Long-term nucleoside analogue therapy can lead effective HBV DNA inhibition in patients with chronic HBV infection in immune tolerate phase

YAYUN LIU
Changhai Hospital
XueSong Liang (liangxuesong2000@163.com)
https://orcid.org/0000-0003-0527-4978
AiJing Xu
changhai hospital department of infectious diseases
Hao Xu
changhai hospital department of infectious diseases
Jiao Yu
Changhai Hospital Department of infectious diseases
Jianya Xue
changhai hospital department of infectious diseases

Research article

Keywords: chronic hepatitis B (CHB), immune tolerate, liver biopsy, Antiviral therapy, entecavir(ETV), tenofovirdisoproxil fumarate(TDF)

Posted Date: January 30th, 2020

DOI: https://doi.org/10.21203/rs.2.22290/v1

Abstract

Background and Aims

The efficacy and safety of long-term antiviral therapy with nucleoside analogues (NAs) for chronic hepatitis B (CHB) patients in immune tolerate (IT) phase is uncertain.We retrospectively evaluated the efficacy and safety of 61 CHB patients in IT phase receiving NAs therapy from 2013 through 2018.

Methods

We performed a retrospective study of CHB patients who had high HBV DNA, normal or minimum alanine aminotransferase (ALT), liver biopsy confirmed light necro-inflammation and received NAs therapy from 2012 through 2018.All patients received NAs at least 12 months. Patients on-treatment monitoring were in accordance with the roadmap concept and were followed after their treatment start date to the treatment end date (if any) or 6 years at least once every 6 months. The median follow-up time was 36(16; 52) months. We assessed the virological response (the proportions of patients with plasma HBV DNA loads less than the limit of quantification,100IU/ml) and serological endpoints (HBeAg loss and seroconcersion to anti-HBe, and HBsAg loss and seroconversion to anti-HBs). Safety and tolerability, including serious events, were regularly assessed.

Results

At 48 weeks on treatment, 55.6%(95%confidence interval(CI):37.0-70.0%) patients with CHB in IT phase achieved HBV DNA less than quantitative limit(<100IU/ml), and the accumulate proportion of patients who achieved HBV DNA less than quantitative limit was 76.7%(95%CI: 58-90.0%) and 95.8%(95%CI: 79-100%) at 96 weeks and ≥144weeks on treatment, respectively. The HBeAg loss or seroconversion rate in patients with CHB in IT phase on NAs treatment at 48, 96 and ≥144weeks was 2.7%(95%CI: 0-14.0%), 13.3% (95%CI: 4-31.0%) and 29.2%(95%CI: 13-51%), respectively. During the study only one patient achieved HBsAg seroconversion after 3years NAs therapy and only one patient experienced drug-related breakthrough during the study.

Conclusion

Patients with CHB in IT phase have relatively preferable safety and HBV DNA inhibition efficacy on long-term NAs treatment.

Background

Hepatitis B virus (HBV) infection is still the leading cause of chronic liver diseases, with over 250 million chronically infected individuals around the world[1–3], the majority of those affected are from the Asia-Pacific region and are mostly acquired infection prenatally or in the early childhood[4]. Chronic HBV infection related liver inflammation may result in the progression of liver cirrhosis and hepatocarcinoma (HCC), which resulting in more than half a million deaths annually [5, 6]. Natural course studies of chronic

HBV infection suggests that serum HBV DNA levels are strongly correlated to the development of HCC and cirrhosis regardless of ALT levels, HBV genotype and HBeAg status in adults [6–9]. Antiviral therapy with nucleos(t)ide analogues(NAs) can efficaciously inhibit viral replication, relieve liver inflammation, reverse liver fibrosis, reduce HCC incidence and thereby improve the clinical outcomes of patients with chronic HBV infection[10–13].Therefore, the existing national and international guidelines for the prevention and treatment of chronic hepatitis B (CHB) clearly recommend CHB patients during immune active (IA) phase (HBeAg positive or negative)to undergo antiviral treatment as soon as possible [14–16]. However, due to the presence of minimal liver inflammation, no obvious disease activity and poor antiviral efficacy of interferon (IFN) or NAs[17, 18], the current mainstream treatment recommendations for CHB patients in immune-tolerant (IT) phase are close follow-up until the entry into the IA period [14–16].

Given the strong correlation of high HBV DNA levels and the increased risk of HCC [7, 19, 20],and immunological and histological evidence for disease progression inpatients in IT phase [21, 22], attention to antiviral strategies of IT patients had been increased [17, 23, 24]. The safety and efficacy of long-term treatment of NAs or IFN in patients in IT phases is still lacking. In this retrospective study, we evaluated the safety and efficacy of long-term antiviral therapy with NAs in patients in IT phases.

Study Design And Patients

The minimal long-term antiviral therapy in IT patients was a retrospective consecutive study. 119 chronic HBV infected individuals who underwent liver biopsies and routine laboratory tests in shanghai changhai hospital between January 2012 and September2018,were retrospectively screened. Chronic HBV infection was defined as serum hepatitis B surface antigen (HBsAg) was persistently positive for more than 6 months [14-16]. Patients fulfilled the following criteria were eligible for enrollment: 18 years old, hepatitis B envelope antigen(HBeAg) positive; HBV DNA ≥2.0E7 IU/ml; three recorded ALT levels≥2 times upper limit of normal (2×ULN) one year prior to liver biopsy and the ALT monitoring interval was 3 to 6 months; without antiviral therapy 3 months before liver biopsy. The exclusion criteria were that (1) co-infected with hepatitis C virus (HCV), hepatitis D virus (HDV)and human immunodeficiency virus (HIV);(2) tested positive for markers such as ceruloplasmin, anti-nuclear antibodies and anti-mitochondrial antibodies; (3) with HCC;(4)had heart diseases, thyroid diseases and kidney disease;(5) alcohol consumption >40g/day for man and >20g/day for woman.Figure1 summarized the flow diagram of the study population.

The serum ALT ULN in the patients in this study differed for men(30 U/L) and women (20 U/L). ALT normal reference intervals in Chinese apparently healthy adults were recommended as 55U/L for men and45 U/L for women[13, 25-27]

This studywas approved by the Ethics Committee of Changhai Hospital.As a retrospective study, written informed consent was exempted.

Data collection

The original medical records of the patients enrolled, including demography, family history, personal history, HBV-related antiviral treatment history, etc., were independently reviewed by two specialist physicians(AJ Xu and XS Liang).Baseline laboratory parameters of all patients were defined as one week following liver biopsy. Virological and biochemical assessments were performed at least every 6 months after liver biopsy. The levels of HBsAg, HBeAg, anti-HBs, anti-HBc, anti-HBe, were measured using commercially available kits (Abbot Laboratories, North Chicago, IL) in our clinical lab. Serum HBV-DNA level was quantified using commercially available kits(Sansure Biotech, China) with a threshold of the HBV DNA detection limit was 100 IU/ml.Data double entry method we used to reduce data input bias.

Liver biopsy and histology score

Percutaneous liver biopsy guided by B-ultrasound were performed using disposable needle (16G×10MM, Precisa, MS Hospital Service S.p.A). Liver necroinflammation and fibrosis were classified into 4 grades,G0-4 and S0-4, respectively according to the Scheuer scoring system [28].

Treatment

According to national or international treatment recommendations for CHB patients in IT phase[14-16] and combined the wishes of patients, 46patients (23patients with G ≥2 or S≥ 2, and 23 with G<2) initially received NAs (n=39) or pegIFN(n=7)antiviral therapy. Another 15patients in IT phase (1 patient with G ≥2, and 14 patients with G<2) did not receive antiviral therapy. All the patients were followed every 6 months. The median follow-up time was 36(16, 52) months.In the antiviral treatment progress, we implemented response-guiding-treatment (RGT) on all patients undergoing antiviral therapy following the roadmap concept [29, 30]. The baseline characteristics of patients in treatment and non-treatment groups were summarized in tableS1. There were no significant differences of the baseline data between the two groups.

Efficacy outcome measures

Definitions of response: Virological response (VR): undetectable serum HBV DNA, <100IU/ml.Partial virological response(PVR): serum HBV DNA decreased more than 2.0 Log $_{10}$ IU/ml from baseline, but detectable in a real-time PCR assay(≥100IU/ml) at 48 weeks of NAs therapy. Virological breakthrough: serum HBV DNA increased >1Log $_{10}$ IU/ml from the nadir in two consecutive measurements. Biochemical response: A serum ALT level of 5-55 for man and 5-45 for woman was considered normal.

Primary endpoints

The proportions of patients achieving the loss of HBeAg and plasma HBV DNA loads less than the limit of quantification, 100IU/ml at the treatment point 48 weeks(year1), 96weeks(year 2)and more than 144 weeks (year 3) respectively.

Secondary endpoints

The proportions of HBeAg loss and seroconversion to anti-HBe, namely HBeAg clearance and HBeAb positive rate, and ALT normalization rate (male: <55U/L,femal:<45U/L) at the treatment point 48 weeks(year1), 96weeks(year 2)and more than 144 weeks (year 3) respectively.

Safety outcome measures

The frequency, nature and severity of adverse events during the treatment duration.

Statistical analysis

Normal distribution quantitative data was expressed as X±SD, and non-normal distribution quantitative data was expressed as the median and interquantile range(IQR).The normal distribution variables were compared between groups using t test for univariate comparisons, and Mann-Whitney U tests were used for non-normal distribution variables. Logistic regression was used to analysis the risk factors. Receiver operating characteristic (ROC) curves and area under the ROC curves (AUROC) were used to estimate diagnostic performance. The best cutoff for maximal diagnostic accuracy was selected based upon the highest sum of sensitivity and specificity.

A P-value (2-tailed) of 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (ver.21.0.0; Chicago, IL, USA).

Results

Basic characteristics of patients

We screened 119 CHB patients who received liver biopsy in Shanghai Changhai hospital from 2012 to 2018, at last 61 treatment naïve CHB patients who were HBeAg positive, HBV DNA ≥ 2E7 IU/ml and ALT ≤ 2 × ULN before liver biopsy were enrolled in this study(Figure1).

The baseline demographics and clinical characteristics of these CHB patients were summarized in Table1. The mean age of these patients was 32 years.32(52.46%) of these patients had family history of HBV infection and 6 (9.84%) of them had a history of HBV-related liver cirrhosis or HCC in close relatives.All patients were positive for HBeAg and the median serum HBV DNA load was 8.70 Log_{10}IU/ml. The median ALT levels of these CHB patients in IT phase were 67 IU/L and the median ALT level in men was significantly higher than that in women (Z=-3.033,p = 0.002). 39.34% (24/61) patients had liver inflammation grade ≥ 2, and only 6 (9.84%)patients had moderate liver fibrosis (S ≥ 2).

Table 1

Baseline characteristics of the study patients.

Characteristics	Total(n = 61)
Age,y	31.95 ± 8.38
Male:Femal	61:11
Family history of HBV infection,n (%)	32(52.46)
Family history of HBV related HCC or cirrhosis,n(%)	6(9.84)
BMI	23.74 ± 3.41
Total cholesterol,mmol/L	4.61 ± 0.91
HBV DNA, Log10 (IU/mL)	8.70(7.75,8.70)
HBsAg, Log10 (U/L)	4.52 ± 0.35
HBeAg, Log 10 (S/CO)	3.10(2.99,3.17)
ALT,IU/L	67(39.5,98)
males	67(43,113)
Females	39(25,56)
AST,IU/L	36(27,54.5)
APRI	0.30(0.19,0.44)
WBC, 10^9/L	5.99 ± 1.34
PLT, 10^9/L	208.24 ± 48.13
Liver inflammation grading	
G0	0
G1	37
G2	21
G3	3
G4	0
Liver fibrosis grading	
S0	36

Note: BMI: body mass index; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; APRI: AST-to-platelet ratio index; WBC: white blood cells; PLT: platelet.

Characteristics	Total(n = 61)
S1	19
S2	4
S3	2
S4	0
Note: BMI: body mass index; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; APRI: AST-to-platelet ratio index; WBC: white blood cells; PLT: platelet.	

Factors related to the liver inflammation grade in patients with chronic HBV infection in immune tolerance phase

To determine the predictors of liver inflammation grade in CHB patients in IT phase, the baseline clinical and demographic characteristics of CHB patients in IT phases, stratified by the liver inflammation grade, were compared. As shown in Table2, compared to patients with mild liver inflammation, patients with moderate liver inflammation (n = 24) had higher serum ALT level (P = 0.03). Univariate and multivariate analysis showed that ALT level was a unique independent factor which was associated with liver inflammation in patients with CHB in IT phase (Table3). ROC analysis found that the AUROC of ALT level was 0.651, 95%CI: 0.510 to 0.792, P = 0.04. At a cutoff value of 83 U/ml, the serum ALT level predicted IT phase CHB patients with moderate liver inflammation with 65.6% accuracy, 75.0% specificity, 57.1% sensitivity. The positive predictive value (PPV) was 57.1% and the negative predictive value (NPV) was 69.2%.

Table 2

Characteristics of patient with chronic HBV infection by liver inflammation grade

Characteristics	Necro-inflammation		P value
	G ≥ 2(n = 24)	G<2(n = 37)	
Fibrosis grade ≥ 2,n/N(%)	5/24(16.67)	1/37(2.70)	0.031
Age ≥ 30y,n/N(%)	12/24(50)	16/37(43.2)	0.79
M:F	21:3	29:8	0.502
BMI > 24.0,n/N(%)	13/24(54.2)	23/37(62.2)	0.60
Total cholesterol, mmol/L	4.69 ± 0.94	4.54 ± 0.89	0.54
HBV DNA, Log10 (IU/mL)	8.69(8.20,8.69)	8.20(7.63,8.69)	0.05
HBsAg, Log10 (U/L)	4.49 ± 0.45	4.53 ± 0.27	0.63
HBeAg, Log 10 (S/CO)	3.08(2.95,3.14)	3.14(3.08,3.19)	0.06
ALT,IU/L	91 ± 54.35	64.7 ± 37.57	0.03
AST,IU/L	39.50(30.00,69.25)	32.00(25.00,52.00)	0.08
APRI	0.34(0.21,0.54)	0.17(0.11,0.28)	0.08
WBC, 10^9/L	6.12 ± 1.10	5.92 ± 1.48	0.57
PLT, 10^9/L	199.38 ± 39.97	214 ± 52.47	0.25

Note: M:male;F: female; BMI: body mass index; ALT: Alanine aminotransferase; AST:aspartate aminotransferase;HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; APRI: AST-to-platelet ratio index; WBC:white blood cells; PLT: platelet.

Table 3
Factors associated with liver inflammation in patients with CHB in IT phase

Virological relapse	Univariate analysis			Multivariated modelling		
	OR	95%CI	P value	OR	95%CI	P value
Demographic characteristics						
sex	0.518	0.123–2.188	0.371			
Age	1.312	0.468–3.681	0.605	0.997	0.933–1.066	0.930
BMI > 24.0 or ≤ 24	1.062	0.912–1.238	0.437	0.854	0.286–2.549	0.778
Family history of HBV related HCC	3.594	0.393–32.854	0.257			
Virology characteristics						
HBsAg(Log $_{10}$IU/ml)	1.173	0.603–2.283	0.638			
HBeAg (Log$_{10}$ S/CO)	0.189	0.021–1.662	0.133			
Chemical characteristics						
ALT, U/L	1.013	1.001–1.025	0.039			
ALT ≥ 2 × UNL or 2 × UNL	0.321	0.107–0.963	0.043	0.333	0.108–1.032	0.057
AST,U.L	1.027	1.001–1.054	0.038			
WBC, ×10^9/mL	1.122	0.760–1.656	0.563			
PLT, ×10^9/mL	0.993	0.982–1.005	0.248			
APRI	19.867	1.138-346.782	0.040			

Note: AST-to-platelet ratio index; WBC:white blood cells; PLT: platelet. HCC: hepatocellular carcinoma; BMI: body mass index; UNL: Upper normal limit;

Long-term Follow-up Evaluation And Treatment Outcomes

The median duration of follow-up for our cohort of 61 CHB patients in IT phase was 36months (IQR, 16, 60), with a total of 2355 person-months of follow-up. 46 patients received NAs therapy and the median therapy duration of these patients was 36 (16, 52) months. Among the 46 patients on NAs therapy, 6 patients were excluded for the following reasons: prevention of mother-to-child transmission(n = 1), therapy duration shorter than 6 months (n = 2), lack of efficacy evaluation data at least one test after

antiviral treatment(n = 2) and irregular medication (n = 1).At last 40 patients were eligible for antiviral response analysis.

Among the 40 patients, 37 patients had been treated with NA for 48wees, 30 been treated for 96 weeks and 24 patients been treated for more than 3 years, 6 patients had interferon (IFN) treatment experience and 4 patients adjusted the original antiviral strategy according to the road map concept during the long-term NAs antiviral therapy progress.

Primary Response

The proportion of patients who achieved both the loss of HBeAg and plasma HBV DNA ≤ 100 IU/ml at 48w,96w and ≥ 144w were 2.7,13.3 and 29.2%, respectively. Further stratified by liver inflammation grade, patients with different liver inflammation grade at baseline achieved similar primary response at all therapy points (Table4 and tableS2).

Table 4
Efficacy Endpoints

	48weeks		96weeks		≥ 144weeks	
	Total(N = 37)		Total(N = 30)		Total(N = 24)	
	n(%)	95%CI	n(%)	95%CI	n(%)	95%CI
Endpoints						
HBsAg loss or seroconversion	0(0)	0–10	1(3.33)	0–17	1(4.17)	0–21
HBeAg loss or seroconversion	1(2.7)	0–14	4(13.3)	4–31	7(29.2)	13–51
HBV DNA□100 IU/ml	20(55.6)	37–70	23(76.7)	58–90	23(95.8)	79–100
ALT□1 × ULN	25(69.4)	52.0–84.0	28(92.9)	76.0–99.0	23(95.8)	79.0-100
Primary endpoint						
HBeAg loss and HBV DNA□100 IU/ml	1(2.7)	0–14	4(13.3)	4–31	7(29.2)	13–51

HBV DNA response

Twenty of the 37 patients who completed 48 weeks NA treatment achieved virological response (HBV DNA<100IU/ml) at 48wand the virological response rate was 55.6%(95% CI: 37-70%)(Table4).All of the other 17patients achieved partial virological response (PVR), which means that all of them achieved HBV

DNA decreases at least 2 Log_{10} IU/ml from baseline but still detectable in a real-time PCR test(≥100IU/ml) at 48 weeks of NAs therapy.In addition, stratified by baseline liver inflammation grade, there was no significant difference in the HBV DNA response between the two groups(tableS2). As the treatment duration increased, the HBV DNA undetectable rate gradually increased,when the treatment time ≥144weeks nearly all patients achieved HBV DNA<100IU/ml in a real-time PCR assay (Table4).The median time to achieve a undetectable serum HBV DNA level in total population was 7 (6-18.75) months and there was no significant difference in the median time to obtain a undetectable serum HBV DNA level between patients with different baseline liver inflammation grade (G≥2 or 2), 12(6-29)Vs 6(6-12.5)months,P=0.15.

HBeAg response

The HBeAg loss rate of all patients at 48w, 96w and ≥144w was 2.7%(95%CI: 0-14%), 13.3%(95%CI:4-31%) and 29.2%(13-51%), respectively (Table4). Interestingly, when stratified by baseline liver inflammation grade, at 96w of NAs therapy the HBeAg response rate in patients with liver inflammation G 2 was significantly higher than that in patients with liver inflammation G≥2(P=0.037) (tableS2).

HBsAg response

Only one patient obtained HBsAg seroconversion during the study. As shown in FigureS1, 24weeks after NAs therapy initial HBsAg achieved nearly 1Log10 IU/ml decline from baseline and at 84 week of NAs therapy HBsAg clearance occurred. The total antiviral therapy duration of this patient was 112weeks and 24 weeks after drug withdraw the HBsAg response sustained.

ALT response

During the antiviral therapy progress ALT levels decreased compared to that of the therapy baseline. The normalization rate (female ALT45IU/ml, Male ALT55IU/ml) of ALT of patients in IT phase on long-term NAs therapy at 48W,96W and≥144W was 69.4% (95%CI:52.0-84.0%), 92.9% (95%CI:76.0-99.0%) and 95.8%(95%CI:79-100%), respectively (Table 4).When stratified by baseline liver inflammation grade, there was no significant difference in the ALT response between the two groups (tableS2).

Safety response

Of the 40 patients who received long term NAs therapy and with at least one response evaluation data, only one patient had ETV related gene mutation and viral breakthrough. Furthermore, one patient switch TDF to ETV due to drug related itch.

Discussion

Given the limited efficacy of current available therapies on patients with CHB in IT phases [18], national and international guidelines for the prevention and treatment of chronic HBV infection do not recommend treatment during the IT phase[14–16, 31]. However, understanding the correlation between high HBV DNA levels and HBeAg persistence and an increased risk of HCC, as well as the existence of HBV-specific T cells and their ability to proliferate and cytokine production during the IT phase [32], there were increased interest in treating patients in the IT phase. In this study we evaluated efficacy and safety of long-term NAs treating patients with CHB in IT phase that had been confirmed with liver biopsy, and found most patients achieved potent HBV DNA inhibition on treatment duration increased, when treatment duration more than 144 weeks 95.8% patients achieved HBV DNA response (< 100 IU/ml), as well as the HBeAg loss or seroconversion rate of these patients was 29.2%. In addition, during the long-term NAs treatment one patient achieved HBsAg clearance at 84 week of NA therapy and the HBsAg response sustained at 24 weeks off therapy. These results confirmed that patients with CHB in IT phases who receiving long-term NAs treatment achieved a similar virological response rate as patients with CHB in IA phase: HBeAg-positive patients treated with TDF for 5 years, the HBV DNA negative rate (< 300 copies/ml) was 98%, and the HBeAg seroconversion rate was 31%[33, 34]. Some other clinical research also got similar results. Wu ZX[24] evaluated the efficacy of TDF and LDT combination therapy for chronic hepatitis B patients in IT phase and found that TDF and LDT combination therapy shows a rapid antiviral response in patients with CHB in IT phase. In another ETV and Peg IFN combination therapy study also shows potent antiviral response in patients with CHB in IT phase [17]. However, HBeAg seroconversion rate of patients in these studies was unsatisfactory.

Baseline ALT level (2–10 × ULN) or liver inflammation grade(G2), genotype A or B, low baseline HBsAg level (< 25 000 IU/mL) and high quantitative baseline core antibody (qAnti-HBc), etc. are all the predictor of the efficacy of IFN[14, 16]. Therefore, national and international guidelines suggest that patients in IT phase with liver tissue inflammation and necrosis ≥ G2 or S2 are indications to initiate antiviral therapy [14, 15, 31]. However, in this study, it was found that IT patients with liver tissue inflammation and necrosis G1 or S0-1 received long-term NA treatment obtained a similar virological response as patients with necrotic ≥ G2. In addition, univariate and multivariate analysis showed that baseline liver tissue inflammation and necrosis ≥ G2 or ≥ S2 was inadequate for predictor of efficacy of long-term NAs treatment in patients with CHB in IT phase (data were not shown).

Immune tolerate means that patients with high viral load, minimal or no hepatic inflammation, suggesting a lack of immune recognition or tolerance to the virus. However, the current biochemical, serological and molecular markers are inadequate for differentiating immune tolerance phase from HBeAg positive chronic hepatitis B phase. In this study, 39.34% patients who were clinically determined to be in the IT phase based on biochemical test, liver imaging test, and virological markers showed histological inflammation grade ≥ 2 by liver biopsy. Multivariate analysis showed that ALT ≥ 2 × UNL was the only independent predictor for liver inflammation grade, however, the PPV and NPV was only 57.1% and 69.2%, respectively. The results suggested that use of only current biochemical ,serological and molecular markers without resorting to liver biopsy to define immune tolerate phase in chronic HBV infection may miss histological significant disease in a proportion of patients.

As a retrospective real-time study there were some limitations. First, the retrospective design of study might have caused selective bias resulting uncertainty in predictor of liver inflammation and necrosis in patient with CHB in IT phase. Second, because the data was not always complete, we cannot determine the predictive effect of HBeAg or HBsAg level dynamic change on the efficacy of IT patients with CHB in long-term NAs treatment.

Conclusions

Our study showed that liver biopsy is still the gold standard to determine the extent of liver inflammation and necrosis in patients with CHB in IT phase. Patients with CHB in IT phase received long-term NAs antiviral treatment can achieve potent virological response and not limited to patients with liver tissue inflammation and necrosis G2.

Declarations

Declarations

The authors declare that they have no competing interests

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Changhai Hospital. (CHEC2019-056).

Written informed consent was exempted.

Consent for publication

Not applicable

Availability of data and material

All data and analysis results are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Beijing Medical Health Foundation-Medicine Science Study (No.YWJKJJHKYJJ-G17007) and Shanghai Natural Science Fund, (No.16ZR1400400) only provide financial support, but not participating in the study design, data collection and analysis

Authors' contributions

LXS conceived the study and participated in the design of the study; performed the data analysis and drafted the manuscript;

XAJ recruited the patients and collected data;

LYY checked the data and performed the data analysis;

XJY treated and followed the patients.

YJ treated and followed the patients

XH treated and followed the patients

All authors have read and approved the final manuscript.

Acknowledgment

The authors thank Dr Qian He (Department of Health Statistics, Second Military Medical University, Shanghai) for giving helpful comments in data analysis and confirmation of statistical methods.

Abbreviations

NAs, nucleoside analogues；CHB：chronic hepatitis B；IT, immune tolerate; ALT, alanineamino transferase; CI, confidence interval ;HBV, Hepatitis B virus；HCC,hepatocarcinoma;IA：immune active；IFN：interferon； HCV：hepatitis C virus；HDV：hepatitis D virus；HIV：human immunodeficiency virus；RGT, response-guiding-treatment; VR, Virological response;PVR, Partial virological response;ROC, Receiver operating characteristic;AUROC,area under the ROC curves；PPV,positive predictive value;NPV, negative predictive value

References

1. JL. D: **Hepatitis B virus infection.** . *N Engl J Med* 2008, **359**:1486-1500.
2. Liaw YF, CM. C: **Hepatitis B virus infection.** *Lancet* 2009, **373**:582-592.
3. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, JJ. O: **Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013.** . *Lancet* 2015, **386**:1546-1555.
4. Lavanchy D: **Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures.** . *J Viral Hepat* 2004, **11**:97-107.
5. Chou R, Dana T, Bougatsos C, Blazina I, Khangura J, B. Z: **Screening for hepatitis B virus infection in adolescents and adults: a systematic review to update the U.S. Preventive Services Task Force recommendation.** . *Ann Intern Med* 2014, **161**:31-45.
6. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, Risk Evaluation of Viral Load E, Associated Liver Disease/Cancer-In HBVSG: **Predicting cirrhosis risk based on the level of circulating hepatitis B viral load**. *Gastroenterology* 2006, **130**(3):678-686.
7. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH: **Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level**. *Jama* 2006, **295**(1):65-73.
8. Yim HJ, Lok AS: **Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005**. *Hepatology* 2006, **43**(2 Suppl 1):S173-181.
9. Chen CJ, Yang HI: **Natural history of chronic hepatitis B REVEALed**. *J Gastroenterol Hepatol* 2011, **26**(4):628-638.
10. Chang T-T, Liaw Y-F, Wu S-S, Schiff E, Han K-H, Lai C-L, Safadi R, Lee SS, Halota W, Goodman Z *et al*: **Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B**. *Hepatology* 2010, **52**(3):886-893.
11. Papatheodoridis GV, Idilman R, Dalekos GN, Buti M, Chi H, van Boemmel F, Calleja JL, Sypsa V, Goulis J, Manolakopoulos S *et al*: **The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or tenofovir in Caucasians with chronic hepatitis B**. *Hepatology* 2017, **66**(5):1444-1453.
12. Patrick Marcellin, Edward Gane, Maria Buti, Nezam Afdhal, William Sievert, Ira M Jacobson, Mary Kay Washington, George Germanidis, John F Flaherty, Raul Aguilar Schall *et al*: **Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study**. *Lancet* 2013, **381**(9865):468-475.
13. Wu WC, Wu CY, Wang YJ, Hung HH, Yang HI, Kao WY, Su CW, Wu JC, Chan WL, Lin HC *et al*: **Updated thresholds for serum alanine aminotransferase level in a large-scale population study composed of 34 346 subjects**. *Aliment Pharmacol Ther* 2012, **36**(6):560-568.
14. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, American Association for the Study of Liver D: **AASLD guidelines for treatment of chronic hepatitis B**. *Hepatology* 2016, **63**(1):261-283.
15. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN *et al*: **Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update**. *Hepatol Int* 2016, **10**(1):1-98.

16. . GWHO: **Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection.** . 2015 Available from: https://www.ncbi.nlm.nih.gov/books/NBK305553/.
17. Feld JJ, Terrault NA, Lin HS, Belle SH, Chung RT, Tsai N, Khalili M, Perrillo R, Cooper SL, Ghany MG *et al*: **Entecavir and Peginterferon Alfa-2a in Adults With Hepatitis B e Antigen-Positive Immune-Tolerant Chronic Hepatitis B Virus Infection**. *Hepatology* 2019, **69**(6):2338-2348.
18. Chan HL, Chan CK, Hui AJ, Chan S, Poordad F, Chang TT, Mathurin P, Flaherty JF, Lin L, Corsa A *et al*: **Effects of tenofovir disoproxil fumarate in hepatitis B e antigen-positive patients with normal levels of alanine aminotransferase and high levels of hepatitis B virus DNA**. *Gastroenterology* 2014, **146**(5):1240-1248.
19. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, Iloeje UH, Su J, Hsiao CK, Wang LY, You SL *et al*: **Changes in serum levels of HBV DNA and alanine aminotransferase determine risk for hepatocellular carcinoma**. *Gastroenterology* 2011, **141**(4):1240-1248, 1248 e1241-1242.
20. Kennedy PTF, Litwin S, Dolman GE, Bertoletti A, Mason WS: **Immune Tolerant Chronic Hepatitis B: The Unrecognized Risks**. *Viruses* 2017, **9**(5).
21. Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, Chauhan R, Bose S: **Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT**. *Gastroenterology* 2008, **134**(5):1376-1384.
22. Mason WS, Gill US, Litwin S, Zhou Y, Peri S, Pop O, Hong ML, Naik S, Quaglia A, Bertoletti A *et al*: **HBV DNA Integration and Clonal Hepatocyte Expansion in Chronic Hepatitis B Patients Considered Immune Tolerant**. *Gastroenterology* 2016, **151**(5):986-998 e984.
23. Rosenthal P, Ling SC, Belle SH, Murray KF, Rodriguez-Baez N, Schwarzenberg SJ, Teckman J, Lin HS, Schwarz KB, Hepatitis BRN: **Combination of Entecavir/Peginterferon Alfa-2a in Children With Hepatitis B e Antigen-Positive Immune Tolerant Chronic Hepatitis B Virus Infection**. *Hepatology* 2019, **69**(6):2326-2337.
24. Wu ZX, Chen FS, Zhou XL, Huang Q, Zhang SA, Wu HC, Cai LR, Zeng ZY, Li YH, Li DL: **Tenofovir and telbivudine combination therapy rapidly decreases viral loads in immune-tolerant chronic hepatitis B patients awaiting assisted reproduction: an open-label, randomized, controlled study**. *Eur J Gastroenterol Hepatol* 2019, **31**(7):832-835.
25. Park HN, Sinn DH, Gwak GY, Kim JE, Rhee SY, Eo SJ, Kim YJ, Choi MS, Lee JH, Koh KC *et al*: **Upper normal threshold of serum alanine aminotransferase in identifying individuals at risk for chronic liver disease**. *Liver Int* 2012, **32**(6):937-944.
26. Yang S, Qiao R, Li Z, Wu Y, Yao B, Wang H, Cui L, Yang Y, J. Z: **Establishment of reference intervals of 24 chemistries in apparently healthy adult Han population of Northern China**. *Clinical Biochemistry* 2012, **45**:1213-1218.
27. Ceriotti F, Henny J, Queraltó J, Ziyu S, Özarda Y, Chen B, Boyd James C, Panteghini M: **Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study**. In: *Clinical Chemistry and Laboratory Medicine.* vol. 48; 2010: 1593.

28. V. J. Desmet, M. Gerber, J. H. Hoofnagle, M. Manns, P. J.Scheuer: **Classification of chronic hepatitis: diagnosis, grading and staging**. *Hepatology* 1994, **19**(6):1513-1520.
29. Chang TT: **On-treatment monitoring of HBV DNA levels: predicting response and resistance to oral antiviral therapy at week 24 versus week 48**. *Hepatol Int* 2009, **3 Suppl 1**:16-23.
30. Gane EJ: **The Roadmap concept: using early on-treatment virologic responses to optimize long-term outcomes for patients with chronic hepatitis B**. *Hepatol Int* 2008, **2**(3):304-307.
31. Chinese Society of Infectious Diseases, Chinese Medical Association, Chinese Society of Hepatology, Association CM: **The guideline of prevention and treatment for chronic hepatitis B(2019 version)**. *Chin J Clin Infect Dis* 2019, **12**(6): E001-E001.
32. Bertoletti A, PT. K: **The immune tolerant phase of chronic HBV infection: new perspectives on an old concept.** *Cell Mol Immunol* 2015, **12**:258-263.
33. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R *et al*: **Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study.** *Lancet* 2013, **381**:468-475.
34. Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z *et al*: **Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B**. *HEPATOLOGY* 2010, **52**:886-893.

Supplemental Figure

FigureS1: Virological index dynamic change in patients who achieved HBsAg loss during NAs therapy. ALT: Alanine aminotransferase; AST:aspartate aminotransferase; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen

Figures

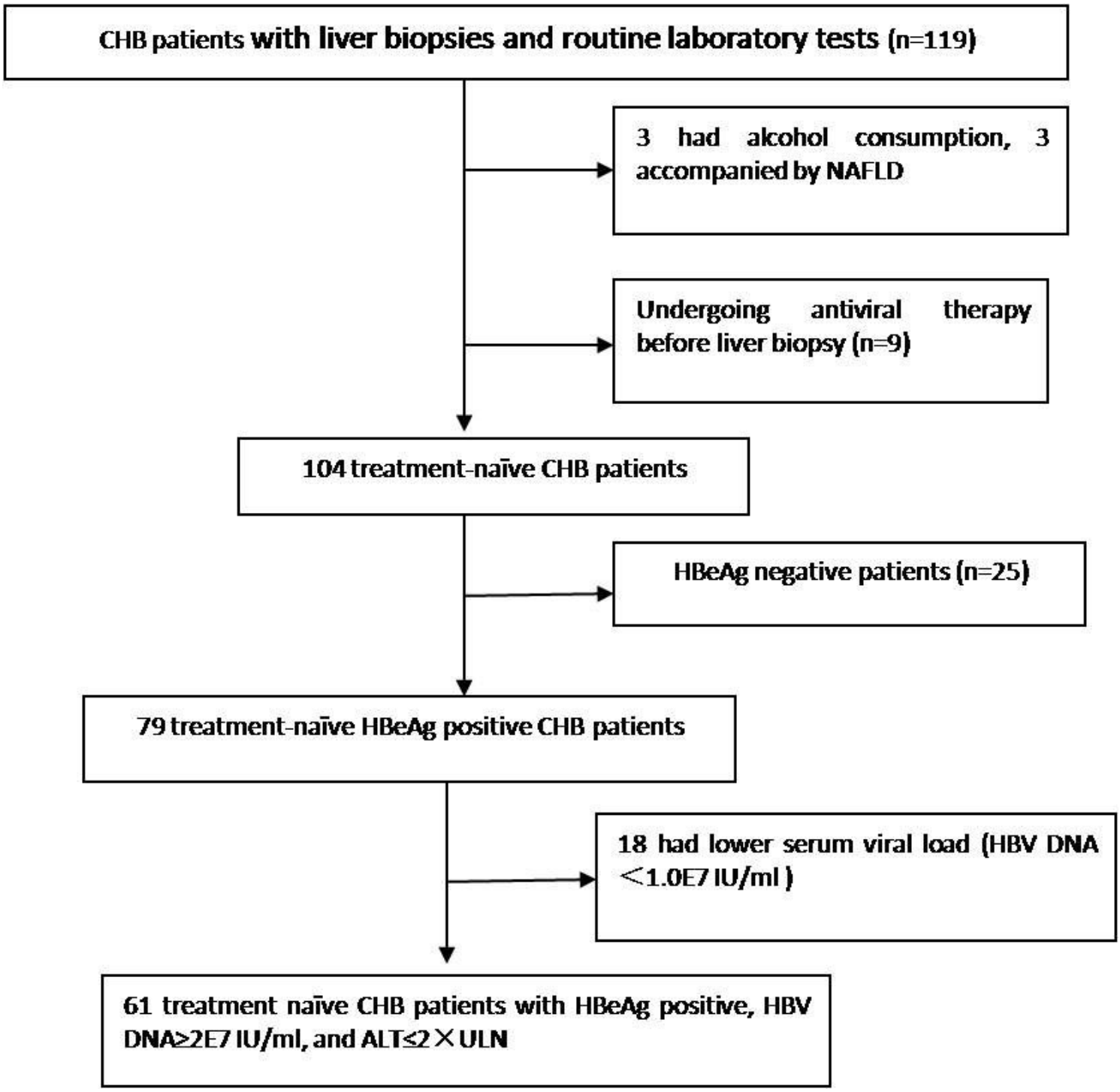

Figure 1

Flow diagram of the study population.ALT: Alanine aminotransferase;CHB:chronic hepatitis B, HBeAg: hepatitis B e antigen; NAFLD: nonalcoholic fatty liver disease;ULN: upper limit of normal.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tablesupple.docx
- FigureS1.jpg