NEJMra1001683
26. Chinese Society of Hepatology CMA, Chinese Society of Infectious Diseases CM. The guideline of prevention and treatment for chronic hepatitis B:a 2015 update. *Chin J Hepatol*. 2015;23:888–905. doi:10.3760/cma.j.issn.1007-3418.2015.12.002

27. Yuen MF, Seto WK, Fung J, Wong DK, Yuen JC, Lai CL. Three years of continuous entecavir therapy in treatment-naive chronic hepatitis B patients: VIRAL suppression, viral resistance, and clinical safety. *Am J Gastroenterol*. 2011;106:1264–1271. doi:10.1038/ajg.2011.45

28. Cai S, Cao J, Yu T, Xia M, Peng J. Effectiveness of entecavir or telbivudine therapy in patients with chronic hepatitis B virus infection pre-treated with interferon compared with de novo therapy with entecavir and telbivudine. *Medicine (Baltimore)*. 2017;96:e7021. doi:10.1097/MD.0000000000007021

29. Zheng C, Yan H, Zeng J, Cai S, Wu X. Comparison of pegylated interferon monotherapy and de novo pegylated interferon plus tenofovir combination therapy in patients with chronic hepatitis B. *Infect Drug Resist*. 2019;12:845–854. doi:10.2147/IDR.S195144

30. Cai S, Li Z, Yu T, Xia M, Peng J. Serum hepatitis B core antibody levels predict HBeAg seroconversion in chronic hepatitis B patients with high viral load treated with nucleos(t)ide analogs. *Infect Drug Resist*. 2018;11:469–477. doi:10.2147/IDR.S163038

31. Liu F, Wang X, Wei F, et al. Efficacy and resistance in de novo combination lamivudine and adefovir dipivoxil therapy versus entecavir monotherapy for the treatment-naive patients with chronic hepatitis B: a meta-analysis. *Virol J*. 2014;11:59. doi:10.1186/1743-422X-11-59

32. Veenstra DL, Sullivan SD, Clarke L, et al. Cost effectiveness of entecavir versus lamivudine with adefovir salvage in HBeAg-positive chronic hepatitis B. *Pharmacoeconomics*. 2007;25:963–977. doi:10.2165/00019053-200725110-00006