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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Low Hepatitis C Viral Load Predicts Better Long-Term Outcomes in Patients Undergoing Resection of Hepatocellular Carcinoma Irrespective of Serologic Eradication of Hepatitis C Virus

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ABSTRACT

Purpose
Hepatitis C virus (HCV) infection has been recognized as a potent risk factor for the postoperative recurrence of hepatocellular carcinoma (HCC). However, little is known about the impact of HCV viral load on surgical outcomes. The study objective was to investigate clinical significance of HCV viral load on long-term outcomes of HCC.

Patients and Methods
Three hundred seventy patients who were classified as Child-Pugh class A and underwent curative liver resections for HCV-related HCC were divided into low and high viral load groups (≤ or > 5.3 $\log_{10}$IU/mL) based on the results of a minimum *P* value approach to predict moderate to severe activity of hepatitis; the clinical outcomes were then compared.

Results
The 5-year recurrence-free survival rate was 36.1% in the low viral load group and 12.4% in the high viral load group ($P < .001$). The 5-year overall survival rate was 76.6% in the low viral load group and 57.7% in the high viral load group ($P < .001$). Multivariate analysis confirmed significant correlation between high viral load and tumor recurrence with a hazard ratio of 1.87 (95% CI, 1.41 to 2.48; $P < .001$). Subanalysis revealed that the favorable results in the low viral load group were not attributed to whether or not serologic eradication of HCV was obtained both in primary and recurrent lesions.

Conclusion
Low HCV viral load predicts better long-term surgical outcomes in patients with HCC regardless of the serologic eradication of HCV.

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INTRODUCTION

Recent developments in medical and surgical treatments have significantly improved the long-term outcomes of patients with hepatocellular carcinoma (HCC).[1] However, the cumulative recurrence rate remains as high as 50% to 60% at 3 years and 70% to 100% at 5 years, even after curative liver resection.[2-7]

Hepatitis C virus (HCV), a major cause of chronic hepatitis and liver cirrhosis, has been recognized as a potent risk factor of carcinogenesis[8] and/or the recurrence of HCC.[9,10] Because postoperative persistent viremia is thought to be the main cause of sustained liver dysfunction and the high tumor recurrence rate in patients with HCV, adjuvant antiviral therapy using interferon (IFN) has recently been attempted, and favorable outcomes have been reported in several studies.[11-13]

Conventionally, the eradication of HCV and a sustained status of undetectable HCV-RNA have been regarded as the most important factors for obtaining better clinical results after IFN therapy. In recent studies, however, possibly favorable effects of a reduced viral load on long-term outcomes have been suggested in patients with chronic hepatitis.[14,15]

Our hypothesis was that a correlation existed between the HCV viral load and long-term surgical outcomes. Simply labeling patient as having viremia did not sufficiently stratify those who had low viral load versus those who had high viral load. In this study, we tested this hypothesis by examining patients who had undergone curative

liver resection for HCV-related HCC and analyzed the impact of the HCV viral load on postoperative outcomes.

PATIENTS AND METHODS

Study Population

This study was performed in accordance with the ethical guidelines for clinical studies at the University of Tokyo Hospital (Tokyo, Japan). The subject pool consisted of 508 consecutive patients who underwent curative liver resection for HCV-related HCC between January 2002 and December 2011. Patients classified as Child-Pugh class B (n = 49) or patients missing preoperative viral load data (n = 89) were excluded because the goal of this study was to reveal the prognostic impact of the HCV viral load in patients who were considered to be capable of tolerating antiviral therapies. The remaining 370 patients were included in the analysis.

Serum HCV-RNA Quantification

Serum HCV-RNA was quantified within 4 weeks before surgery using a conventional reverse transcriptase polymerase chain reaction (PCR) assay before 2007 and a new commercially available real-time PCR assay (TaqMan PCR; Roche Molecular Systems, Pleasanton, CA) in 2007 and thereafter. In this study, the viral load unit was standardized to a logarithm style ($\log_{10}$IU/mL) for the statistical analysis according to the following equation: Y ($\log_{10}$IU/mL) = $\log_{10}$ [X (kIU/mL) $\times$ 10^3].

Surgical Treatment and Histopathologic Assessments

The indications for hepatic resection and the types of operative procedures were determined as previously described.[16] Briefly, operative decisions were based on an algorithm consisting of the presence of ascites, the serum total bilirubin level, and the results of an indocyanine green tolerance test.[17] Because HCC has a high propensity to invade the portal veins and because intrahepatic metastasis via vascular invasion is one of the major forms of recurrence, tumor-bearing portal regions (ie, the segment or subsegment of the liver) were systematically removed (ie, an anatomic resection) to reduce the risk of local recurrence as long as such resections were feasible given the functional reserve of the liver.[18]

The histologic classifications of the tumor and background liver were described based on the system of the Liver Cancer Study Group of Japan.[19] The histologic differentiation of HCC (well, moderate, or poor) was determined according to the Edmondson grade.[19,20] Both the fibrotic stage and the activity of the hepatitis in the background liver were also recorded according the classification proposed by Desmet et al.[21]

Postoperative Antiviral Therapy

Postoperative adjuvant IFN therapy was performed only in patients who had a good performance status and were capable of tolerating a standard high-dose combination therapy with ribavirin. Specifically, patients who were younger than 65 years of age, had no evidence of cirrhosis, and had a sufficient platelet count ($> 9.0 \times 10^4/\mu$L) were considered good candidates for postoperative antiviral therapy.

Patient Follow-Up

All patients were regularly screened for recurrences through the evaluation of the HCC-specific tumor markers α-fetoprotein (AFP) and des-γ-carboxyprothrombin every 1 to 2 months, with ultrasonography every 2 months, and with dynamic computed tomography every 4 months, as previously reported.[22] The HCV viral load was re-examined after surgery in possible candidates for adjuvant antiviral therapy. The function of the background liver was monitored using the serum ALT levels. If the ALT levels increased beyond 100 IU/L, an appropriate dose of ursodeoxycholic acid and/or monoammonium glycyrrhizinate was administered expecting their liver protective effects.[23,24]

Recurrence was defined as the appearance of a new lesion with radiologic features compatible with HCC, as confirmed using at least two imaging modalities. When a recurrence was detected, the patient received further treatment using a repeated hepatectomy, radiofrequency ablation, transcatheter arterial chemoembolization (TACE), or other treatment options, as indicated. In the present study, recurrence-free survival (RFS) was defined as the interval between the operation and the date of the diagnosis of the first recurrence or the last follow-up examination, and overall survival (OS) was calculated based on the time from surgery to death or last follow-up.

Data Analysis

Statistical analysis was performed using SAS software, version 9.3 (SAS Institute, Cary, NC). Medians and ranges of continuous data were compared using the Mann-Whitney *U* test. Categorical data were compared using Pearson's χ^2 test or Fisher's exact test as appropriate. $P < .05$ was considered statistically significant.

High viral load was defined as HCV viral load to predict moderate to severe activity of hepatitis (grade 2 or 3 in Desmet classification[21]). The cutoff value was determined using the minimum *P* value approach, and clinical outcomes were compared between the patients with a high viral load and those with a low viral load. In addition, the low viral load group was further subclassified according to whether or not HCV-RNA was detectable, and clinical outcomes between these subgroups were also compared.

Survival curves for OS and RFS were generated using the Kaplan-Meier method and were compared using the log-rank test. To identify risk factors for tumor recurrence, multivariate regression analysis was performed with the Cox proportional hazards model using a backward elimination procedure. To prevent overfitting, only factors that showed statistically significant association with tumor recurrence with $P < .10$ were included in the final model. Prognostic value of HCV viral load was

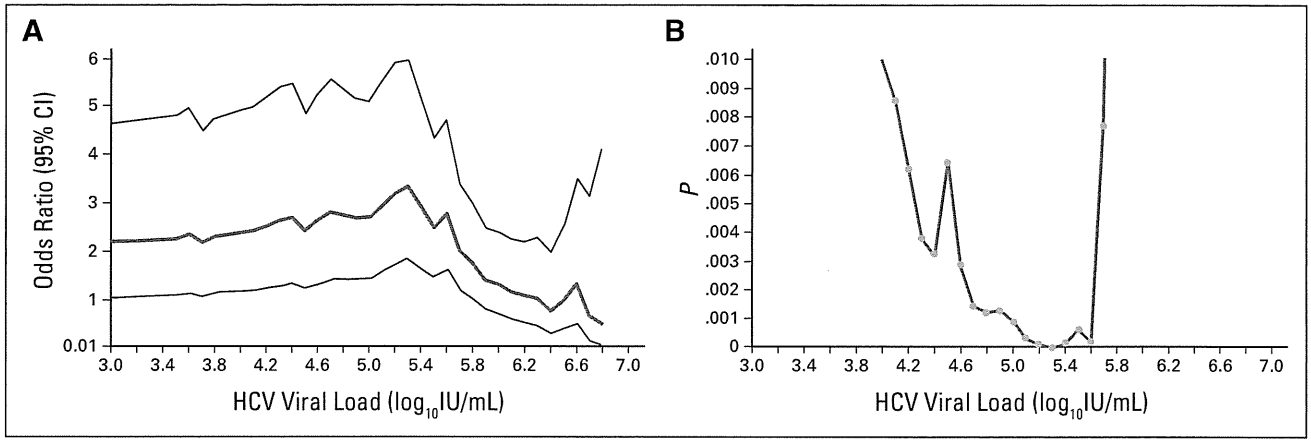

Fig 1. Optimal cutoff value of hepatitis C virus (HCV) RNA viral load to predict moderate to severe activity of hepatitis. (A) Plot of odds ratio. (B) Plot of *P* value in likelihood test (null hypothesis: odds ratio, 1).

Table 1. Baseline Demographics and Clinical Characteristics

Characteristic	Patients With Low Viral Load (n = 168) No.	%	Patients With High Viral Load (n = 202) No.	%	*P*
Age, years					.07
Median	69		70		
Range	47-83		39-85		
Sex					
Male	139	82.7	131	64.9	< .001
Female	29	17.3	71	35.1	
HBsAg					
Positive	7	4.2	4	2.0	.24
Negative	161	95.8	198	98.0	
HBcAb					
Positive	45	28.5	59	33.7	.30
Negative	113	71.5	116	66.3	
HCV genotype					
1b	40	69.0	87	81.3	0.07
Other	18	31.0	20	18.7	
HCV-RNA, $\log_{10}$IU/mL					< .001
Mean	3.0		6.0		
Standard deviation	1.9		0.4		
History of IFN therapy					
Positive	69	41.6	48	24.0	< .001
Negative	97	58.4	152	76.0	
No. of tumors					
Solitary	107	63.7	127	63.2	.87
Multiple	61	36.3	75	37.1	
Maximum diameter of the tumor, mm					.45
Median	24		25		
Range	8-130		6-200		
AST, IU/L					< .001
Median	34		49		
Interquartile range	24-56		36-63		
ALT, IU/L					< .001
Median	32		47		
Interquartile range	20-52		29-67		
Total bilirubin, mg/dL					.12
Median	0.7		0.7		
Interquartile range	0.5-0.9		0.6-0.9		
PT, %					.18
Median	82.9		82.9		
Interquartile range	73.1-94.9		75.6-98.3		
ICG-R15, %					< .001
Median	13.8		16.9		
Interquartile range	8.8-20.8		12.1-24.3		
Platelets, $10^4/\mu L$					.11
Median	14.4		14.0		
Interquartile range	11.4-19.0		10.1-17.1		
AFP, ng/mL					< .001
Median	9		15		
Interquartile range	4-42		7-99		
DCP, mAU/mL					.49
Median	32		35		
Interquartile range	17-177		19-146		

Abbreviations: AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin; HBcAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; ICG-R15, indocyanine green retention rate at 15 minutes; IFN, interferon; LC, liver cirrhosis; PT, prothrombin time.

quantified by comparing Harrell's concordance statistics of prognostic models based on the results of the multivariate analysis.

RESULTS

Characteristics of High and Low HCV Viral Load Groups

The best cutoff value of HCV viral load to predict moderate to severe activity of hepatitis was more than 5.3 $\log_{10}$IU/mL in both the plots of odds ratio and *P* value in the likelihood test (Fig 1). The background characteristics are compared between the high viral load (n = 202) and low viral load (n = 168) groups in Table 1. Female sex was more frequent in the high viral load group than in the low viral load group. The rates of coinfection with hepatitis B were not significantly different between the two groups. A history of IFN therapy was more common in the low viral load group. Number and maximum diameter of lesions were comparable between the two groups. The serum ALT and AST levels, indocyanine green retention rate at 15 minutes, and AFP levels were significantly higher in the high viral load group, whereas the platelet count was almost the same between the groups.

As for surgical factors (Table 2), the initial hepatectomy rates were 51.8% and 67.3% in the low and high viral load groups, respectively ($P < .001$). The remaining patients had repeat hepatectomies for

Table 2. Surgical, Histopathologic, and Postoperative Factors

Factor	Patients With Low Viral Load (n = 168) No.	%	Patients With High Viral Load (n = 202) No.	%	*P*
Surgical factors					
Liver resection					< .001
First HX	87	51.8	136	67.3	
≥ Second HX	81	48.2	66	32.7	
Operation time, minutes					.06
Median	359		330		
Interquartile range	279-461		263-443		
Blood loss, g					
Median	700		660		.77
Interquartile range	370-1,059		350-1,050		
Transfusion	7	4.2	7	3.5	.79
Anatomic resection	73	43.5	80	39.6	.45
Surgical margin, mm					.20
Mean	3.1		3.6		
Standard deviation	5.4		4.9		
Histopathologic factors					
Tumor differentiation*					
Well	35	21.5	42	21.2	.86
Moderate	106	65.0	134	67.7	
Poor	22	13.5	22	11.1	
Major vascular invasion	10	5.9	5	2.6	.39
Microvascular invasion	59	35.3	64	32.0	.50
Fibrosis score†					
F0-2	52	49.1	56	37.1	.06
F3-4	54	50.9	95	62.9	
Postoperative factors					
Adjuvant IFN therapy	6	3.6	7	3.5	1.00
HCV-RNA at 1 year, $\log_{10}$IU/mL‡					< .001
Mean	3.3		6.0		
Standard deviation	2.0		0.4		
ALT after surgery, IU/L					.004
Median	33		42		
Interquartile range	20-56		28-64		
AFP at 1 month, ng/mL					< .001
Median	4		7		
Interquartile range	3-7		4-13		
DCP at 1 month, mAU/mL					.08
Median	14		15		
Interquartile range	10-16		10-19		

Abbreviations: AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin; HCV, hepatitis C virus; HX, hepatectomy; IFN, interferon.
*Based on modification of the Edmondson grade.[19]
†Based on the classification by Desmet et al.[21]
‡Based on data from 31 and 28 patients with low viral load and high viral load, respectively.

recurrent lesions. Type of surgery (anatomic *v* nonanatomic), operating time, blood loss, and surgical margins were comparable between the groups. Histopathologically, no significant difference was observed in histologic grade of tumor or presence of vascular invasions. Fibrotic scores tended to be higher in the high viral load group.

Postoperative IFN therapy was performed in only six patients (3.6%) and seven patients (3.5%) in the low and high viral load groups, respectively. Because the median age of the patients in this study was 70 years and approximately 50% of the patients exhibited marked thrombocytopenia and/or cirrhotic changes in their background livers, the standard combination therapy of IFN with ribavirin was difficult to apply in most of the patients.

In 59 patients in whom postoperative viral load data were available, the HCV-RNA levels did not significantly change from baseline to 1 year after surgery (4.5 ± 2.0 $\log_{10}$IU/mL before surgery *v* 4.6 ± 1.9 $\log_{10}$IU/mL after surgery; $P = .78$). HCV-RNA viral load at 1 year and postoperative mean ALT levels were higher in the high viral load group. Postoperative AFP levels were also higher in the high viral load group even after curative resection.

Patient Survival

The median follow-up time of the studied population was 38.4 months (range, 1 to 120 months), and no hospital deaths occurred. During the study period, recurrence was observed in 108 patients (60.7%) and 137 patients (71.4%) in the low and high viral load groups, respectively.

The 1-, 3-, and 5-year RFS rates were 66.1%, 37.4%, and 36.1% in the low viral load group and 60.2%, 25.8%, and 14.9% in the high viral load group, respectively ($P < .001$; log-rank test). The 3- and 5-year OS rates were 87.6% and 76.6% in the low viral load group and 77.2% and 57.7% in the high viral load group, respectively ($P < .001$; log-rank test; Fig 2). At the time of the first recurrence, multiple intrahepatic recurrences were more frequent in the high viral load group (47.5%) than in the low viral load group (33.8%; $P = .05$). Repeat hepatectomy, radiofrequency ablation, and TACE were performed for intrahepatic recurrence in 26.0% (n = 33), 19.7% (n = 25), and 42.5% (n = 54) of patients in the high viral load group, respectively, and 41.0% (n = 34), 20.5% (n = 17), and 30.1% (n = 25) of patients in the low viral load group, respectively ($P = .06$).

The median HCV viral load of the positive HCV-RNA subgroup in the patients with low viral load was 4.9 $\log_{10}$IU/mL (range, 2.3 to 5.3 $\log_{10}$IU/mL), and it was significantly lower than that in the high viral load group ($P < .001$). Clinicopathologic parameters were almost comparable between the two subgroups in the low viral group except that HCV-RNA titers and serum AST and ALT levels were significantly higher in positive HCV-RNA patients ($P < .001$). The 1- and 3-year RFS rates were similar between the two subgroups (65.6% and 38.8% for the negative HCV-RNA patients and 66.5% and 35.9% for the positive HCV-RNA patients, respectively; $P = .61$; Fig 3A). The RFS rate among the low viral load group with positive HCV-RNA was superior to that of the high viral load group ($P = .009$). A similar tendency was also observed in the OS rates. The positive HCV-RNA patients had relatively favorable results, similar to the negative HCV-RNA patients when the viral load was ≤ 5.3 $\log_{10}$IU/mL. The 3- and 5-year OS rates were 85.8% and 78.1% for the negative HCV-RNA patients, respectively, and 89.0% and 75.8% for the positive HCV-RNA patients, respectively ($P = .94$; Fig 3B). The OS rate of the low viral load group with positive HCV-RNA was superior to that of the high viral load group ($P = .005$). These observations were constant when stratifying the study population according to hepatectomies for primary or recurrent lesions (Appendix Fig A1, online only).

Risk Factors for Postoperative Recurrence

Risk factors for postoperative recurrence were investigated in 357 patients without postoperative antiviral therapy. In the multivariate analysis, we chose 17 potential confounders considering their clinical significance and reported evidences,[4,25-31] as indicated in Table 3. There were no specific combinations of factors suggesting multicollinearity in scatter plots. In multivariate analysis, high HCV viral load (> 5.3 $\log_{10}$IU/mL), macroscopic vascular invasion, repeat resection for recurrent tumor, tumor exposure, and tumor size greater than 2 cm were selected in the final model. The concordance statistic of the four-factor model (macroscopic vascular invasion + repeat resection + tumor exposure + size > 2 cm) was 0.603 (95% CI, 0.559 to 0.647),

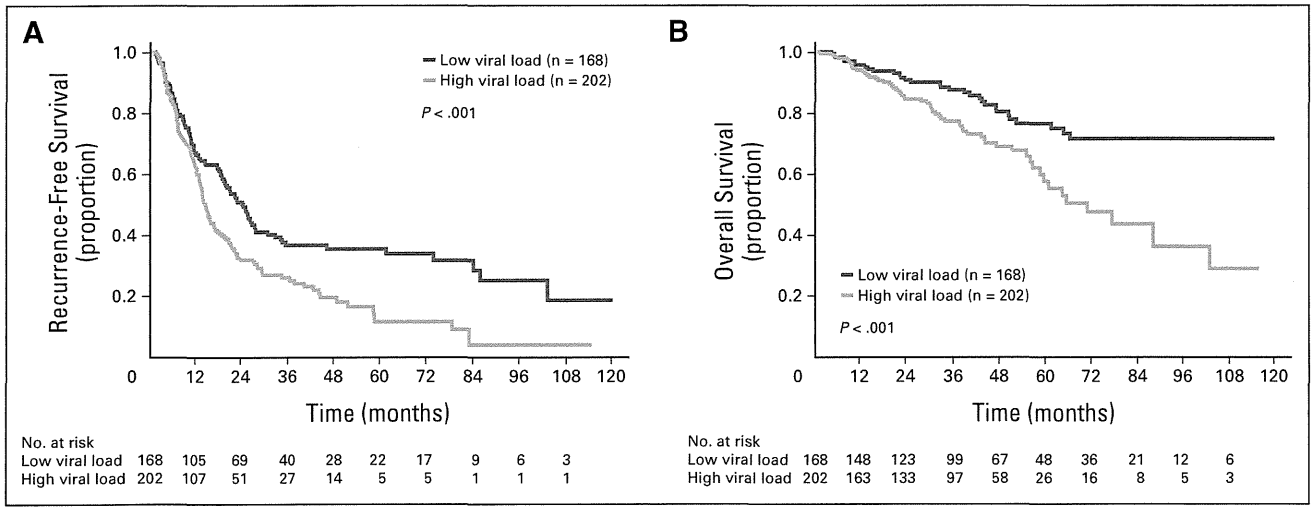

Fig 2. (A) Cumulative recurrence rate and (B) cumulative overall survival curves of low and high viral load groups.

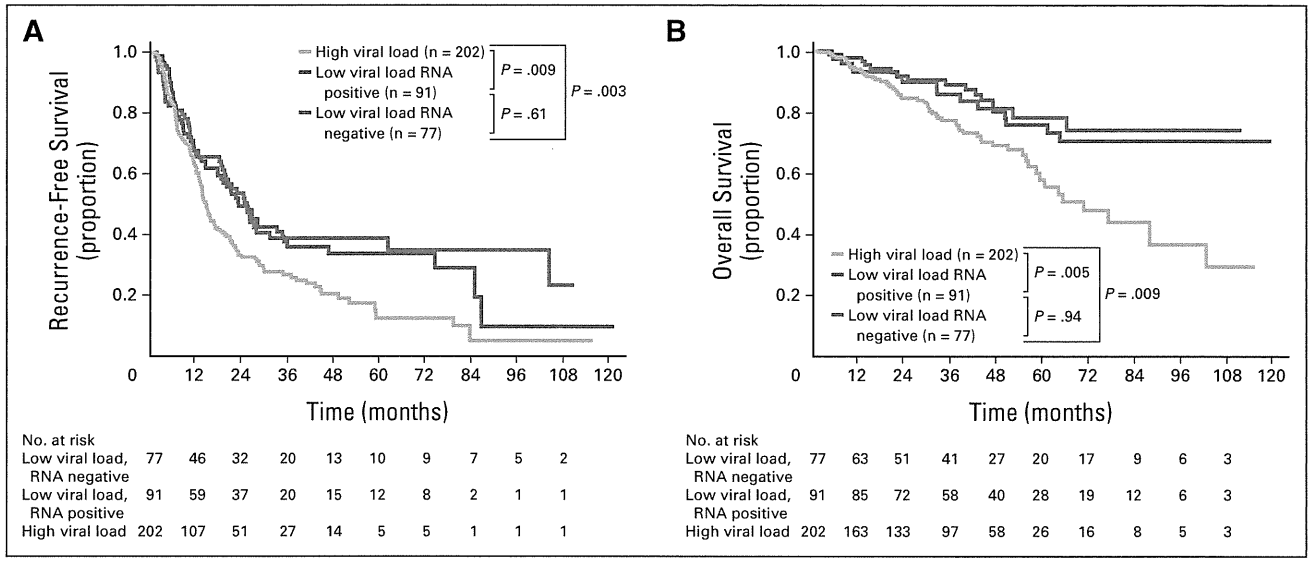

Fig 3. (A) Cumulative recurrence rate and (B) cumulative overall survival curves of low and high viral load groups stratified according to the results of hepatitis C virus RNA quantification.

and it improved to 0.627 (95% CI, 0.590 to 0.665) when including HCV viral load greater than 5.3 $\log_{10}$IU/mL in the prognostic model (Table 3).

DISCUSSION

In this study, we analyzed 370 patients who underwent curative liver resection for HCV-related HCC. The current study indicates that a low viral load ($\leq$ 5.3 $\log_{10}$IU/mL) is strongly associated with lower recurrence rate and better OS regardless of the serologic eradication of HCV. These observations were constant both in initial hepatectomy for primary lesions and repeat hepatectomy for recurrent lesions. Multivariate analysis confirms that a high HCV viral load ($>$ 5.3 $\log_{10}$IU/mL) is an independent factor associated with a 1.84-fold greater risk of tumor recurrence after curative resection of HCC.

In patients with HCV-related HCC, virologic status of HCV has been thought to be a prognostic factor associated with high tumor recurrence rate.[9,10] Adjuvant antiviral therapy is performed with the aim of preventing tumor recurrence by improving the fibrotic status and/or activity of inflammation in the background liver through the reduction of the viral load. Several studies have shown favorable long-term outcomes of adjuvant IFN therapy after locoregional treatments or surgical resections.[11,32-34] However, the effectiveness of antiviral therapy has been discussed mainly from the view point of virus eradication,[11-13,34-36] and little is known about the significance of the viral load itself for tumor recurrence.

Akamatsu et al[37] previously reviewed 371 patients who had undergone locoregional treatments for HCV-related HCC and denied a correlation between the viral load and the recurrence rate of HCC. However, their study contained a heterogeneous population that underwent

Table 3. Factors Associated With Recurrence of Hepatocellular Carcinoma

Factor	P*	Coefficient†	SE	Wald χ^2	HR	95% CI
HCV-RNA > 5.3 $\log_{10}$IU/mL	< .001	0.627	0.144	19.1	1.87	1.41 to 2.48
Macrovascular invasion	< .001	1.384	0.327	17.9	3.99	2.10 to 7.57
Repeat resection for recurrence	< .001	0.505	0.150	11.4	1.66	1.24 to 2.22
Tumor exposure	.013	0.337	0.136	6.1	1.40	1.07 to 1.83
Tumor size > 2 cm	.093	0.252	0.150	2.8	1.29	0.96 to 1.83

NOTE. The concordance statistic for the four-factor model (macrovascular invasion + repeat resection + tumor exposure + size > 2 cm) was 0.603 (95% CI, 0.559 to 0.647). The concordance statistic for the full model (the four-factor model + HCV-RNA > 5.3 $\log_{10}$IU/mL) was 0.627 (95% CI, 0.590 to 0.665). Multivariate Cox regression was applied with stepwise backward selection. Initially, all factors were included in the model. Then factors that showed no or limited statistically significant association ($P > .01$) with tumor recurrence adjusted for the remaining factors in the model were deleted from the model in a stepwise fashion. The 17 factors tested were as follows: sex, primary versus repeat resection, tumor size (> *v* ≤ 2 cm), number of tumors (solitary *v* multiple), hepatitis B core antibody (yes *v* no), HCV viral load (> *v* ≤ 5.3 $\log_{10}$IU/mL), fibrotic status of the underlying liver (F3-4 *v* F0-2), serum ALT level (> *v* ≤ 40 IU/L), indocyanine green retention rate at 15 minutes (> *v* ≤ 15%), serum α-fetoprotein level (> *v* ≤ 20 ng/mL), plasma des-γ-carboxyprothrombin level (> *v* ≤ 40 mAU/mL), type of hepatectomy (anatomic *v* nonanatomic), perioperative transfusion (yes *v* no), tumor exposure (yes *v* no), microvascular invasion (yes *v* no), macrovascular invasion (yes *v* no), and tumor differentiation (well/moderate *v* poor).

Abbreviations: HCV, hepatitis C virus; HR, hazard ratio.

*Based on likelihood test adjusted for the other factors in the final model.

†Estimated coefficient for the variable and the associated SE.

various types of treatments including surgery, ablation, and TACE. Therefore, the true clinical influence of HCV viral load on long-term outcomes of HCV-related HCC is still unclear. In the current study, we carefully reviewed patients who underwent curative surgical resections under a consistent treatment strategy in a single high-volume hepatobiliary center. Major prognostic improvements were observed both in recurrence and survival when a low viral load was obtained according to the cutoff value (5.3 $\log_{10}$IU/mL) that was determined by the minimum *P* value approach to predict moderate to severe activity of hepatitis. Comparison of clinicopathologic factors revealed that high viral load was associated with higher serum ALT and AST levels (both before and after surgery) and higher fibrotic status. These correlations are consistent with previous reports[38,39] and suggest the higher carcinogenic potential in the background liver in patients with high HCV viral load.

Another noteworthy result is that the preferable outcomes in the low viral load group are not significantly influenced by whether or not the serologic eradication of HCV is obtained. As shown in Figure 3, when the survival curves were compared between the RNA-positive and RNA-negative patients, no significant difference was observed, although both curves represented apparently better outcomes than that for the high viral load group. We also confirmed a similar tendency both in initial hepatectomy and repeat hepatectomy in a subset analysis (Appendix Figure 1, online only). These results suggest that a lower viral load might be preferable even if the serologic eradication of HCV is not obtained, supporting the outcomes of previous studies[14,15] and a recent meta-analysis[40] studying the effectiveness of IFN therapy.

Recent introduction of combination therapy consisting of pegylated IFN and ribavirin has dramatically improved the sustained viral response rate in patients with HCV.[32,33] However, the postoperative use of IFN remains a major concern because HCC usually emerges in the liver that has been damaged over the course of decades, and accordingly, patients tend to be elderly and to exhibit cirrhotic changes. Therefore, a high-dose standard combination therapy is not always applicable because of the issue of tolerability. Furthermore, even if IFN therapy is available, a sustained viral response may not always be achievable, especially in female patients or patients infected with HCV genotype 1b, both of which have been reported as factors refractory to antiviral therapy.[41-44] In fact, the median age of the current population was 70 years, and 47.8% of the patients were clinically diagnosed with cirrhosis. The proportion of women was higher in the high viral load group, and 71.4% of the patients had genotype 1b.

Given the current results, a low HCV viral load can be a new clinical end point in adjuvant therapy for HCV-related HCC. In this context, a more tolerable antiviral therapy, including low-dose IFN therapy with prolonged therapeutic duration[45,46] or possibly a combination with protease inhibitors,[47-49] may be a therapeutic option for elderly patients or patients with liver cirrhosis. Given the fact that anatomic resection of the liver was also an independent predictor of recurrence in the multivariate analysis, combination of anatomic resection and adjuvant IFN therapy may enhance the postoperative outcomes in patients with HCV-related HCC by eradicating micrometastases and reducing the carcinogenic potential in the underlying liver.

Because this study was retrospective, prospective/randomized trials are needed to confirm the true influence of the HCV viral load and the effectiveness of adjuvant antiviral therapy on postoperative outcomes. In addition, the results of the Sorafenib as Adjuvant Treatment in the Prevention of Recurrence of Hepatocellular Carcinoma (STORM) trial, if sorafenib is found to be of benefit, will impact the selection of postoperative therapy in HCC in the near future. Given the possibility of drug interactions and competing toxicity between sorafenib and antiviral agents, further investigation on the selection of adjuvant treatment is needed, especially in patients with HCV-associated HCC.

In conclusion, a low viral load may predict lower recurrence and better survival in patients undergoing hepatic resection for HCV-related HCC irrespective of the serologic eradication of HCV. Postoperative antiviral therapy with individually adjusted intensity and incorporation of direct antiviral agents may warrant prospective study to characterize safety and impact on recurrence risk in patients undergoing surgical resection for HCV-associated HCC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The efficacy of nocturnal administration of branched-chain amino acid granules to improve quality of life in patients with cirrhosis

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Abstract
Background Nocturnal administration of branched-chain amino acid (BCAA) granules improves serum albumin levels in patients with cirrhosis. However, it is unclear whether or not this administration method can improve the patients' quality of life (QOL). In this study, we aimed to investigate the efficacy of BCAA granules, given nocturnally, in improving QOL in these patients.
Methods We performed a multicenter, randomized controlled trial examining the comparative effects of BCAA granules given orally for 3 months with daytime or nocturnal administration in patients with compensated cirrhosis. Health-related QOL was measured by a Japanese version of the questionnaire on subjective and objective symptoms, and the Short Form-8 (SF-8) questionnaire.
Results Twenty-one patients received BCAA granules three times a day (one sachet after each meal: the daytime group), and 16 patients received the granules twice a day (one sachet after breakfast, and two sachets before bedtime: the nocturnal group). Baseline characteristics did not differ between the groups (whole cohort: Child-Pugh grade A/B, 21/16; mean age, 68.2 years). There was no significant difference in any of the subjects revealed by the questionnaire regarding subjective or objective symptoms, or by the SF-8 between the daytime group and the nocturnal group after 3 months of treatment. The daytime group showed a significant effect on general health, vitality, social functioning, mental health, and role emotional as revealed on the SF-8. Conversely, the nocturnal group exhibited a significant decrease in the occurrence of muscle cramps in the legs ($P = 0.014$) and significantly improved Fisher's ratio after 3 months ($P = 0.04$).
Conclusions Nocturnal administration of BCAA granules in patients with cirrhosis reduced the occurrence of muscle cramps in the leg but did not improve the patients' QOL.

Keywords Liver cirrhosis · Branched-chain amino acid · Quality of life

Abbreviations

AAA	Aromatic amino acid
BCAA	Branched-chain amino acid
CT	Computed tomography
HCC	Hepatocellular carcinoma
LES	Late evening snack
OGTT	Oral glucose tolerance test
PEM	Protein-energy malnutrition

The trial described in this work has been registered under the following trial number: UMIN 000005274.

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QOL Quality of life
SF-8 Short Form-8

Introduction

It has been reported that nutritional state influences survival in patients with liver cirrhosis [1]. The energy balance in patients with liver cirrhosis is characterized as protein-energy malnutrition (PEM), disorder of glycolysis, decline of glycogenosis, negative nitrogen balance, and hyperlipolysis [2–4]. Patients with advanced liver cirrhosis show a characteristic decrease in the plasma concentration of branched-chain amino acids (BCAAs) and an increase in aromatic amino acids (AAAs). Two large randomized trials have shown that the long-term administration of BCAA supplements decreased the progression of hepatic failure and was associated with improved survival in patients with cirrhosis [5, 6]. Moreover, nocturnal energy supplementation improved nitrogen balance and abnormal fuel metabolism in patients with cirrhosis [7–9]. As an intervention for energy malnutrition, frequent meals or a late evening snack (LES) have been recommended [7–11]. The group of Sakaida et al. (Okamototo et al. [12], Sakaida et al. [13], and Tsuchiya et al. [14]) showed that 1-week administration of a BCAA-enriched nutrient, Aminoleban EN® (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) (210 kcal) in hospitalized patients with liver cirrhosis improved energy malnutrition, but glucose intolerance occurred 12–14. Of note, long-term use of LES for 3 months showed worsened glucose tolerance, as assessed with the 75- oral glucose tolerance test (OGTT) [15]. Fukushima et al. [16] revealed in their study that nocturnal administration of BCAA granules, giving one sachet (L-isoleucine 952 mg, L-leucine 1904 mg, L-valine 1144 mg) after breakfast and two sachets before bedtime, improved the serum albumin level in cirrhotic patients who had shown no improvement in serum albumin level with daytime BCAA administration (given with each meal). However, it is unclear whether the method of BCAA administration can improve quality of life (QOL) in patients with cirrhosis. The objective of this study was to investigate the efficacy of nocturnal administration of BCAA granules in improving QOL in patients with cirrhosis.

Patients and methods

Study design and selection of patients

Between October 2008 and September 2010, this randomized controlled study was conducted in 9 hospitals and 4 medical clinics in Japan. The protocol was undertaken with the approval of the Institutional Review Board of each participating institution and in accordance with the World Medical Association's Declaration of Helsinki (1989). The final protocol was approved by the Ethics Committee of the Kitasato University, Sagamihara, Japan (C-Ethics Committee, ID 08-438). Written informed consent was obtained from each enrolled subject. The trial described in this work has been registered under the following trial number: http://www.umin.ac.jp/ctr/index.htm, UMIN 000005274.

The inclusion criteria were patients with cirrhosis who were aged between 20 and 80 years whose serum albumin level ranged from 3.1 to 3.5 g/dl. Cirrhosis was diagnosed on the basis of clinical, radiological, and laboratory parameters, and/or liver biopsy. The patients underwent endoscopy and computed tomography (CT). Exclusion criteria were: (1) Child-Pugh score $\geq$10; (2) hepatocellular carcinoma (HCC); (3) endoscopically confirmed existing moderate or large varices and post-ligated ulcers 1 month after final esophageal variceal ligation; (4) ongoing pharmacological therapy for portal hypertension with nonselective beta-blockers, nitrates, and angiotensin II type 1 receptor blockers; (5) portal thrombosis; (6) drinking alcohol within 3 months before the start of the study; (7) a history of BCAA supplementation in the previous 3 months; (8) pregnancy; and (9) allergy or past adverse reaction to BCAA.

Instruments for QOL assessment

A disease-specific health-related quality of life (HRQOL) analysis and a cross-sectional analysis of general HRQOL were conducted using the Japanese version of questionnaires on subjective and objective symptoms in patients with liver cirrhosis, and using the Japanese version of the Medical Outcomes Study 8-Item Short-Form Health Survey (SF-8). The validity and reliability of the Japanese versions of both questionnaires have already been confirmed, as described previously [17]. The Japanese version of the questionnaires on subjective and objective symptoms comprises 6 subscales (sluggishness, fatigue, general itching, anorexia, abdominal fullness, and muscle cramps). The SF-8 comprises 8 subscales (GH, general health; PF, physical functioning; RP, role limitation due to physical problems; BP, body pain; VT, vitality; SF, social functioning; MH, mental health; RE, role limitation due to emotional problems), and all of these categories are compatible with those in the SF-8. In the present study, the score of each of the 8 subscales, the physical health component summary score (PCS), and the mental health component summary score (MCS) were measured using the norm-based scoring method, which was based on a large-scale population study conducted in Japan [17]. QOL

scores are shown as mean scores with a 95 % confidence interval (95 % CI), with the higher scores representing better QOL. In order to enhance the potential for unbiased and truthful answers, participants responded anonymously to all questions and returned the questionnaires using return stamped-addressed envelopes.

Intervention protocol

Baseline evaluation included physical examination and routine laboratory tests. Daily food intake was estimated by a self-administered questionnaire. Screening information of possibly eligible patients was transmitted to the registration center. After confirmation of eligibility, the patients were randomly assigned in a 1:1 ratio by the center to either daytime BCAA granule administration [4 g BCAA (Livact® Granules; Ajinomoto Pharmaceutical, Tokyo, Japan): L-isoleucine 952 mg, L-leucine 1904 mg, L-valine 1144 mg] after each meal, or nocturnal BCAA granule administration (4 g after breakfast and 8 g before bedtime) for 3 months. Study randomization was conducted by computer to achieve a balance between the two groups without stratification. Daily food intake was mandated by the patients' physicians in charge at 30 kcal/kg/day and 1.2 g protein/kg/day. We assessed compliance with treatment at each outpatient visit by interview.

Follow up

The initial clinical visit was 2 weeks after the introduction of treatment in both arms, with a clinical visit again after another 2 weeks. The follow-up interval was every 4 weeks. Biochemical (serum albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, free fatty acid, cholinesterase, NH3, glycated albumin, and Fisher's ratio) and hematological profiles (platelets and prothrombin time) were obtained at each consultation after the patients had had an overnight fast. After 3 months of treatment, a clinical examination was performed, and patients underwent CT examinations as part of the HCC surveillance. Adverse events arising from BCAA granule administration were defined according to the Common Terminology Criteria for Adverse Events version 3.0 or 4.0. After recruitment of the last patient, follow up was designed to be continued in both treatment arms for 3 months.

Sample size calculation and statistical analysis

To our knowledge, only one study in the literature has investigated the effect of nocturnal BCAA granule administration in patients with cirrhosis [16]. In that study, nocturnal BCAA administration improved protein metabolism, but its effect on QOL was not examined [16]. Therefore, in the present study, we calculated the sample size by estimating improvement in the serum albumin level. We estimated that the rate of improvement after 3 months of treatment would be not <50 % in the nocturnal group and at least 10 % in the daytime group. At least a 10 % failure rate in both groups was estimated previously. On the basis of a 40 % difference in the improvement rate after 3 months of follow up, a sample size of 10 patients per group would provide 80 % power with a 2-sided alpha of 0.05 by log-rank test.

Values for continuous variables are presented as means ± standard deviation (SD) or medians (ranges). The paired or non-paired Student's *t*-test was used to assess the significance of differences in the comparison of normally distributed data, and the Mann–Whitney *U*-test or the Wilcoxon test was used for the non-normally distributed data, while numerical variables were assessed by Pearson's χ^2test or Fisher's exact test, as appropriate. *P* values of <0.05 were considered to indicate statistical significance. All reported *P* values were two-sided. Analyses were performed using SPSS version 17.0 software (SPSS, Chicago, IL, USA).

Results

Between September 2008 and August 2010, total of 50 patients with liver cirrhosis were refferred for possible randomization after screening from 4 hospitals and 2 clinics of the 13 centers open to the study (Fig. 1). In all, 10 patients were excluded from randomization: 7 patients who had previously received BCAA administration, and 3 patients who were confirmed to have HCC. Forty patients were initially enrolled and randomized. However, after randomization, 3 patients in the nocturnal group opted for other treatments because their regular pharmacy could not keep up our treatment schedule with nocturnal BCAA granule administration. Therefore, 21 patients received BCAA granules three times a day (one sachet after each meal: the daytime group), and 16 patients received the BCAA granules twice a day (one sachet after breakfast and two sachets before bedtime: the nocturnal group). The contributions from the six centers were as follows: Kitasato University East Hospital, $n = 23$; Kitasato University Hospital, $n = 2$; Kutsukake Clinic, $n = 7$; Hiratsuka Kyosai Hospital, $n = 3$; Toshiba Rinkan Hospital, $n = 1$; Kakunaka Clinic, $n = 1$.

Baseline characteristics did not differ between the groups. The main characteristics of the patients are summarized in Table 1. There were no significant differences between patients randomized to the daytime group and those in the nocturnal group in any of the parameters.

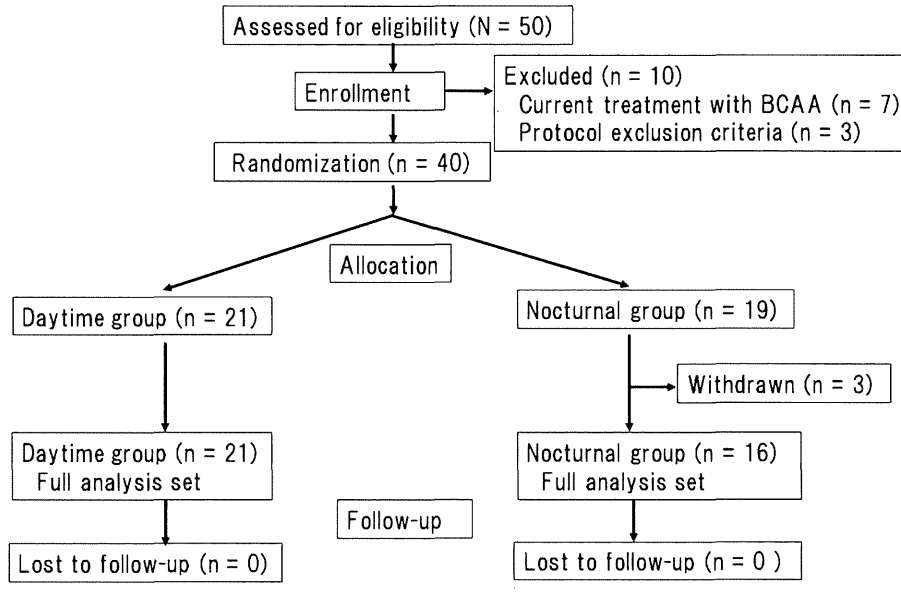

Fig. 1 Flow diagram of study recruitment through follow up. *BCAA* branched-chain amino acid

Table 1 Patients' baseline clinical and biochemical characteristics

Patients	Daytime group $n = 21$	Nocturnal group $n = 16$	P
Age (years)	73 (41–83)	72 (52–79)	0.70
Gender (male/female)	8/13	7/9	0.78
Etiology			0.24
Alcohol	1	2	
HBV	1	1	
HCV	16	7	
Others	3	6	
Liver function			
Child-Pugh class (A/B)	12/9	9/7	0.96
BMI (kg/m^2)	22.2 (14.9–30.3)	23.9 (19.2–29.2)	0.21
Ascites (yes/no)	0/21	0/16	
History of HCC therapy (yes/no)	4/17	4/12	0.16

Values for age and BMI are expressed as medians and ranges

HBV hepatitis B virus, *HCV* hepatitis C virus, *BMI* body mass index

QOL assessment

The therapy was well accepted and tolerated. There were no significant differences in any of the subjects according to the results of the questionnaire survey on either subjective or objective symptoms, or on the SF-8 between the daytime group and the nocturnal group after 3 months of treatment.

All the patients had a significant decrease in the occurrence of muscle cramps in the legs ($P = 0.004$) (Fig. 2a) and all exhibited significant effects on general health scores as revealed by the SF-8 after 3 months ($P = 0.01$) (Fig. 3a). After 3 months of treatment, the daytime group had a decrease in the occurrence of muscle cramps in the legs (Fig. 2b), and exhibited significant effects on general health ($P = 0.005$), vitality ($P = 0.049$), social functioning ($P = 0.016$), mental health ($P = 0.037$), and role emotional ($P = 0.029$) as revealed by the SF-8 (Fig. 3b). On the other hand, although the nocturnal group had a significant decrease in muscle cramps in the legs ($P = 0.014$) (Fig. 2c), there were no significant effects on any parameter of the SF8 in this group (Fig. 3c).

Changes in laboratory data

After 3 months' treatment, there were no significant differences in any laboratory parameters between the daytime group and the nocturnal group. After 3 months, Fisher's ratio was significantly increased in the nocturnal group (1.48 ± 0.04 vs. 2.32 ± 0.71, $P = 0.04$) compared with the value in the daytime group (1.42 ± 0.24 vs.

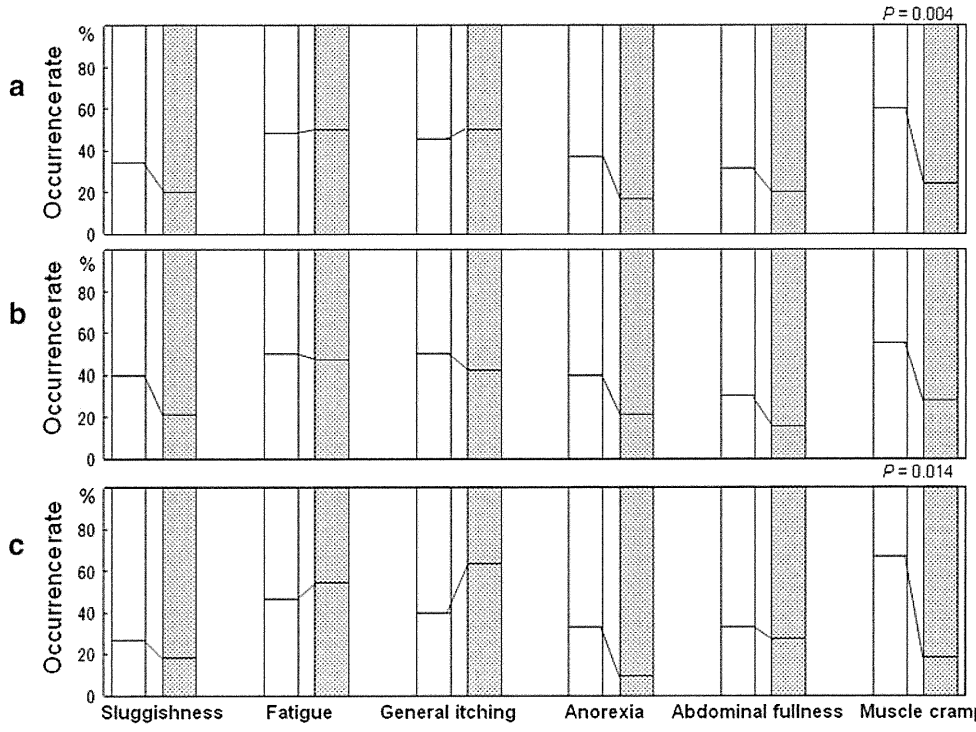

Fig. 2 Occurrence rates from the Japanese version of questionnaires on subjective and objective symptoms in patients with liver cirrhosis. All the patients had a significant decrease in the occurrence of muscle cramps in the legs ($P = 0.004$) (nocturnal group, $P = 0.014$). *White bars*, pretreatment; *dotted bars*, after 3 months of treatment. **a** All the patients, **b** daytime group, **c** nocturnal group

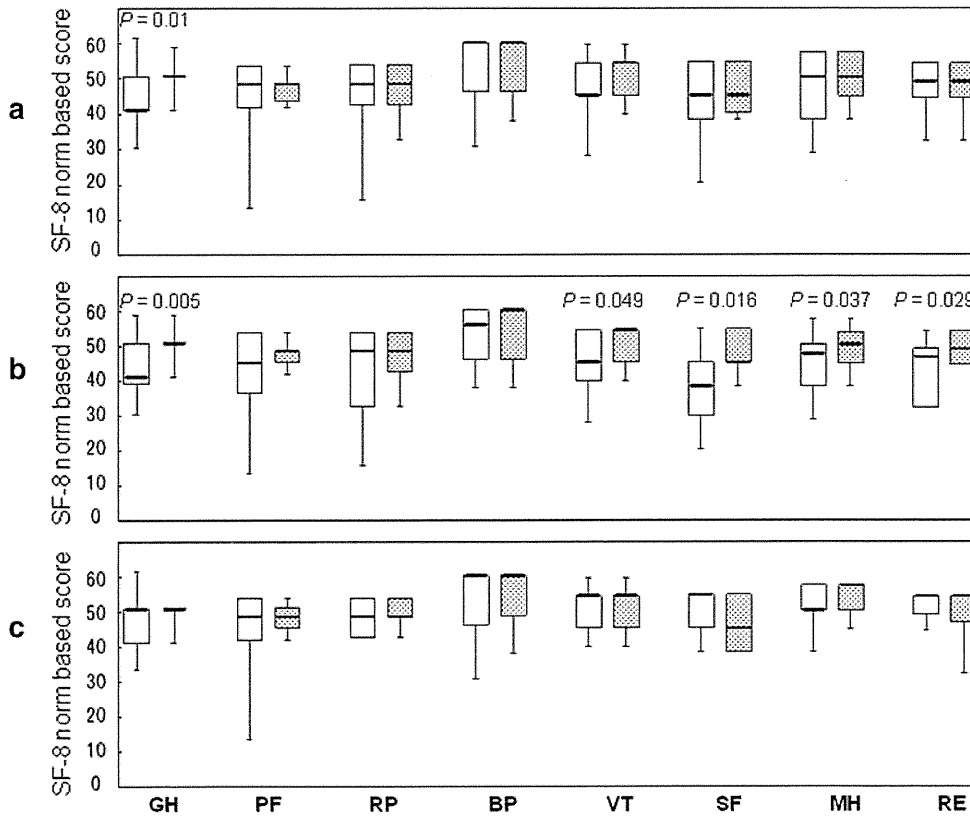

Fig. 3 Results from the Japanese version of the Medical Outcomes Study Short-Form 8-Item Health Survey (SF-8). All the patients showed a significant effect revealed by the general health scores on the SF-8 ($P = 0.01$). The daytime group showed a significant effect on general health ($P = 0.005$), vitality ($P = 0.049$), social functioning ($P = 0.016$), mental health ($P = 0.037$), and role emotional ($P = 0.029$) as revealed on the SF-8. Conversely, the nocturnal group did not show any significant effects on any parameters of the SF8. *GH* general health, *PF* physical functioning, *RP* role limitation due to physical problems, *BP* body pain, *VT* vitality, *SF* social functioning, *MH* mental health, *RE* role limitation due to emotional problems. *White bars*, pretreatment; *dotted bars*, after 3 months of treatment. **a** All the patients, **b** daytime group, **c** nocturnal group

1.75 ± 0.16, $P = 0.18$). However, serum albumin after 3 months was not changed in either group (3.34 ± 0.15 vs. 3.42 ± 0.19 g/dl, $P = 0.85$ in the nocturnal group, and 3.35 ± 0.18 vs. 3.40 ± 0.35 g/dl, $P = 0.59$ in the daytime group) (Table 2). Moreover, glycated albumin was not changed by BCAA administration.

Table 2 Changes in chemical markers in the daytime and nocturnal groups

	Daytime group			Nocturnal group		
	Baseline	3 Months	*P*	Baseline	3 Months	*P*
Albumin (g/dl)	3.4 (3.1–3.8)	3.4 (3.0–4.0)	0.59	3.4 (3.1–3.5)	3.4 (3.0–3.8)	0.85
Platelets ($\times 10^4/\mu l$)	6.8 (3.8–38.4)	7.35 (3.3–16.6)	0.17	7.95 (4.0–12.8)	9.05 (4.4–18.7)	0.46
AST (IU/l)	65 (24–139)	72 (26–149)	0.28	60 (21–139)	53 (31–115)	0.99
ALT (IU/l)	42 (11–105)	49 (17–123)	0.32	41 (14–112)	32 (17–96)	0.57
T. Bil. (mg/dl)	1.1 (0.4–2.8)	1.1 (0.4–2.2)	0.46	1.0 (0.5–2.7)	0.9 (0.4–2.8)	0.08
PT (%)	73.5 (58.1–90.9)	76.0 (54.0–87.0)	0.65	63.4 (50.0–81.4)	70.8 (54.5–82.2)	0.32
NH_3 (μg/dl)	70 (19–108)	51 (19–104)	0.33	29 (10–138)	19 (17–158)	0.61
Free fatty acid (Eq/l)	652 (99–2437)	261 (87–2736)	0.83	281 (39–2685)	579 (39–2850)	0.72
Cholinesterase (mg/dl)	144 (59–187)	149 (41–252)	0.59	128 (87–273)	150 (87–246)	0.79
Glycated albumin (%)	21.0 (15.5–44.8)	19.3 (16.6–47.8)	0.91	20.0 (16.0–42.3)	19.5 (16.8–39.4)	0.73
Fisher's ratio	1.27 (1.00–2.23)	1.60 (1.33–2.30)	0.18	1.57 (1.14–1.72)	2.12 (1.15–3.52)	0.04
AFP (ng/ml)	5.65 (3.6–222.0)	9.0 (2.9–59.5)	0.35	11.1 (2.1–432.0)	14.7 (1.8–954.0)	0.36

All data values are expressed in medians and ranges

T. Bil. total bilirubin, *PT* prothrombin time, *AFP* alpha-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

Treatment compliance

Overall, 20 (95.2 %) patients in the daytime group and 15 (93.8 %) patients in the nocturnal group received more than 80 % of the planned daily dose of the study drug.

Adverse reactions

Adverse reactions to BCAA administration were identified in 4 patients in the nocturnal group: 2 patients developed abdominal distension (grade 2), and 2 patients developed general itching (grade 1). However, all 4 patients recovered with conservative treatment.

HCC and esophageal varices

Two patients in each arm of the present study developed HCC after 3 months of follow up. There were no patients who bled from esophageal varices during the observation period.

Discussion

To our knowledge, this is the first multicenter randomized controlled trial to have investigated the efficacy of nocturnal BCAA granule administration in improving QOL in patients with cirrhosis. In this study, the administration of BCAA granules significantly reduced the occurrence of muscle cramps in the leg for all the patients and the nocturnal group. Generally, muscle cramps are caused by a variety of factors, including diuretic treatment, intolerance of amino acids, and deficiency of vitamin E and taurine [18, 19]. Marchesini et al. [6] reported that the frequency of muscle cramps, which was associated with poor QOL in patients with decompensated cirrhosis, was dramatically reduced by BCAA supplementation over a period of 3 months; however, the BCAA supplementation was associated with poor QOL in patients with decompensated cirrhosis. Of note, in our study, QOL in the daytime group was significantly improved after 3 months of treatment compared with that in the nocturnal group. In particular, the components of GH, VT, SF, MH, and RE improved significantly in the daytime group. We could not explain this discrepancy between the QOL findings in our study and those of Marchesini et al. [6]. However, in our nocturnal group, Fisher's ratio, which is the most important index of an amino acid imbalance, was significantly increased compared with that in the daytime group. In the matter of the results of the plasma Fisher's ratio, it is necessary to consider the influence of the interval between the last BCAA intake and blood sampling in the fasting state. In a single-administration study of BCAA in healthy rats, the mean maximum blood concentration of BCAA was observed after about 4 h and the concentration gradually decreased after that, and the mean half-life was about 58 h [20]. However, it is considered that the preservation of a higher BCAA concentration until morning is particularly important in patients with decompensated cirrhosis. Patients with liver cirrhosis cannot constantly maintain the concentration of BCAA administered in the daytime until nighttime because the BCAA is consumed for their

daytime physical activity [16]. We speculated that nocturnal BCAA administration would complement the deficiency experienced during the night. However, we found that nocturnal administration tended to induce more abdominal distension and general itching at night than daytime administration, thus lowering the QOL in nocturnal group.

In the present study, the glycated albumin level was not changed by BCAA administration. Sako et al. [18] reported that the administration of the BCAA supplement, Aminoleban EN® (210 kcal), in the evening decreased the number of muscle cramps in 8 outpatients with advanced liver cirrhosis. However, excess elevation of blood glucose occurs after meals in liver cirrhosis patients [21]. Aoyama et al. [15] reported that patients with a glucose level of more than 200 mg/dl 2 h after a 75-g OGTT experienced impaired glucose tolerance after 3 months' administration of BCAA supplements. However, impaired glucose tolerance did not occur with the administration of BCAA granules that consisted of only 4 g BCAA. A previous clinical trial has reported that BCAA administration decreased plasma glucose levels in patients with advanced liver cirrhosis [22]. Furthermore, Kawaguchi et al. [23] reported that BCAA improved insulin resistance in patients with chronic liver disease. Therefore, it is possible that BCAA granules are more useful for muscle cramps in patients with glucose intolerance than are BCAA supplements. On the other hand, Nakaya et al. [24] reported that BCAA supplements improved the nutrition state (serum albumin, energy metabolism) in patients with decompensated cirrhosis. Accordingly, in decompensated patients who cannot eat enough, it seems to be more feasible for them to take BCAA supplements.

HCC was noted in 2 patients with hepatitis C in each arm of the present study after 3 months of treatment with BCAA granules. It seems that the HCC occurrence rate (11.1 % per 3 months) in this study was much higher than the general rate (2–5 %) in Japan [25]. All 4 patients for whom HCC was noted had received HCC therapy previously, and their serum albumin levels had decreased after 3 months of the BCAA administration. The present study might have been better if we had not included these 4 patients who had previously received HCC therapy. However, a recent clinical study reported that long-term BCAA administration inhibited the development of HCC in liver cirrhosis patients with hepatitis C [5]. Furthermore, one study from Japan showed that BCAA administration in patients with liver cirrhosis suppressed HCC recurrence after treatment with radiofrequency ablation [26]. Further studies are needed to clarify whether or not there are clinical benefits of BCAA administration that would help prevent HCC.

There are three limitations of the present study. First, we could not establish a control group (i.e., a no-treatment group). However, large trials of BCAA supplements show that they decrease the progression of hepatic failure and are associated with improved survival in patients with decompensated cirrhosis [5, 6]. Therefore, we believe that it is not necessary to compare the BCAA administration results with a no-treatment control group. The second limitation is the short-term (3 months) administration. If we had administered BCAA granules for a longer term (e.g., 1 year), the serum albumin level might have increased significantly. On the other hand, the high rate of compliance in our study might have been due to the short-term administration. Finally, after the randomization, 3 patients in the nocturnal group chose early withdrawal because their regular pharmacy could not keep up with our treatment schedule. Accordingly, our study did not follow ITT (intention-to-treat) analyses because we omitted these patients from analysis in this study. However, it is considered that this withdrawal was caused by institutional error and not because of the patients' lack of compliance.

In conclusion, the daytime administration of BCAA granules significantly improved the QOL in patients with cirrhosis. And, while nocturnal administration significantly reduced patients' leg muscle cramps, it did not seem to improve their QOL.

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Conflict of interest H. Hidaka has served as a consultant and speaker for Ajinomoto Pharmaceutical Co., Inc., Tokyo, Japan; all the other authors have no personal interests to disclose in relation to this article.

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