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ORIGINAL ARTICLE

Quantitative HBV DNA and AST are strong predictors for survival after HCC detection in chronic HBV patients

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ABSTRACT

Hepatitis B virus infection (HBV) is an important co-factor in the development of hepatocellular carcinoma (HCC). We studied whether quantitative HBV DNA at time of HCC detection influences survival of HCC patients.

All diagnosed HCC cases between 2000 and 2008 at our university-based reference centre were analysed to determine the influence of hepatitis B viral load on overall survival. Clinical and virological findings were evaluated in univariate and multivariate analyses, survival rates were assessed for HCC patients with a high viral load (HBV DNA ≥10^5 copies/ml) and low viral load (HBV DNA <10^5 copies/ml).

HCC was diagnosed in 597 patients, including 98 patients with HBV. The group of 37 patients (38%) who had a high viral load contained more HBeAg-positive patients, had lower serum albumin levels and higher serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. The one- and five-year survival rates of HCC patients with a high viral load were 58% and 11% and for HCC patients with a low viral load 70% and 35%, respectively. In multivariate analysis a higher AST level and higher viral load were significantly associated with shorter overall survival (HR=2.30; p=0.018, HR=1.22; p=0.015, respectively).

HBeAg positivity, low albumin level or high AST or ALT levels in HCC patients are associated with a higher HBV DNA. HBV DNA level at detection is associated with overall survival of HCC patients. These findings support the concept that after HCC detection adequate suppression of HBV DNA by nucleoside analogue therapy may improve survival.

KEYWORDS

HBV DNA, hepatitis B virus (HBV), hepatocellular carcinoma (HCC), survival, viral load

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality.[1] In many patients, HCC occurs against the background of a chronic viral infection. Chronic hepatitis B virus (HBV) infection, chronic hepatitis C viral (HCV) infection and cirrhosis are major aetiologies of HCC.[2,3] Worldwide approximately 400 million people are chronically infected with HBV.[4]

In the last 15 years, reliable quantification of HBV DNA over a large dynamic range has become feasible. Several hospital-based and community-based studies have subsequently found significant associations between the level of serum HBV DNA and the risk to develop liver cirrhosis or HCC.[5] After an HCC has developed and surgery is performed, recurrence of HCC is associated with original tumour size, number of tumours, grade of differentiation, level of alpha-fetoprotein (AFP), alcohol consumption and HCV co-infection.[6-9]

The impact of viral load on survival of HCC patients after surgery with curative intent may be overshadowed by tumour-related factors or stage of the liver disease at detection of HCC. Understanding the respective role of tumour and viral factors in HCC survival may provoke new treatment strategies to increase HCC survival. It has been hypothesised that anti-viral therapy for HCC patients with

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active HBV replication along with HCC treatment might reduce the recurrence rates for HBV-associated HCC.[10]
A few recent studies have evaluated HBV replication status as a predictor of HCC recurrence.[9,11,12] However, to our knowledge only a few reports from high endemic areas published to date, often with a limited number of patients, have suggested a relation between viral status and prognosis in patients with HBV-associated HCC.[13-16] In the current study in a low endemic area, univariate and multivariate analyses of the prognostic factors, including serum HBV DNA level, were performed to determine whether the HBV DNA levels at the time of HCC appearance are associated with overall survival.

MATERIAL AND METHODS

Study design

A hospital-wide registry, including data from patient files and virological records of all patients diagnosed with HCC at the Erasmus MC in Rotterdam, the Netherlands during the period from 1 January 2000 to 31 December 2008, was used. The diagnosis of HCC was made from radiological and biochemical findings and, if necessary, confirmed by histological examination. Within the group of nodules larger than 2 cm, with the typical features of HCC on a dynamic imaging technique, no biopsy was performed. Nodules between 1-2 cm were investigated further with two dynamic studies imaging modalities, computed tomography (CT) scan or magnetic resonance imaging (MRI) with contrast. If the appearances were typical of HCC (i.e., hypervascular with washout in the portal/venous phase) in two techniques the lesion was treated as HCC. If the findings were not characteristic or the vascular profile was not coincidental among techniques, the lesion was biopsied, according to the AASLD guidelines.[17]
All HBsAg(+) patients were included in this study. Follow-up of HCC recurrence by an alpha-fetoprotein (AFP) test and ultrasound, CT, or MRI was done every three to six months for up to two years after potential curative treatment. After two years, follow-up was continued annually for up to five years after treatment. Recurrence of tumour in the treated area or elsewhere was defined as re-appearance of vascular enhancement.[17] In the presence of underlying liver cirrhosis, lifetime follow-up was performed. If HCC recurred, the size, number, and localisation of the recurrent disease were registered. Verification of living patients was done using information obtained from the general physician or the civil registration.

Biochemical and serological markers

Data were collected on patient age, gender, nucleotide or nucleoside analogue therapy (lamivudine, adefovir, telbuvidine, tenofovir or entecavir or a combination of these drugs), AFP, size and number of lesions, and the presence of lymph node enlargement or metastases. The collected liver parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and albumin. In addition, the Model For End-Stage Liver Disease (MELD) score was calculated. Cirrhosis was diagnosed using established clinical, biochemical, and histological criteria. Patients with cirrhosis were classified according to the Child-Pugh classification.
At time of HCC diagnosis, the serum HBV DNA level was assessed using in-house Taqman PCR (detection limit 400 copies/ml) based on the Eurohep standard, HBeAg (AxSYM, Abbott, Abbot Park, IL, USA) and hepatitis B surface antigen (HBsAg) (AxSYM, Abbott) status were measured.[18] A high HBV load was considered to be HBV DNA $\geq 10^5$ copies/ml, HBV DNA $<10^5$ copies/ml was considered a low viral load.[19] All patients were negative for anti-hepatitis C virus antibody and did not report alcohol abuse at time of diagnosis and commencement of this study.

Statistical analysis

Variables were compared using the Mann-Whitney U test, t-test or with the χ^2 test whenever appropriate. Statistical significance was considered if the p value was <0.05. Univariate analysis was used to assess the importance of prognostic factors on overall survival. Survival curves were drawn using the Kaplan-Meier method. The difference between Kaplan-Meier curves was tested using the log-rank test. The baseline characteristics age, gender, log bilirubin, log albumin, log AST, log ALT, log HBV DNA, log AFP, MELD score, Barcelona Clinic Liver Cancer (BCLC) score, HBeAg and anti-viral therapy were considered. Multivariate Cox regression analysis was performed with all characteristics with a p value <0.20 in univariate analysis and known factors associated with survival to determine the independent contribution of each variable. Analysis was performed using SPSS software.

RESULTS

Clinical, biochemical and virological data

A total of 597 patients were diagnosed with HCC. Out of these 597 patients, 98 patients (16%) fulfilled the inclusion criteria. The patient characteristics at presentation with HCC are shown in *table 1*. Median follow-up was 22 months (1-114). One year after presentation, 60% of the patients were still alive, and the five-year survival rate of this cohort was 21%. In 50 patients (51%), treatment with curative intent was initiated; this included surgical resection (wedge resection, segment resection, or hemihepatectomy), liver transplantation or radio

Table 1. *Patient characteristics at first presentation with HCC*

Characteristic	(n=98)
Age (years)*	55 (23-80)
Gender (male)	79 (81%)
Total bilirubin (µmol/l)*	18 (4-481)
Albumin (g/l)*	38 (22-49)
AST (U/l)*	69 (21-1278)
ALT (U/l)*	54 (19-670)
AFP (ng/ml)*	70 (1-652660)
MELD score*	6 (6-25)
Non-cirrhotic	22 (22%)
Child-Pugh classification	
A	49 (64%)
B	20 (26%)
C	7 (9%)
HBV DNA ≥10^5 copies/ml	37 (38%)
HBeAg‡	24 (25%)
Anti-viral (nucleoside or nucleotide analogue) therapy	50 (51%)
Number of tumours*	1 (1-7)
Tumour size (mm)*	34 (8-227)
Metastases	29 (30%)
BCLC	
Stage A	30 (31%)
Stage B	37 (38%)
Stage C	7 (7%)
Stage D	24 (24%)

AST = aspartate aminotransferase; ALT = alanine aminotransferase; AFP = alpha-fetoprotein; BCLC = Barcelona Clinic Liver Cancer score; *median (range); ‡ positive value.

Table 2. *Differences between patients with high and low HBV load*

Characteristic	HBV DNA <10.5 (n =61)	HBV DNA ≥10.5 (n=37)	p value
Age (year)*	56 (23-77)	54 (27-80)	0.650
Gender (male)	49 (80%)	30 (81%)	0.928
Total bilirubin (µmol/l)*	15 (4-481)	24 (6-372)	0.058
Albumin (g/l)*	39 (25-49)	35 (22-48)	0.032
AST (U/l)*	62 (21-328)	95 (33-1278)	0.002
ALT (U/l)*	50 (19-356)	67 (32-670)	0.039
AFP (ng/ml)*	70 (1-652660)	70 (2-121000)	0.640
Non-cirrhotic	15 (25%)	7 (19%)	0.516
MELD score*	6 (6-23)	7 (6-25)	0.253
HBeAg‡	11 (18%)	13 (35%)	0.047
Anti-viral (nucleotide or nucleoside analogue) therapy	32 (53%)	18 (49%)	0.716
Number of tumours*	1 (1-7)	1 (1-4)	1.000
Tumour size (mm)*	34 (8-200)	34 (11-227)	0.714
Metastases	15 (25%)	14 (38%)	0.166
BCLC			0.466
Stage A	21 (34%)	9 (24%)	
Stage B	23 (38%)	14 (38%)	
Stage C	5 (8%)	2 (5%)	
Stage D	12 (20%)	12 (32%)	

AST = aspartate aminotransferase; ALT = alanine aminotransferase; AFP = alpha-fetoprotein; BCLC = Barcelona Clinic Liver Cancer score; *median (range); ‡ positive value.

frequency ablation. In eight patients (8%) transarterial (chemo)embolisation (TACE) or another therapy such as radiotherapy or systemic chemotherapy with palliative intent was started. The remaining group of 40 patients (41%) received no therapy.

Fifty patients (51%) received oral anti-viral therapy. Nine patients had an increase of HBV DNA during the study period but none of these patients switched from the low HBV DNA group to the high HBV DNA group.

In 21 out of 50 patients (42%) a recurrence of HCC was documented after potentially curative treatment. The median time to recurrence was 12 (1-50) months. Recurrence of HCC presented as local recurrence in four patients (19%), a new lesion in 11 patients (52%) and metastases in six patients (29%).

Factors associated with HBV viral load

Among the 98 patients, 37 (38%) had a high viral load. As expected, the group of patients with a high viral load contained more HBeAg(+) patients, had a lower serum albumin level and had a higher serum AST, ALT, and total bilirubin level compared with the group of patients with a low viral load (*table 2*). Treatment with curative intent was not significantly different between patients with high and low viral load (p=0.188). Treatment of HCC was independent of the level of HBV DNA (p=0.202). Patients with a higher viral load more often had a recurrence of HCC after treatment with curative intent (p=0.025).

Univariate and multivariate analyses were performed to determine HBV-related predictors for overall survival (*table 3*). Multivariate Cox regression analysis was performed with all characteristics with a p value <0.20 in univariate analysis and known factors associated with survival to determine the independent contribution of each variable.

The strong correlation between AST and HBV DNA made it impossible to join them in one model. Separately, multivariate analysis confirmed both a high AST level and a high viral load (HBV DNA) to be significantly associated with a shorter survival (HR=2.30; p=0.018, HR=1.22; p=0.015, respectively). A higher AFP, a higher MELD score and a higher BCLC classification were also associated with a shorter survival (HR=1.30; p=0.008, HR=1.08; p=0.021, HR=1.95; p<0.001, respectively).

Association of serum HBV DNA levels at time of HCC diagnosis and overall survival

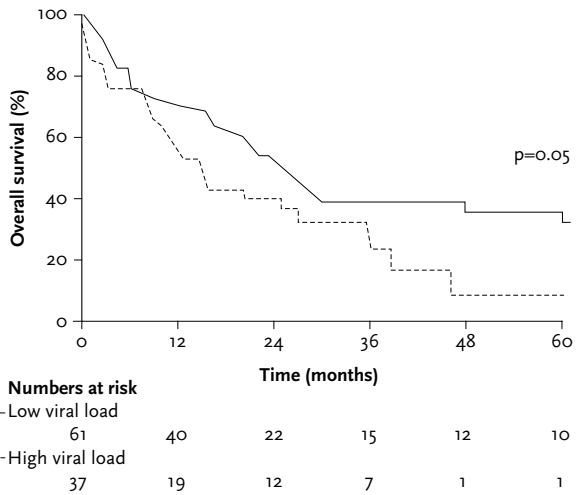
The median survival time of HCC patients with a high viral load was 15 months (1-62), and 25 months (1-114) in

Table 3. *Univariate analysis of factors associated with survival*

Variable	Hazard ratio (95% confidence limit)	p value
Age (10 years)	1.00 (0.78-1.27)	0.97
Gender (female:male)	0.80 (0.41-1.57)	0.50
log total bilirubin (10 µmol/l)*	1.02 (0.99-1.05)	0.31
log albumin (10g/l)*	0.68 (0.44-1.03)	0.07
log AST (U/l)*	2.30 (1.15-4.60)	*0.018*
log ALT (U/l)*	0.75 (0.31-1.80)	0.51
log AFP (ng/ml)*	1.32 (1.09-1.60)	*0.006*
MELD score	1.07 (1.01-1.14)	*0.033*
HBV DNA	1.22 (1.04-1.43)	*0.015*
HBeAg‡	1.47 (0.84-2.55)	0.19
Anti-viral (nucleotide or nucleoside analogue) therapy	0.69 (0.42-1.14)	0.15
BCLC	1.95 (1.54-2.48)	*<0.001*

AST = aspartate aminotransferase; ALT = alanine aminotransferase; AFP = alpha-fetoprotein; BCLC = Barcelona Clinic Liver Cancer score; *continuous value; ‡ positive value.

Figure 1. *Overall survival of HCC patients with high and low HBV DNA*



HCC patients with a low viral load (*figure 1*). The one-, three- and five-year survival rates of HCC patients with a high viral load were 58%, 32% and 11%, respectively. For HCC patients with a low viral load, the one-, three- and five-year survival rates were 70%, 39% and 35%, respectively (*figure 1*). Patients with higher serum HBV DNA levels at the time of tumour presentation had a shorter overall survival compared with patients with lower serum HBV DNA levels (p=0.05). Including HCC treatment into the total multivariate analysis, a high viral load continued to be significantly associated with a shorter survival (HR=1.18 (95% CL; 1.01 to 1.38); p=0.042).

DISCUSSION

In our study we showed in multivariate analysis that a high AST level and high viral load were two independent factors associated with poor survival. A unique and important finding of this study is that it demonstrates the impact of high viral load on overall survival of HCC patients despite the treatment they received. Consistently, we observed that biochemical profiles indicative of active inflammation in our data were worse in patients who had high viraemia than in patients who had low viraemia, further supporting the theory of the potential carcinogenic process through active inflammation associated with high viraemia.

Localised HCC tumours can be subjected to potentially curative treatments such as surgical resection, liver transplantation or radiofrequency ablation.[17] In our study 50 patients (51%) were able to receive treatment with curative intent. Only 8% of the study population received treatment with palliative intent; this low percentage is due to a limited availability of TACE treatment during the study period. Patients without treatment were often unable to receive treatment due to more advanced liver disease.

In many patients, HCC occurs against a background of advanced fibrosis or cirrhosis.[20-22] Cirrhosis decreases the regenerative capacity of the liver and therefore not every HCC patient is a suitable candidate for local surgical resection. Although many surgical and nonsurgical options have been developed for the treatment of HCC, the prognosis for these patients remains poor. Even in those who receive radical therapy, prevention of post-treatment recurrence remains a medical challenge.[23]

Several factors have been reported to be associated with poor survival after surgical resection or local ablation therapies, including tumour characteristics, such as multiplicity, size, AFP levels, portal invasion, surgical tumour findings, parameters related to liver function such as albumin levels, and Child-Pugh classification.[11,15]

Taking into account the fact that HCC arises in cirrhotic livers, evaluation of the detailed oncogenic process in patients with cirrhosis is an important subject for cancer prediction.[17]

Liver cirrhosis due to hepatitis C virus usually shows a rather steady and constant clinical course, which enables us to estimate the future carcinogenesis rate only from clinical information at the time of the diagnosis of cirrhosis.

However, in contrast to hepatitis C and other risk factors, it is known that HBV-related HCC is less associated with the presence of cirrhosis, and this trend becomes more obvious in younger patients often infected at birth whose duration of infection is not long enough to develop full-blown cirrhosis.[23] This observation has prompted the suggestion that HBV itself has direct carcinogenic potential.[23] The detailed mechanism of HBV-related liver carcinogenesis

is still unclear.[18,24] It is possible that active viral replication and HBx-protein expression contribute to the carcinogenic process.[11] Prospective studies have indicated a very strong correlation between the height of the viral load and the risk of developing HCC. Lamivudine therapy in patients with HBV-related compensated cirrhosis reduced the incidence of HCC in patients when viral suppression was sustained, but no previous report has studied the relationship of these viral factors and survival of HCC patients.[11,15,25,26]

When we investigate the relationship between hepatocellular carcinogenesis and its affecting and contributing factors, explanatory parameters should include not only tumour-related factors but also data on the extent of the liver disease, as e.g. included in the Child classification, BCLC and MELD classification. We also suggest including quantitative virological data in this prognostic modelling.

In the current study, patients with a higher viral load also had more elevated liver enzymes. Oral nucleoside or nucleotide analogue therapy has developed over the last years. The profile of drugs such as entecavir or tenofovir combines high efficacy with a low potential for resistance. Therefore, a logical next step is to treat all HBV-related HCC patients with nucleoside or nucleotide analogue therapy. A meta-analysis also suggested a potential efficacy of adjuvant interferon after curative therapy for HCC.[27] Two recent prospective studies focusing primarily on the correlation between hepatitis B viral load and recurrence of small HCC after curative resection revealed that HBV DNA level at resection was an important risk factor for recurrence of small HCC after surgery.[9,28]

A potential limitation of the present study is that the data were based on a retrospective cohort study. A large-scale prospective trial should be conducted in the future to elucidate the effect of sustained viraemia on survival of HCC patients and the prospective roles of antiviral treatment.

In theory, treating high viral load patients with antiviral drugs both pre- and post-operatively is reasonable. Current treatment in patients with advanced HCC is sorafenib, where median survival can increase by nearly three months.[29] In this study high HBV viral load and hepatic inflammatory activity were both significantly associated with a poor prognosis; median survival was ten months longer in HCC patients with a low viral load.[30]

Given the strong association between HBV viral load and overall survival, it is anticipated that the implementation of strategies for the use of antiviral therapy in this setting will result in a durable suppression of HBV replication and ultimately will lead to an increase of survival in HCC patients. We suggest that, for HCC patients with high serum HBV DNA levels, inhibition of viral replication may decrease inflammation and improve survival.

In conclusion, a lower albumin level or a higher serum AST or ALT activity are liver-related factors that are closely associated with a higher hepatitis B viral load. In our dataset as well as in the data of Qu et al. high HBV DNA shortened overall survival.[28] In the current analysis serum AST and viral load independently affected overall survival. This association supports the role for antiviral treatment for patients with a high HBV DNA together with treatment of HCC to increase overall survival. Further clinical trials with this endpoint are required to confirm the beneficial effect of hepatitis B viral suppression after HCC treatment to improve survival.

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None

REFERENCES

1. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362:1907-17.
2. El-serag HB, Marrero JA , Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 2008;134:1752-63.
3. Huo TI, Lin HC, Hsia CY, et al. The model for end-stage liver disease based cancer staging systems are better prognostic models for hepatocellular carcinoma: a prospective sequential survey. Am J Gastroenterol. 2007;102:1920-30.
4. Lee W. Hepatitis B virus infection. N Engl Med. 1997;337:1733-45.
5. Chen CJ, Yang HI, Iloeje UH; REVEAL-HBV Study Group. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. Hepatology. 2009;49:S72-S84.
6. Toyama T, Hiramatsu N, Yakushijin T, et al. A new prognostic system for heptocellular carcinoma including recurrent cases a study of 861 patients in a single institution. J Clin Gastroenterol. 2008;42:317-22.
7. Koike Y, Shiratori Y, Sato S, et al. Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus-an analysis of 236 consecutive patients with a single lesion. Hepatology. 2000;32:1216-23.
8. Sasaki Y, Yamada T, Tanaka H, et al. Risk of recurrence in a long-term follow-up after surgery in 417 patients with hepatitis B-or hepatitis C-related hepatocellular carcinoma. Ann Surg. 2006;244:771-80.
9. Hung IF, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at time of resection. Am J Gastroenterol. 2008;103:1663-73.
10. Xu J, Liu L, Tang H. Antiviral therapy for hepatitis B virus-associated hepatocellular carcinoma: potential to reduce the tumor recurrence rates and/or improve overall survival. Med Hypotheses. 2011;76(3):457-9.
11. Jang JW, Choi JY, Bae SH, et al. The impact of hepatitis B viral load on recurrence after complete necrosis in patients with hepatocellular carcinoma who receive transarterial chemolipiodolization: implications for viral suppression to reduce the risk of cancer recurrence. Cancer. 2007;110:1760-7.
12. Huang Y, Wang Z, An S, et al. Role of hepatitis B virus genotypes and quantitative HBV DNA in metastasis and recurrence of hepatocellular carcinoma. J Med Virol. 2008;80:591-7.
13. Kubo S, Hirohashi K, Tanaka H, et al. Effect of viral status on recurrence after liver resection for patients with hepatitis B virus-related hepatocellular carcinoma. Cancer. 2000;88:1016-24.

14. Kubo S, Hirohashi K, Tanaka H, et al. Usefulness of viral concentration measurement by transcription-mediated amplification and hybridization protection as a prognostic factor for recurrence after resection of hepatitis B virus-related hepatocellular carcinoma. Hepatology Research. 2003;25:71-7.

15. Wu JC, Huang YH, Chau GY, et al. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol. 2009;51:890-7.

16. Ikeda K, Arase Y, Kobayashi M, et al. Consistently low hepatitis B virus DNA saves patients from hepatocellular carcinogenesis in HBV-related cirrhosis. Intervirology. 2003;46:96-104.

17. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208-36.

18. Pas SD, Fries E, de Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol. 2000;38(8):2897-901.

19. 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.

20. Verhoef C, de Man RA, Zondervan PE, Eijkemans MJ, Tilanus HW, Ijzermans JN. Good outcomes after resection of large hepatocellular carcinoma in the non-cirrhotic liver. Dig Surg. 2004;21:380-6.

21. Trevisani F, D'Intino PE, Caraceni P, et al. Etiologic factors and clinical presentation of hepatocellular carcinoma. Differences between cirrhotic and noncirrhotic Italian patients. Cancer. 1995;75:2220-32.

22. Van Roey G, Fevery J, van Steenbergen W. Hepatocellular carcinoma in Belgium: clinical and virological characteristics of 154 consecutive cirrhotic and non-cirrhotic patients. Eur J Gastoenterol Hepatol. 2000;12:61-6.

23. Hoshida Y. Risk of recurrence in hepatitis B-related hepatocellular carcinoma: impact of viral load in late recurrence. J Hepatol. 2009;51:842-4.

24. Chuma M, Hige S, Kamiyama T, et al. The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma. J Gastroenterol. 2009;44:991-9.

25. Eun JR, Lee HJ, Kim TN, Lee KS. Risk assessment for the development of hepatocellular carcinoma: according to on-treatment viral response during long-term lamivudine therapy in hepatitis B virus-related liver disease. J Hepatol. 2010;53:118-25.

26. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351(15):1521-31.

27. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): A meta-regression approach. J Hepatol. 2010;52:889-94.

28. Qu LS, Jin F, Huang XW, Shen XZ. High hepatitis B viral load predicts recurrence of small hepatocellular carcinoma after curative resection. J Gastrointest Surg. 2010;14:1111-20.

29. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378-90.

30. Nakai T, Shiraishi O, Kawabe T, Ota H, Nagano H, shiozaki H. Significance of HBV DNA in the hepatic parenchyma from patients with non-B, non-C hepatocellular carcinoma. World J Surg. 2006;30:1338-43.