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Original Article

Baseline prognostic factors and statistic model to predict early virological response in lamivudine-treated patients with chronic hepatitis B

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Abstract: Lamivudine is a potent nucleoside analogue used in treating chronic hepatitis B (CHB). However, resistance to the drug remains a problem. We analyzed all lamivudine recipients in this trial to determine the baseline characteristics and a model to predict early virological response reflecting the long-term effect of lamivudine. In this prospective trial, 230 patients who had not treated with nucleotide analogue with chronic HBV infection were assigned to receive 100 mg of lamivudine once daily for 24 weeks at least. All patients were followed up every 2 week. Cox proportional hazard regression model analyses were employed to evaluate baseline variables and to develop a statistical model. Female (P = 0.042), baseline higher serum aspartate aminotransferase (AST) (P = 0.002), and lower level of HBV-DNA (P = 0.016) were identified to be associated with higher possibility of early virological response. A model was established based on these variables to calculate the risk scores (R) for CHB patients. R > -0.45 suggested early virological response to lamivudine. The model was validated among an independent set of 40 patients. The gender as well as baseline AST and HBV-DNA levels can predict early virological response. The model provides a better tool for response prediction based on the three prognostic factors.

Keywords: Hepatitis B, chronic, lamivudine, proportional hazards model, virology

Introduction

Chronic hepatitis B virus (HBV) infection is a major health problem [1-3]. About 400 million people with chronic hepatitis B virus (HBV) infection, the risk of progression to end-stage complications such as cirrhosis and hepatocellular carcinoma has been correlated with persistent HBV replication, as reflected by serum HBV-DNA levels [4]. Correspondingly, prolonged suppression of HBV replication with antiviral therapy has been linked to reduced risks of end-stage disease manifestations, a finding that underscores the importance of long-lasting HBV suppression as a primary treatment goal [5-12].

Seven drugs are licensed for the treatment of chronic HBV infection: lamivudine [13], interferon alfa [14, 15], adefovir dipivoxil [16], peginterferon alfa-2a [17], entecavir [18], telbivudine [19], and tenofovir disoproxil fumarate. Interferons are not recommended for use in patients with compensation or immunosuppression; they may have treatment limiting side effects, and they require parenteral administration. Oral nucleosides, although potent, have been limited by the development of resistance mutations in the HBV polymerase-reverse transcriptase [20, 21].

In the developed countries, for the lower ratio of resistance mutations and better long-term efficacy, entecavir and tenofovir disoproxil fumarate maybe the first choice for the CHB patients. However, in the developing countries such as China, lamivudine maybe the first choice for most of the CHB patients because of the eco-

nomic reasons. So lamivudine has been widely used in clinical practice. Nonetheless, development of resistance mutations in the HBV polymerase-reverse transcriptase has limited its application. Therefore, it is very important to identify those who benefit most from this therapy.

Multiple studys have shown that early virological response could predict the outcomes of nucleotide analogue treatment for patients with chronic HBV infection [18, 19]. We can derive two information from the above conclusion: firstly, the variable of time, from baseline to the virological response, may affect the long-term effect of lamivudine; secondly, the variable of time may affect the long-term effect of lamivudine during the early stage of the antiviral treatment. And if we study the association between the variable of time and long-term effect in the later stage of the antiviral treatment, many patients may withdraw from the study because of resistance mutations and other reasons. This situation may affect the result of the study. So we analyzed all lamivudine recipients to determine the baseline characteristics and a model to predict early virological response and may predict the long-term therapeutic outcomes indirectly.

Materials and methods

Ethics statement

The present study was an observational study, and ethical issues were considered during the data collection and preservation. The Ethics Committee of Infectious Disease Hospital, Mengchao Hepatobiliary Hospital, Fujian Medical University approved the study; all patients gave a written informed consent.

Patients

A total of 270 CHB patients receiving lamivudine treatment in Infectious Disease Hospital, Mengchao Hepatobiliary Hospital, Fujian Medical University from June 2001 until May 2011 were recruited into the present study after a review of medical records and the completion of screening procedures to establish their eligibility for the trial. All patients were HBeAg-positive or HBeAg-negative, men or women between 16 and 65 years of age. All patients met the following diagnostic criteria for CHB and had indications for lamivudine treatment: Patients were positive for serum HBsAg for longer than six months; A serum alanine aminotransferase level, 2 to 10 times of the upper limit of normal; A serum HBV-DNA level more than 10^4 IU per milliliter.

Exclusion criteria included

Coinfection with hepatitis C, hepatitis D, or the human immunodeficiency virus; Evidence of hepatic decompensation, pancreatitis, or hepatocellular carcinoma; Previous treatment for hepatitis B with nucleoside or nucleotide analogues or both; Treatment with interferon or other immunomodulators within the previous 12 months; Other forms of liver disease, such as NAFLD, alcoholic hepatitis, and drug induced hepatitis; A serum creatinine level greater than 1.5 mg per deciliter (133 μmol per liter); A serum amylase or lipase level greater than 1.5 times the upper limit of normal; A prothrombin time prolonged by more than 3 seconds; A serum albumin level less than 33 g per liter; and a bilirubin level greater than 2.0 mg per deciliter (50 μmol per liter). Eligible patients with a serum alpha fetoprotein level greater than 50 ng per milliliter required exclusion of underlying hepatocellular carcinoma.

Study design

Eligible subjects were assigned to receive lamivudine therapy (100 mg, q.d., PO) for at least 24 weeks. Primary data analyses were specified for a 24-week treatment period. Patient histories were obtained, physical examinations were conducted, and laboratory assessments were performed at baseline, and week 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), HBeAg, creatinine, amylase, lipase, and prothrombin time were tested by standardized methods in Infectious Disease Hospital, Mengchao Hepatobiliary Hospital, Fujian Medical University Clinical Laboratory. ABI 7500 Real-Time PCR was used to quantitatively detect serum HBV-DNA levels using HBV PCR kit (FuXing, Shanghai, China); the detection limit was 420 IU/mL.

The definition of early virological response

Early virological response was defined as having a plasma HBV-DNA level of less than 420 IU

Table 1. Baseline demographic, clinical, biochemical and virological features

Variables	Model (n = 230)[a]	Validation (n = 40)[b]	*P* value
Demographic			
Age, mean ± SD, y	30.1±10.4	35.6±11.7	0.003
Gender, %			0.629
1 Man	76.5	80	
2 Women	23.5	20	
Clinical			
History of HBV infection, mean ± SD, y	5.5±4.4	5.5±5.0	0.244
Biochemical, mean ± SD			
ALT[c] U/L	138.9±81.7	124.7±65.3	0.297
AST[c] U/L	111.7±67.2	108.8±60.0	0.798
Virological			
log_{10}, HBVDNA, IU/mL, mean ± SD	7.2±1.0	7.6±0.8	0.01
HBeAg, %			0.642
1 Positive	85.7	88.3	
2 Negative	14.3	11.7	

[a]Patients studied to establish prognostic factors and statistical model to predict early virological response; [b]Patients studied to validate the model; [c]Abbreviations: SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen.

Table 2. Variables assessed among 230 patients to establish prognostic factors and statistical model for early virological response

Variables	B[a]	SE[b]	df	*P* value
Age, y	-0.001	0.008	1	0.908
Gender	0.369	0.181	1	0.042
History of HBV infection	0.007	0.016	1	0.673
ALT[c]	-0.001	0.001	1	0.559
AST[c]	0.004	0.001	1	0.002
HbeAg[c]	-0.287	0.270	1	0.288
log10 (HBV-DNA)	-0.222	0.092	1	0.016

[a]Partial regression coefficient; [b]Standard error of partial regression coefficient; [c]Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen.

per milliliter at week 24 according to PCR analysis.

Statistical analysis

In our study all lamivudine-treated patients were considered for the analyses. Enrolled patients were randomly assigned into two groups by using a table of random number with an allocation ratio of 85%:15%. Eighty five percent of patients were studied to determine predictors and establish a statistical model, and the rest for the model validation.

The demographics of patients in the model group and model validation group were compared by Student's t test for continuous data, and x^2 test for dichotomous variables. Cox proportional-hazards regression was adopted to screen predictors from the baseline parameters and develop a model for early virological response. Receiver operating characteristic (ROC) curve and area under curve (AUC) were applied to assess the optimal cutoff values of the model through maximizing the Youden's index.

A $P < 0.05$ was considered statistically significant. A 95% confidence interval accompanied all estimates when it was appropriate. All the analyses were performed by SPSS version 13.0 (SPSS Inc., IL, The USA).

Results

Demographic characteristics and baseline parameters

270 patients were recruited. **Table 1** presents descriptive statistics on patient features recorded at baseline. No patient withdrew from the study before the 24th week. At 24th week, 65.2% of patients showed suppression of plasma HBV-DNA level to < 420 IU/mL.

Predictors of early virological response

Two hundred and thirty patients were studied to determine predictors of early virological response and to develop a prediction model. Cox Proportional Hazard Regression Model analyses were employed to evaluate baseline variables, including gender, age, history of HBV infection, serum concentrations of ALT, AST, HBV-DNA level, and HBeAg.

The results showed that female patients had a 5.52 time ($P = 0.042$) greater chance of ear-

ly virological response. Patients with higher serum concentrations of AST (P = 0.002) were more likely to achieve early virological response. On the contrary, lower level of HBV-DNA (P = 0.016) suggested higher possibility of early virological response. Other factors had no predictive value (**Table 2**).

Early virological response modeling

Risk scores (R) for individual patients were estimated by combining the three prognostic variables with the Partial Regression Coefficient reported in **Table 2**. That is, R = 0.369 × LN (gender of patient, 1 = male, 2 = female) + 0.004 × LN (AST U/L) -0.222 × LN (log10 HBV-DNA IU/mL). For example, a hypothetical CHB patient is female and serum AST of 146 U/L, and HBV-DNA Level of 8.1E + 8I U/mL, the risk score would be calculated as follows: R = 0.369 × LN (2) + 0.004 × LN (146) -0.222 × LN (log_{10}8.1E + 8) = -0.183.

Model validation

Forty independent patients were studied to validate the model accuracy. Their risk scores (R) were calculated according to the equation mentioned above. The receiver operating characteristic (ROC) curve was drawn by combining the risk scores and the actual occurrence of early virological response at week 24. Actually there were 19 patients achieved early virological response at that time point. The area under the curve (AUC) was 71.3%, with a 95% confidence interval (CI) of 55.0% to 87.7% (P = 0.023). The optimal cutoff was determined through maximizing the Youden's index (J), J = sensitivity + specificity-1. The optimal cutoff value was -0.45, with a sensitivity of 100% and a specificity of 45%.

Discussion

Lamivudine is a safety oral drug with potent and specific anti-HBV activity. Lamivudine treatment could reduce risks of end-stage disease manifestations such as cirrhosis and hepatocellular carcinoma. Due to its low cost, lamivudine probably remains the most widely used NA worldwide. However, it has been limited by the development of resistance mutations in the HBV polymerase-reverse transcriptase. There were studies reported that one year of lamivudine monotherapy achieves virological remission in 36-44% of HBeAg-positive and 72-75% of HBeAg-negative chronic hepatitis B (CHB) patients [13, 18-20, 22, 23]. Other studies reported that long-term lamivudine monotherapy results in progressively accumulating rates of viral resistance due to YMDD mutations (10-25% at year-1, 65-80% at year-5) followed by virological and biochemical breakthroughs and clinical worsening of liver disease, including severe exacerbations, decompensation and liver failure [13, 19, 20, 24-26]. Lamivudine-resistance is rather a big problem: additional drugs such as adefovir are required to suppress the virus, and the patients would have to bear an increased economic burden and higher risk of developing end-stage disease. If we can predict the effect of lamivudine before the treatment, many medical resources would be saved, and many subsequent problems would be avoided. Therefore, we conducted this study. In this study, a model using serum concentrations of aspartate aminotransferase, a plasma HBV-DNA level, and whether the patient is male or female accurately predicted the early virological response (R > -0.45). The model accuracy was validated in an independent patients group. As early virological response could predict the outcomes of nucleotide analogue treatment for patients with chronic HBV infectio [18-20], so our model seems to be reliable to help lamivudine therapy decision making.

As a useful predictor of prognosis, early virological response has been adopted to guide clinical pratice. During lamivudine treatment, for exemple, we can decide whether to continue the therapy, or to add on or switch to another drug according to the response. However, if none virological response or incomplete virological response occures at Week 24, it indicats that resistance mutations may have happened or is more likely to happen in the future, subsequent therathy would be quite difficult. On this situation, early virological response is not an ideal predictor for nucleotide analogue treatment.

It was reported that Baseline ALT and baseline HBV-DNA could predict the virological response of lamivudine treatment at At week 52 and 156 [27]. Another study showed that baseline HBV-DNA levels < 9 logs copies/mL and ALT level ≥ 2 × ULN are associated with better long-term outcome and lower risk of YMDD mutations

compared to HBV-DNA levels > 9 logs copies/mL or ALT level < 2 × ULN during lamivudine treatment for chronic hepatitis B [28]. Similarly, Baseline ALT and HBV-DNA could predict the therathy outcome of other nucleoside analogue and interferon treatment. However, several drawbacks existed in these studies. First, they did not use the multivariate analysis to evaluate all candidate variables, hence the accuracy of the results was not guaranteed. Secondly, they did not combine the multivariate analysis with time as a variable to evaluate the predictor of outcomes. Thirdly, they did not provide a model to predict the outcomes of lamivudine treatment.

In a word, baseline ALT, baseline HBV-DNA and early virological response are not perfect predictors in prediction of lamivudine treatment effect in clinical pratice.

Cox proportional-hazards regression [29] was the main statistical tool for survival modeling, it were employed to evaluate baseline variables and to develop a statistical model to predict early virological response in the study. The candidate variables included: the gender, age, history of HBV infection, serum concentrations of ALT, AST, HBV-DNA level, eAg positive or nagtive at baseline. The early virological response time was defined as the time period from the beginning of lamivudine treatment to the onset of the response. The term "censored" indicates the absence of early virological response at week 24 or lost to follow-up. We analyzed the association between early virological response and the candidate variables at baseline in all lamivudine recipients in our study to determine the predictors of early virological response. Then the predictors and partial regression coefficient were combined to develop a model for response prediction, and risk scores (R) were calculated for validation of the model. The Receiver Operating Characteristic (ROC) curve was plotted by combining the risk scores and the actual occurrence of early virological response at week 24 actually. To validate the nomograms, we compared the actual occurrence of the response with the predicted response rates. Theoretically, an area under the curve greater than 50% suggests the model utility, and greater than 70% indicates moderate utility. Further, we calculated standard indices of validity such as sensitivity, specificity, and AUC to determine the optimal cutoff.

The results showed that female (P = 0.042), higher serum AST (P = 0.002), and lower level of HBV-DNA level (P = 0.016) were predictive values of early virological response.

The F bonino's study showed that combined response rates at 24 weeks post-treatment of peginterferon α-2a, lamivudine and the two combined were consistently higher in female patients compared with male patients [30]. The study of jinjun chen showed that the male gender was independently associated with higher HBsAg level [31]. The ERIK H.C.J. BUSTER's study showed that female sex predicted a sustained response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa [32]. In a word, female patients may have a good prognosis of antiviral treatment. Our results showed that gender was the predictor for the early virological response. It may because of the sex hormones level between male and female patients.

The study of Brook MG pointed out that the loss of HBsAg in addition to HBeAg and HBVDNA was more likely to occur in patients with chronic infection of less than 2 years duration after alpha-interferon therapy [33]. It explained that the lesser time of HBV infection has a association with the better outcomes of antiviral therapy. To our surprise, however age and history of HBV infection were not identified as predictive factors in our study, but many of the patients may have infected with HBV long before the diagnosis was made. Therefore, the actual significance of age and HBV infection history in prognosis of lamivudine-treated patients is still to be determined.

It is known by liver biopsy that active CHB patients with higher level of viral replication are more likely to respond to alpha-interferon therapy. As serum aspartate aminotransferase (AST) reflect the activity of chronic hepatitis B to a certain degree, it is easy to understand that the factors were identified as predictors of the early virological response. However, alanine aminotransferase (ALT), which also reflects the grade of hepatitis activity on liver biopsy, was not found to be associated with early virological response in the present study. The possible explanation is that ALT is more susceptible than AST to be affected by some drugs. It was reported that CHB patients with lower level of HBV-DNA are more likely to respond to alpha-

interferon and lamivudine therapy [34]. Our study also demonstrated that lower baseline level of HBV DNA was associated with significantly higher possibility of early virological response.

Factors used to establish our model were baseline levels of AST, HBV-DNA and gender. All these are objective and relatively stable variables. By combining the there variables with the partial regression coeffi-cient, we developed a model to predict early virological response. That is, R = 0.369 × LN (gender of patient, 1 = male, 2 = female) + 0.004 × LN (AST U/L)-0.222 × LN (log10 HBV-DNA IU/mL). The model was validated in an independent set of patients. The area under the ROC curve was 71.3% (P = 0.023), and the optimal cutoff was -0.45. It means that patients with R > -0.45 are most likely to achieve early virological response to lamivudine treatment, or good outcomes of the therapy. Patients with R > -0.45 are most suitable for lamivudine treatment in clinical practice.

The model and optimal cutoff value were established from a short-term follow-up study of only lamivudine-treated CHB patients, which makes the conclusion a little weaker. Whether the model and cutoff value can be applied to other nucleos(t)ide analogs therapy for CHB needs to be determined. Furthermore, developing a more accurate model and cutoff values to predict the outcome of lamivudine treatment in CHB patients requires a large-scale, multicenter and long-term follow-up study. But, in the present study, the data were all from a single center, with a small sample size and a short follow-up period (only 24 weeks). Lastly, route of HBV transmission (Horizontal vs. vertical), and the viral characteristics (genotype, HBeAg status) could affect the predictive model. However, as the exact route of HBV transmission for each patient is rather difficult to determine, and also for economic reasons, these factors were not included in the study. Therefore, whether the model and optimal cutoff value can accurately predict the early virological response and long-term outcome of lamivudine treatment should be further studied.

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Disclosure of conflict of interest

None.

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