Journal of Clinical Virology 48 (2010) 22–26

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

On-treatment serum HBsAg level is predictive of sustained off-treatment virologic response to telbivudine in HBeAg-positive chronic hepatitis B patients

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ARTICLE INFO

Article history:
Received 21 December 2009
Received in revised form 10 February 2010
Accepted 17 February 2010

Keywords:
Telbivudine
HBsAg
Sustained virologic response
Hepatitis B
Predictor

ABSTRACT

Background: Effective management of chronic hepatitis B infection is still very challenging, despite decades of clinical research. Telbivudine is one of the most frequently used antiviral drug at the current stage, but its long-term effectiveness, particularly at off-treatment, is still unclear.
Objectives: To assess on-treatment HBsAg kinetics in patients treated with telbivudine for 2 years, and predicting sustained virologic response (SR) at 2 years off-treatment.
Study design: Serum HBV DNA/HBsAg levels were assessed from 17 HBeAg+ patients treated with telbivudine 600 mg/day for 104 weeks, at baseline, weeks 24, 52 and 104, as well as during off-treatment follow-up.
Results: HBsAg levels <2 $\log_{10}$ IU/ml at treatment week 104 were highly predictive of SR (i.e., HBV DNA <300 copies/ml, HBeAg seroconversion, ALT normalization) at 2 years off-treatment (positive predictive value [PPV], 93%; negative predictive value [NPV], 100%). HBsAg levels consistently declined from baseline only in patients achieving SR during 2 years off-treatment. At weeks 24 and 52, HBsAg decline rate was a better predictor of off-treatment response than HBV DNA decline rate. HBsAg decline rates of >0.8 and >1 $\log_{10}$ IU/ml at treatment weeks 24 and 52 were predictive of SR (PPV, 75%; NPV, 86% at week 24; PPV, 75%; NPV, 86% at week 52).
Conclusions: Serum HBsAg levels <2 $\log_{10}$ IU/ml at treatment week 104 are highly predictive of SR to telbivudine at 2 years off-treatment. HBsAg decline rate at on-treatment weeks 24 and 52 from baseline were also more predictive of SR than HBV DNA decline rate.

1. Background

Oral nucleos(t)ide analogs (NAs) are widely used in the treatment of chronic hepatitis B (CHB) patients. The suppression of serum HBV DNA to low/undetectable levels and hepatitis B e antigen (HBeAg) seroconversion are associated with durable response to oral antiviral therapy and the clinical remission.[1–4] Current guidelines for CHB recommend a finite course of NA for HBeAg-positive patients, that achieved these end points on two separate occasions with ≥6 months apart.[5,6] However the persistence of covalently closed circular DNA (cccDNA), despite HBeAg seroconversion, can contribute to low-level viral replication that may ultimately lead to hepatitis relapse.

It remains challenge to determine that these patients have sustained virologic response (SR) to oral therapy, due to the invasive nature of quantifying cccDNA levels in hepatocytes. A noninvasive method to assess cccDNA levels with serum HBV DNA and HBeAg levels would be useful for monitoring patients' response and optimizing strategies. Previous studies have demonstrated a positive correlation between levels of intrahepatic cccDNA and serum hepatitis B surface antigen (HBsAg),[7] suggesting that HBsAg levels may be a useful marker of the level of hepatocyte infection by HBV, and could serve as a surrogate indicator of the ability of the host immunity to inhibit viral replication. The usefulness of on-treatment HBsAg levels as a surrogate marker of efficacy and a predictor of outcomes for pegylated interferon (PegIFN) alfa-2a has been demonstrated in CHB patients.[8–12] In these studies, early

Abbreviations: SR, sustained virologic response; NAs, nucleos(t)ide analogs; cccDNA, covalently closed circular DNA; VR, virologic response; CP, consolidation period; pts, patients.

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1386-6532/$ – see front matter.
doi:10.1016/j.jcv.2010.02.014

decline in HBsAg levels was a strong predictor of SR and HBsAg clearance.[8,10,11] In contrast, the relationship between on-treatment with NAs HBsAg levels and long-term response in CHB patients is unclear.

Telbivudine, a potent oral NA for CHB treatment, induces rapid and profound inhibition of HBV DNA replication.[4,13] Telbivudine continuously induces high rates of HBeAg seroconversion in HBeAg-positive patients, particularly among those who have undetectable serum HBV DNA at week 24 of treatment.[4,13,14] Telbivudine may modulate the immune system through a similar pathway with IFN, inhibiting viral replication directly.[15,16]

2. Objectives

To determine the usefulness of quantitative on-treatment HBsAg levels as predictors of SR during and after telbivudine therapy for up to 2 years.

3. Study design

3.1. Patients and study design

The retrospective study consisted of 17 patients from Ruijin Hospital, China. The CHB patients had HBeAg-positive, alanine aminotransferase (ALT) levels between 1.3- and 10-fold the upper limit of normal, and HBV DNA levels ≥106 copies/ml (Table 1; Fig. 1). All the selected patients were HBsAg positive for ≥6 months, with histopathology confirmation of CHB 12 months prior to study entry. They had participated in the large, multinational, randomized, phase III registration study and received telbivudine 600 mg/day for 104 weeks.[13,14,17] Exclusion criteria included prior oral NAs treatment, other liver diseases and pregnancy. At the week 104, patients who met the criteria for virologic response (VR) for ≥24 weeks to treatment discontinued telbivudine. VR was defined as ALT normalization, serum HBV DNA levels undetectable, and HBeAg seroconversion. The remaining patients continued to receive telbivudine 600 mg/day for an additional 104 weeks. VR was monitored in all patients for an additional 104 weeks on/off-treatment. Among patients with VR, SR during the 104 weeks off-treatment follow-up period was defined as ALT normalization, serum HBV DNA levels undetectable, and HBeAg seroconversion.

Table 1
Patient characteristics and treatment efficacy at week 104 with telbivudine.

Parameter		Baseline (n = 17)
Male gender, *n*		12
Age, year, median		29 (16–53)
HBV genotype, *n*		
B		5
C		12
BMI, kg/m^2, median		22 (17–27)
	Baseline	End of treatment
HBV DNA, $\log_{10}$ copies/ml	9.48 (6.18–11.88)	2.18 (2.18–10.02)
ALT level, IU/l	178 (66–257)	23 (9–89)
HBsAg level, $\log_{10}$ IU/ml	3.63 (2.24–4.69)	3.52 (0.00–4.05)*
Undetectable HBV DNA, *n*(%)	0(0)	14 (82)
ALT normalization, *n*(%)	0(0)	15 (88)

* $P = 0.031$.

3.2. Quantitative analysis of serum HBV DNA and HBsAg levels

Serum HBV DNA levels and quantitative serum HBsAg levels were determined at baseline, weeks 24, 52, 76 and 104 of treatment, and during the 104 weeks of follow-up, using the Roche COBAS HBV Amplicor Monitor™ assay (with a low limit of detection of 300 copies/ml), and the ARCHITECT i2000SR HBsAg QT assay,[18] respectively.

3.3. Statistical analysis

Statistics were performed, using SPSS 13.0 (Chicago, IL). It is determined if age, sex, body mass index (BMI), ALT, HBV DNA, and genotype from the patients at baseline can predict the SR of the treatment, using multivariate logistical analysis. Continuous variables were shown as median (range) due to small sample size, and the data were compared using Mann–Whitney *U* test. The decline in serum HBsAg and HBV DNA levels to predict SR was assessed, using area under receiver operating characteristic (AUROC) curves. *P* value <0.05 would be considered statistically significant.

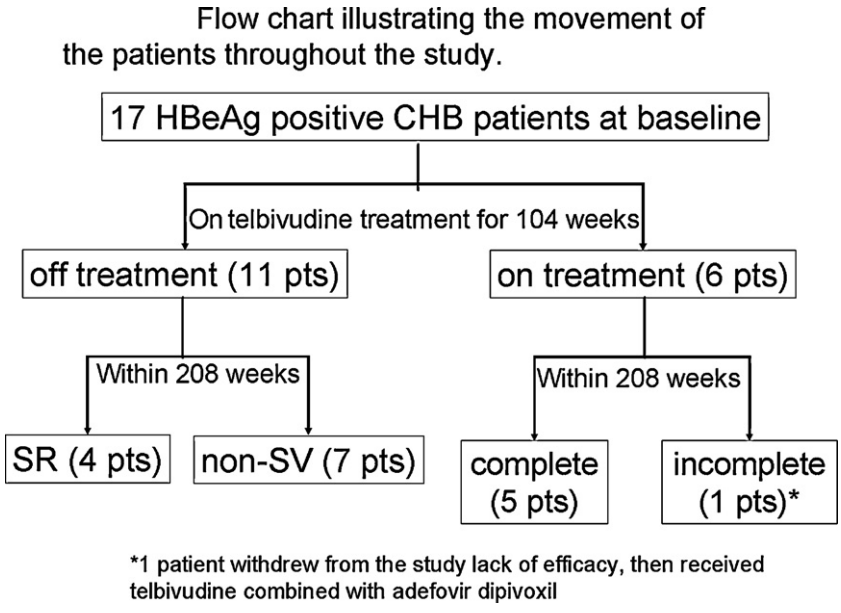

Fig. 1. Flow chart illustrating the movement of the patients throughout the study.

4. Results

4.1. Clinical characteristics

At week 104 of telbivudine treatment (Table 1), 82% and 88% of patients had undetectable serum HBV DNA and ALT normalization, respectively. The median of HBsAg levels declined steadily during treatment from baseline to week 104 (3.63 $\log_{10}$ vs 3.52 $\log_{10}$ IU/ml; $P = 0.031$), but no HBsAg loss/seroconvertion was found during the treatment. Sixty-five percent of (11/17) patients had VR ≥24 weeks and discontinued telbivudine at week 104, but 35% (6/17) patients did not have VR ≥24 weeks and continued telbivudine for further 104 weeks. Moreover, of these 6 non-VR patients for another 104 weeks treatment, 3 of them presented continuously with HBeAg-positive, but serum HBV DNA levels were undetectable and ALT normalization; 3 of the 6 developed virologic breakthrough; 2 of the 3 had the genotypic resistance with rtM204I and biochemical breakthrough, and 1 withdraw and combined with adefovir. The rest 1 of the 3 developed HBeAg-negtive though virologic breakthrough, and none of them developed HBsAg loss.

Within these 11 patients after discontinued treatment, 36% (4/11) met the criteria for SR during the 104 weeks of off-treatment follow-up. The median of consolidation period (CP) of these 4 patients, defined as duration between confirmed HBeAg seroconversion until end of treatment, was 80 (64–92) weeks; whereas the median of CP of other 7 non-SR patients was 68 (44–92) weeks. After discontinued treatment all of these 7 non-SR patients, 2 of them had biochemical breakthrough, all of them had virologic rebound, but developed HBeAg-negative CHB. There is no significant difference of CP between patients with and without SR ($P = 0.203$).

4.2. Characterization of on-treatment HBsAg levels and HBV DNA levels

There were significant differences of median quantitative HBsAg levels between VR and non-VR patients at the weeks 24 ($P = 0.048$), 52 ($P = 0.027$), 76 ($P = 0.015$) and 104 ($P = 0.007$) of telbivudine treatment (Fig. 2A), but was not at baseline ($P = 0.462$). In contrast, significant difference of the HBV DNA levels were detected at the week 24, but not at the weeks 52, 76 and 104 of telbivudine treatment.

Further analysis of HBsAg levels among these 11 VR patients, showed that the significant differences of median quantitative HBsAg between SR and non-SR were observed only at weeks 76 and 104, but was not at weeks 24 and 52.

Significant differences in median quantitative serum HBsAg decline from baseline between SR and non-SR patients were observed at treatment weeks 24, 52, 76 and 104 (Fig. 2B). Surprisingly, 1 of the 4 patients with SR demonstrated on-treatment serum HBsAg levels declined by >3 $\log_{10}$ IU/ml from baseline at week 24, with HBsAg seroconversion occurring at week 100 off-treatment. In contrast, a decline of <1 $\log_{10}$ IU/ml in serum HBsAg levels was observed on the rest of 3 patients with SR at treatment week 24, no serum HBsAg loss was detected until the end of follow-up.

Interestingly, no significant difference of the HBV DNA levels and the decline of the HBV DNA levels from baseline between SR and non-SR patients were detected at the weeks 24, 52, 76 and 104 of telbivudine treatment.

4.3. On-treatment HBsAg as a predictor of off-treatment SR

On-treatment serum HBsAg levels were used as predictive of the probability of achieving SR after discontinued treatment in these 11 patients at week 104 (AUROC, 0.952; 95% confidence interval [CI], 0.844–1.060; $P = 0.008$) (Fig. 3A). Specifically, serum HBsAg levels

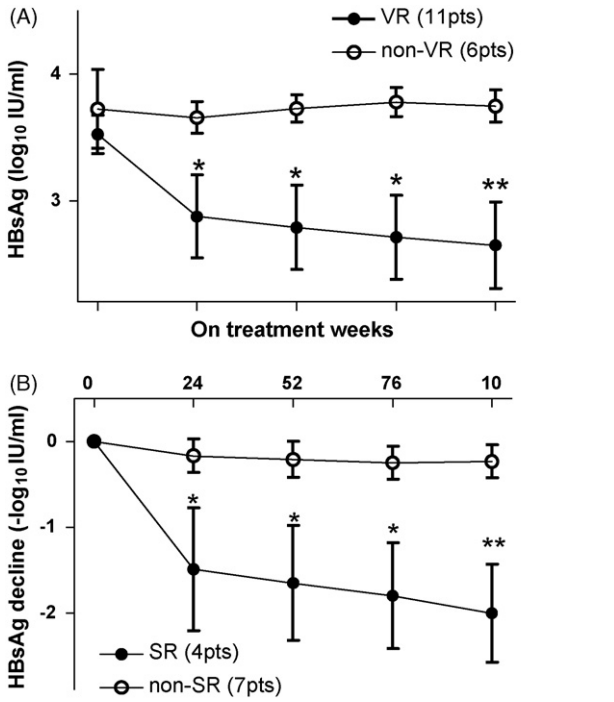

Fig. 2. On-treatment quantitative serum HBsAg levels in CHB patients (A) achieved VR —●— and non-VR —○—, (B) declining from baseline achieve SR, —●— and non-SR, —○—. *$P < 0.05$, **$P < 0.01$.

<2 $\log_{10}$ IU/ml had a sensitivity, specificity, PPV, and NPV for predicting off-treatment SR to telbivudine of 75%, 100%, 100% and 93%, respectively, at treatment week 104.

Using multivariate logistic regression analyses, the basal level of age, sex, body mass index (BMI), ALT and HBV DNA, and genotype were analyzed, but no predictive of the probability of achieving SR was observed.

4.4. Comparison of on-treatment HBsAg and HBV DNA level as predictors of off-treatment SR

AUROCs, designed to aid in the prediction of off-treatment SR, were derived from the serum HBsAg and HBV DNA levels at week 24 of treatment. At treatment week 24, a decline in serum HBsAg levels had a greater power (AUROC, 0.981; 95% CI, 0.923–1.039; $P = 0.005$) to predict off-treatment SR than did serum HBV DNA decline (AUROC, 0.679; 95% CI, 0.368–0.978; P=0.308) (Fig. 3B). When the cutoff value of serum HBsAg decline from baseline was >0.8 $\log_{10}$ IU/ml, the sensitivity, specificity, PPV and NPV for predicting off-treatment SR were 75%, 86%, 75% and 86%, respectively. Furthermore, HBsAg decline from baseline was more predictive (AUROC, 0.942; 95% CI, 0.832–1.053; $P = 0.009$) of off-treatment SR than were serum HBV DNA levels at treatment week 52 (AUROC, 0.519; 95% CI, 0.148–0.891; $P = 0.910$). When the cutoff value of serum HBsAg decline from baseline was >1 $\log_{10}$ IU/ml, the sensitivity, specificity, PPV and NPV for predicting SR were 75%, 86%, 75% and 86%, respectively. However, the absolute HBsAg levels at weeks 24 and 52 of treatment do not show a power to predict off-treatment SR. The patients with HBsAg < 2 log at week 104 are the same patients as those with HBsAg decline >0.8 log at week 24 and >1 log at week 52.

4.5. Characterization of off-treatment HBsAg levels

There was no significant difference of the median quantitative HBsAg levels and the decline median quantitative HBsAg levels

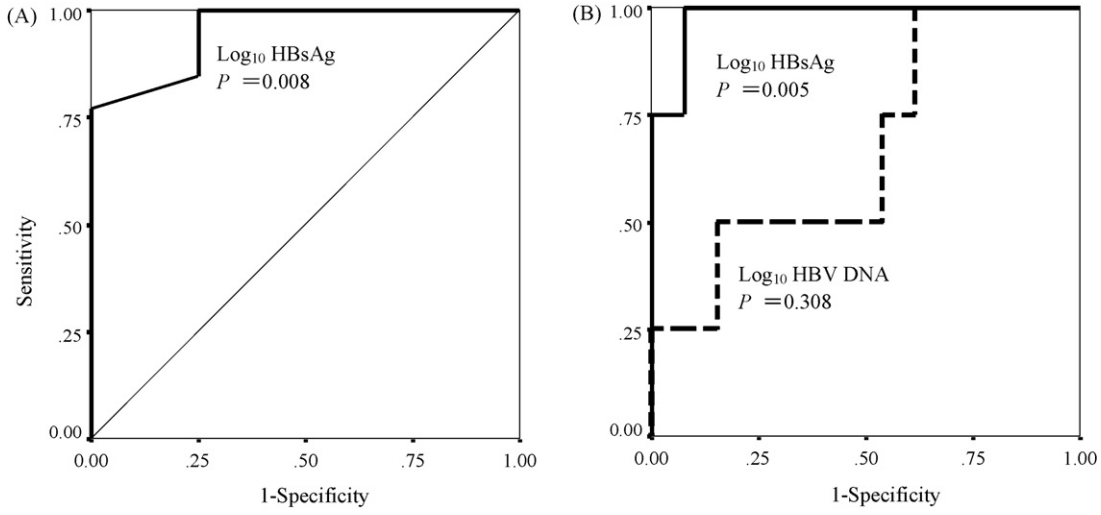

Fig. 3. Analysis of on-treatment HBsAg and HBV DNA levels as predictors of off-treatment SR. (A) AUROC curve of $\log_{10}$ serum HBsAg levels at treatment week 104 in patients with SR for up to 104 weeks off-treatment. (B) AUROC curves of $\log_{10}$ serum HBsAg decline (solid line, –) and $\log_{10}$ serum HBV DNA decline (dashed line, ——) at treatment week 24 from baseline for up to 104 weeks off-treatment.

from baseline between VR and non-VR patients at the weeks 156, 208, respectively, during the follow-up period in VRs and non-VRs. During the follow-up period in SRs and non-SRs, there were significant differences of the median quantitative HBsAg levels between SR and non-SR patients at the weeks 52 (P = 0.014), 76 (P = 0.014) and 104, (P = 0.014) after telbuvidine withdrawal, but was not at week 24. However, the significant differences of the decline median quantitative HBsAg from baseline between SR and non-SR were observed at weeks 24, 52, 76 and 104 after telbuvidine withdrawal.

5. Discussion

In the current study, a higher rate (65%) of HBeAg seroconversion was observed in 17 CHB patients treated with telbivudine for 104 weeks than previous report (~30%).[14] Such discrepancy may be due to different genetic backgrounds and/or environments. The patients in our study are 100% Chinese; whereas there were ~50% Chinese.[14] The assessment of VR is a pivotal for management of CHB patients, serum HBV DNA level is considered the most common surrogate marker for assessment of antiviral therapy,[5,6] especially prior to or 24 week after the treatment.[4,14] Serum HBsAg levels correlate well with the cccDNA and intrahepatic HBV DNA.[7] HBsAg level prior to the treatment[7] and/or decline HBsAg level early antiviral therapy[8,11] is a better predictor for PegIFN efficacy in CHB patients, than serum HBV DNA level. These findings support our data that serum HBsAg levels in CHB patients achieved VR status to telbivudine were significantly lower than that non-VR, at weeks 24, 52, 76, and 104.

Furthermore in our current study there is ~40% (4 out of 11) patients achieved SR 104 weeks after cessation of telbivudine. Other reports, however, demonstrated that high proportion of patients achieved SR after withdrawal of telbivudine (80%) and lamivudine (88%),[14] the median durations of the post-treatment follow-up was 29.1 and 32.6 weeks respectively. The discrepancy of SR achievement in our study and that from others[14] may be due to longer durations of the post-treatment follow-up and/or different genetic backgrounds. It is supported by the report[19] that showed the SR was substantially low in Asians. Interestingly Marcellin et al.[20] reported that, the proportion of HBeAg-negative CHB with Peginterferon or lamivudine treatment achieved SR (HBV DNA <400copies/ml) was 16% or 4%, respectively, in the 2 year follow-up. Such rates are substantially lower than that in the current study. A number of possible reasons can be used for the explanation: (1) the different responses between NAs and Peginterferon; (2) the patients in Marcellin's studies received 48 weeks treatment only, discontinued whether they got VR (most patients did not get VR, so the relapse is higher and SR is lower); (3) different populations reflecting genetic backgrounds and/or different genotypes of viruses. Furthermore, there is no significant difference of CP between patients who achieved SR with (80 weeks) and without SR (68 weeks). Interestingly, others found that CP reached only at 40 or 47 weeks, respectively for telbivudine or lamivudine. CP maybe independent to SR outcomes, and might not be used as predictor in the outcome of SR after withdrawal telbivdine.

Our data show HBsAg is a predictor of SR after telbuvidine withdrawal, whereas others found that there was no correlation between HBsAg and SR after withdrawal of lamivudine.[21] This may be due to different pharmacological pathways and/or immunological modulations, in response to telbuvidine or lamivudine treatment. The serum HBsAg levels only in SR, but not in non-SR, patients declined significantly during 104 weeks treatment. Thus the data from the current study indicate that serum HBsAg decline rate, but not the absolute HBsAg level, is a more reliable predictor of SR for drug withdrawal than HBV DNA decline rate at the week 24 of telbivudine treatment. These findings in telbuvidine are supported by the findings from Moucari et al. in HBV patients treated with PegIFN.[11] Moreover, in the current study, we found that the HBsAg levels in patients who achieved SR still decline during the 104 weeks after drug withdrawal. Such interesting observation is the first report up-to-date, certainly warrant further investigation in a large scale fashion.

HBsAg level <2 $\log_{10}$ IU/ml at the time drug withdrawal, it could serve as a predictor for SR in CHB patients, especially in these patients whose HBsAg at the baseline or other treatment points cannot be obtained, after the cessation of 104 weeks of treatment (PPV,100%; NPV, 93%).

We acknowledge that the number of patients for the current study is small, but it is simply for proof of concept. The long-period treatment and large number of the patients are currently being investigated.

In conclusion, serum HBsAg, a surrogate marker, provides a reliable prediction for achievement of VR during the treatment and SR after telbuvidine withdrawal. Serum HBsAg levels <2 $\log_{10}$ IU/ml at treatment week 104 are highly predictive of SR to telbivudine at 2 years off-treatment. HBsAg decline rate at on-treatment weeks 24 and 52 from baseline are also more predictive of SR than HBV DNA

decline rate. Thus data provide useful information for both basic research and/or guideline for clinical treatment.

Acknowledgments

This was supported by grants from the *National Eleven-Five Project of China* (2008ZX10002-005, 2008ZX10002-007, and 2008ZX09312-007) and *Shanghai Municipal Education Commission of China* (09YZ85).

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