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Original Article

Effects of branched-chain amino acid granules on serum albumin level and prognosis are dependent on treatment adherence in patients with liver cirrhosis

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Aim: To test if the treatment adherence to branched-chain amino acid (BCAA) granules influences the serum albumin level and prognosis in prospective 2984 patients with decompensated liver cirrhosis who were prescribed BCAA granules containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine at 4.15 g/sachet three times a day after meals.

Methods: The primary end-point was the time to the event defined as "hospital admission due to progression of hepatic failure", and factors affecting this outcome were explored. Changes in serum albumin level were evaluated as the secondary end-point.

Results: Patients were divided into the good adherence group (those who reported to have taken "nearly all" prescribed doses) and the poor adherence group (those who reported to have taken "approximately half" or "less" doses), because such stratification was validated by treatment responses in plasma BCAA/tyrosine ratio. Factors related to the primary end-point were age, drug adherence during 6 months of study treatment, previous hepatic cancer, current clinical manifestations, previous clinical manifestations, baseline serum albumin level, platelet count and total bilirubin level. The cumulative event-free survival was significantly higher in the good adherence group. Increase in the serum albumin level was also greater in the good adherence group.

Conclusion: Higher BCAA treatment adherence better raised the serum albumin level, leading to improvement of event-free survival. These results indicate the importance of patient instruction for the adequate use of BCAA granules.

Key words: branched-chain amino acids, hepatic failure, liver cirrhosis, prognosis, serum albumin, treatment adherence

INTRODUCTION

ALTHOUGH LIVER CIRRHOSIS is caused by any of a wide variety of etiologies,[1] clinical features of the disease share complications such as ascites, edema, hepatic encephalopathy and esophageal varices.[2] Some of these complications are attributable to decreased serum concentrations of albumin and other proteins,[3–5] and oral supplemental branched-chain amino acid (BCAA) therapy with BCAA granules or BCAA-enriched nutrients is recommended, in addition to dietary treatment with adequate protein and energy intake, for the management of these complications.[6–8]

Branched-chain amino acid granules are used for the improvement of hypoalbuminemia in patients with decompensated liver cirrhosis,[4,9–11] and several studies have demonstrated their efficacy in reducing complications of liver cirrhosis.[4,12–14] Furthermore, a reduction in

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the risk of hepatic cancer is also reported in patients taking BCAA granules.[12,15–17]

On the other hand, the patients' treatment adherence was not so favorable owing to the size of individual doses and unpleasant taste, causing interruption of treatment[13] or reduction of doses.[4] Although serum albumin level has been shown to improve in a dose-dependent manner based on the prescribed BCAA doses,[10] no studies have investigated exactly how treatment adherence may influence the serum albumin level and prognosis of patients with liver cirrhosis.

We conducted the present analysis to evaluate how treatment adherence may affect the serum albumin level and prognosis in a prospective cohort of 5042 patients with liver cirrhosis who had started BCAA treatment at a fixed dose of three sachets/day in a preceding study.[18]

METHODS

Study design and protocol

THIS WAS A multicenter prospective observational study to determine the incidence of adverse events, including hepatocellular carcinoma (HCC) and cirrhosis-related events, under the actual condition of treatment in patients with decompensated liver cirrhosis who were prescribed BCAA granules between June 2003 and December 2006,[18] and were further followed up thereafter.

A total of 5042 patients with decompensated liver cirrhosis, who presented hypoalbuminemia despite adequate dietary intake, were enrolled in this study at 929 medical institutions in Japan. These patients were p.o. administrated BCAA granules containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine (Livact Granules, Ajinomoto Pharmaceutical, Tokyo, Japan) at 4.15 g/sachet three times a day after meals.

Patient flow is shown in Figure 1. Of the 5042 patients enrolled, the medical records were not available for 222 patients, and 123 patients were lost to follow up after the initial hospital visit. Thus, the remaining 4697 patients constituted the prospective cohort. Patients meeting any of the following criteria were then excluded, and the remaining 2984 patients were subjected to the analysis: (i) a baseline serum albumin level higher than 3.5 g/dL; (ii) a baseline serum total bilirubin level of 3.0 mg/dL or higher; (iii) unknown duration of study observation; (iv) baseline dosage of prescribed BCAA granules other than three sachets/day; or (v) unknown BCAA treatment adherence for 6 months after the start of study observation.

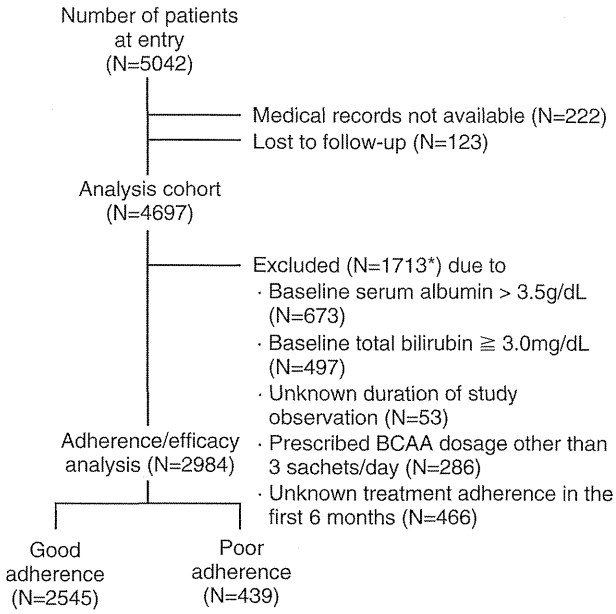


Figure 1 Patient flow. *Among the 1713 patients, 262 were excluded by meeting two or more conditions of the exclusion criteria. BCAA, branched-chain amino acid.

The patients' treatment adherence was evaluated by a questionnaire analysis at the end of the 6-month surveillance period. The questionnaire provided three answer arms who took "nearly all", "approximately half" and "less" of the prescribed dose of BCAA granules at three sachets/day. Each patient was instructed to select one of the above three answer arms that best reflected his/her drug adherence status in the preceding study period.

The primary end-point was the time to onset of the event, defined as hospital admission due to progression of hepatic failure, including ascites, edema, jaundice and hepatic encephalopathy. Changes in liver function during the 6 months were evaluated as the secondary end-point.

This study was conducted in accordance with the Japanese Good Post-Marketing Surveillance Practice.

Statistical analysis

Continuous data were expressed as mean ± standard deviation, and differences in mean values were statistically tested using paired or unpaired Student's *t*-test as appropriate. Categorical variables were compared by Wilcoxon signed rank test, Wilcoxon rank sum test or χ^2-test as required. The cumulative event-free survival rates were estimated using the Kaplan–Meier method

and compared by log–rank test. Any risk factors contributing to the primary end-point were investigated by univariate and multivariate analyses using a Cox proportional hazards model. Data analysis was performed using JMP ver. 9.02 and SAS ver. 9.2 (both SAS Institute, Cary, NC, USA). The level of significance was assessed as two-sided $P < 0.05$.

RESULTS

Patients' characteristics and flow

OF THE PROSPECTIVE cohort consisting of 4697 patients, 1713 were excluded by meeting the exclusion criteria (Fig. 1). Among them, 673 patients had a baseline serum albumin level higher than 3.5 g/dL, 497 patients had a baseline serum total bilirubin level of 3.0 mg/dL or higher, 53 patients had an unknown duration of study observation, 286 patients were prescribed BCAA granules of a dosage other than three sachets/day, and 466 patients reported unknown treatment adherence during the 6 months of study observation. Two hundred and sixty-two patients were excluded by fulfilling two or more conditions of the exclusion criteria. Thus, the remaining 2984 patients were subjected to the adherence/efficacy analysis (Fig. 1). Clinical characteristics of these patients are shown in Table 1. The observation period ranged 6.0–47.9 months, with a median of 21.6 months.

Risk factors for the primary end-point

For the primary end-point, univariate and multivariate analyses using a Cox proportional hazards model identified the following independent factors to influence the development of the event: age, treatment adherence for the 6 months of study observation, previous hepatic cancer, current clinical manifestations, previous clinical manifestations, baseline serum albumin level, platelet count and serum total bilirubin level (Table 2).

Treatment adherence and plasma BCAA/tyrosine ratio

All these variables except treatment adherence have already been documented as risk factors in patients with liver cirrhosis.[19,20] Taking notice of treatment adherence, therefore, 2545 patients who reported to have taken "nearly all" the prescribed doses during the 6-month period comprised the good adherence group and 439 patients who reported to have taken "approximately half" or "less" of the prescribed doses during that period comprised the poor adherence group for further analysis.

Table 1 Clinical characteristics of patients

Characteristics		$n = 2984$
Sex	Male	1584 (53.1%)
	Female	1400 (46.9%)
Age (years)	20–29	1 (0.0%)
	30–39	24 (0.8%)
	40–49	165 (5.5%)
	50–59	530 (17.8%)
	60–69	1038 (34.8%)
	70–79	1024 (34.3%)
	80–89	195 (6.5%)
	>90	7 (0.2%)
	Mean ± SD	66.1 ± 10.1
Cause of liver cirrhosis	HBV	217 (7.3%)
	HCV	1755 (58.8%)
	Alcohol	487 (16.3%)
	PBC	74 (2.5%)
	AIH	63 (2.1%)
	HBV + HCV	16 (0.5%)
	HBV + alcohol	29 (1.0%)
	HCV + alcohol	92 (3.1%)
	HBV + HCV + alcohol	2 (0.1%)
	Other	57 (1.9%)
	Unknown	192 (6.4%)
Treatment adherence (during 6 months)	All	2545 (85.3%)
	Half or less	439 (14.7%)
Previous hepatic cancer	Yes	504 (16.9%)
	No	2454 (82.2%)
	Unknown	26 (0.9%)
Current clinical manifestations	Yes	1568 (52.6%)
	No	1410 (47.3%)
	Unknown	6 (0.2%)
Previous clinical manifestations	Yes	1291 (43.3%)
	No	1670 (56.0%)
	Unknown	23 (0.8%)
Diabetes	Yes	536 (18.0%)
	No	2448 (82.0%)
Serum albumin (g/dL)		3.04 ± 0.36
Platelet (×10 000/μL)		9.73 ± 6.15
AST (IU/L)		67.1 ± 62.8
ALT (IU/L)		47.8 ± 40.9
Serum total bilirubin (mg/dL)		1.30 ± 0.62
BTR		2.95 ± 1.37

For categorical variables, the number of patients and percentage are shown. For continuous variables, the mean ± SD is presented. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; SD, standard deviation.

Table 2 Risk factors for the event

Explanatory variable		Univariate				Multivariate			
		Hazard ratio	*P*-value	95% CI		Hazard ratio	*P*-value	95% CI	
				Lower limit	Upper limit			Lower limit	Upper limit
Sex	Male/female	1.18	0.0625	0.99	1.40	1.18	0.0685	0.99	1.42
Age (years)		1.01	0.0190	1.00	1.02	1.02	<0.0001	1.01	1.03
Cause of liver cirrhosis	HBV (yes/no)	1.01	0.9673	0.74	1.34				
	HCV (yes/no)	0.86	0.0928	0.72	1.03				
	Alcohol (yes/no)	1.16	0.1775	0.93	1.42				
Treatment adherence (during 6 months)	Half or less/all	1.74	<0.0001	1.39	2.15	1.94	<0.0001	1.54	2.42
Previous hepatic cancer	Yes/no	1.53	<0.0001	1.25	1.86	1.76	<0.0001	1.42	2.16
Current clinical manifestations	Yes/no	2.21	<0.0001	1.84	2.65	1.66	<0.0001	1.36	2.04
Previous clinical manifestations	Yes/no	1.88	<0.0001	1.59	2.24	1.45	<0.0001	1.20	1.74
Diabetes	Yes/no	1.24	0.0488	1.00	1.52				
Serum albumin (g/dL)	Lower level	2.51	<0.0001	2.02	3.10	2.00	<0.0001	1.57	2.54
Platelet (×10 000/μL)	Lower level	1.04	<0.0001	1.02	1.06	1.03	0.0010	1.01	1.05
AST (IU/L)	Higher level	1.00	0.8840	1.00	1.00				
ALT (IU/L)	Higher level	1.00	0.0156	0.99	1.00				
Serum total bilirubin (mg/dL)	Higher level	1.68	<0.0001	1.47	1.92	1.49	<0.0001	1.29	1.72
BTR	Lower level	1.22	0.0839	0.98	1.59				

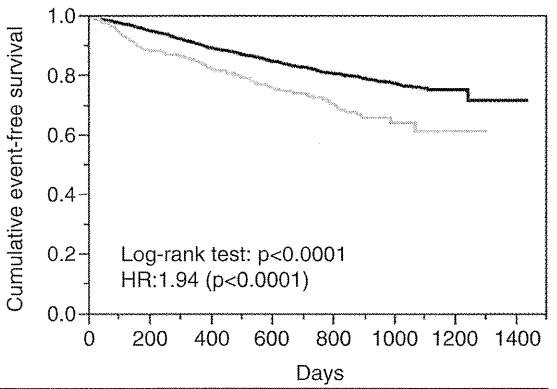
Univariate and multivariate analyses were performed using a Cox proportional hazards model, and hazard ratios, *P*-values and 95% CI of the hazard ratios are shown. For the multivariate analysis, variables were selected and determined by backwards selection ($P = 0.2$) using a model incorporating all factors except BTR. BTR was excluded from the multivariate analysis because a considerable proportion of patients lacked BTR data.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus.

As treatment adherence was judged based on patients' self-reports, we further attempted to validate the treatment adherence by changes in the BCAA/tyrosine ratio (BTR) as an indicator reflecting true BCAA treatment adherence. Although the number of patients with BTR data was limited ($n = 185$ and 19, respectively), both absolute BTR and relative increase in BTR (increase in BTR/baseline BTR) were higher in the good adherence group (absolute BTR, 4.26 ± 0.65 for the good adherence group and 3.79 ± 0.52 for the poor adherence group; and relative increase in BTR, 0.53 ± 0.8 for the good adherence group and 0.30 ± 0.68 for the poor adherence group; $P < 0.1$ for both) at 6 months of treatment, while there was no significant difference in baseline BTR between the two groups (2.94 ± 0.49 and 2.86 ± 0.46). A comparison between the two groups was thus considered to be feasible.

Treatment adherence and event-free survival

Regarding the primary end-point, Kaplan–Meier analysis and log-rank test showed a significantly higher cumulative event-free survival rate for the good adherence group as compared with the poor adherence group (Fig. 2).



	0	200	400	600	800	1000	1200	1400
Good adherence	2545	2201	1856	1545	981	345	30	2
Poor adherence	439	301	231	182	100	32	4	0

Figure 2 Comparison of cumulative event-free survival rate by treatment adherence status. Cumulative event-free survival rates were estimated for the good adherence and poor adherence groups using the Kaplan–Meier method, and are shown along with the number of patients at risk. Two curves were compared by log-rank test, and hazard ratio (HR) was calculated by Cox proportional hazards model. (—) Good adherence; (---) poor adherence.

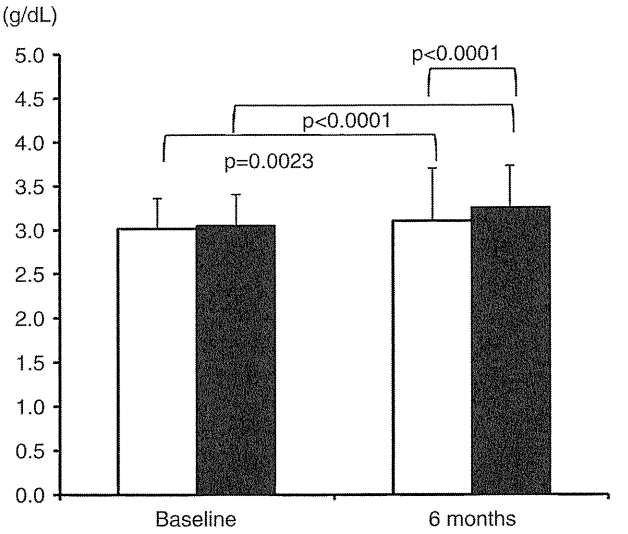


Figure 3 Comparison of serum albumin levels by treatment adherence status. Columns and bars indicate mean and standard deviation of serum albumin levels obtained at baseline and at 6 months of study treatment, respectively. Statistical assessment within each adherence group was carried out by paired Student's *t*-test. For differences between the groups at baseline and at 6 months, Student's *t*-test was conducted. (□) Poor adherence ($n = 366$); (■) good adherence ($n = 2378$).

Treatment adherence and blood biochemistry

Changes in liver function-related parameters during 6 months of the study treatment were examined for each of the good adherence and poor adherence groups. No significant difference was noted in platelet count, aspartate aminotransferase or alanine aminotransferase (ALT) between these groups. At 6 months of study treatment, serum total bilirubin level significantly increased in the poor adherence group but not in the good adherence group. Serum albumin level rose significantly in both of these groups at 6 months of study treatment, and the increase was significantly greater for the good adherence group (Fig. 3).

Comparison of clinical characteristics between good adherence group and poor adherence group

Baseline clinical characteristics were compared between the good adherence group and poor adherence group as shown in Table 3. Patients of the poor adherence group showed a significantly younger age, lower proportion of

Table 3 Clinical characteristics of patients by adherence status

Characteristics		Good adherence, $n = 2545$	Poor adherence, $n = 439$	P-value
Sex	Male	1334 (52.4%)	250 (56.9%)	$P = 0.0789$
	Female	1211 (47.6%)	189 (43.1%)	
Age (years)	20–29	1 (0.0%)	0 (0.0%)	
	30–39	16 (0.6%)	8 (1.8%)	
	40–49	135 (5.3%)	30 (6.8%)	
	50–59	445 (17.5%)	85 (19.4%)	
	60–69	894 (35.1%)	144 (32.8%)	
	70–79	888 (34.9%)	136 (31.0%)	
	80–89	161 (6.3%)	34 (7.7%)	
	>90	5 (0.2%)	2 (0.5%)	
	Mean ± SD	66.3 ± 9.9	65.2 ± 11.1	$P = 0.0344$
Cause of liver cirrhosis	HBV	184 (7.2%)	33 (7.5%)	$P = 0.0111$
	HCV	1539 (60.5%)	216 (49.2%)	
	Alcohol	393 (15.4%)	94 (21.4%)	
	PBC	59 (2.3%)	15 (3.4%)	
	AIH	52 (2.0%)	11 (2.5%)	
	HBV + HCV	13 (0.5%)	3 (0.7%)	
	HBV + alcohol	23 (0.9%)	6 (1.4%)	
	HCV + alcohol	77 (3.0%)	15 (3.4%)	
	HBV + HCV + alcohol	2 (0.1%)	0 (0.0%)	
	Other	46 (1.8%)	11 (2.5%)	
	Unknown	157 (6.2%)	35 (8.0%)	
Previous hepatic cancer	Yes	448 (17.6%)	56 (12.8%)	$P = 0.0110$
	No	2078 (81.7%)	376 (85.6%)	
	Unknown	19 (0.7%)	7 (1.6%)	
Current clinical manifestations	Yes	1321 (51.9%)	247 (56.3%)	$P = 0.1545$
	No	1218 (47.9%)	192 (43.7%)	
	Unknown	6 (0.2%)	0 (0.0%)	
Previous clinical manifestations	Yes	1094 (43.0%)	197 (44.9%)	$P = 0.6969$
	No	1432 (56.3%)	238 (54.2%)	
	Unknown	19 (0.7%)	4 (0.9%)	
Diabetes	Yes	457 (18.0%)	79 (18.0%)	$P = 0.9844$
	No	2088 (82.0%)	360 (82.0%)	
Serum albumin (g/dL)		3.04 ± 0.36	3.01 ± 0.35	$P = 0.1519$
Platelet (×10 000/μL)		9.56 ± 5.85	10.76 ± 7.63	$P = 0.0002$
AST (IU/L)		67.2 ± 66.1	66.2 ± 38.1	$P = 0.7578$
ALT (IU/L)		48.4 ± 42.7	44.2 ± 28.7	$P = 0.0518$
Serum total bilirubin (mg/dL)		1.29 ± 0.61	1.33 ± 0.66	$P = 0.2430$
BTR		2.98 ± 1.42	2.82 ± 1.07	$P = 0.4400$

For categorical variables, the number of patients and percentage are shown. For continuous variables, the mean ± SD is presented. Statistical analysis was conducted by χ^2-test or by Student's t-test.
AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; SD, standard deviation.

hepatitis C virus positivity and higher proportion of alcoholic cirrhosis, lower incidence of previous hepatic cancer, and higher platelet count (Table 3). Also, they tended to be male patients with lower serum ALT activity (Table 3).

DISCUSSION

THE LOTUS STUDY demonstrated that the outcome of patients with advanced liver cirrhosis was improved by the treatment with BCAA granules at three

sachets/day, compared with the dietary treatment.[4] As utilized in that study, the recommended dosage of BCAA granules is one sachet three times a day p.o. after meals; however, some patients may not take all three sachets in a day due to problems such as treatment adherence. We therefore conducted the present prospective cohort study to examine how differences in the actual intake of BCAA granules may influence the prognosis of patients with liver cirrhosis.

Assessment of clinical characteristics of the patients included in the present study indicated that these patients shared average clinical features of liver cirrhosis in Japanese patients such as accountable etiologies.[1] Logistic analysis revealed that none of these causes was an independent risk factor for patients' outcome. Indeed, the prognosis of patients with liver cirrhosis was determined by eight factors including treatment adherence, regardless of the cause of liver cirrhosis (Table 2).

We focused on the treatment adherence among the eight independent risk factors in the present study, because the clinical significance of the other seven factors has already been described.[19,20] For this concern, patients were divided into the good adherence group (those who reported to have taken "nearly all" prescribed doses) and the poor adherence group (those who reported to have taken "approximately half" or "less" doses), because such stratification was validated by treatment responses in plasma BCAA/tyrosine ratio. Actually, 85.3% of patients reported to have taken "nearly all" three sachets of BCAA granules/day as prescribed. This result was comparable to the 86% adherence in the patients of the LOTUS study.[4] In the present study, treatment adherence was monitored longer after the first 6 months continuously, and remained similar: 81.1% for 7–12 months, 80.6% for 13–18 months and 79.7% for 19–24 months. These data indicate that treatment adherence observed for the first 6-month period was kept over longer treatment periods and, therefore, suggest that it is reasonable to monitor the treatment adherence of the first 6-month period for the long-term prognosis.

Improvement of hypoalbuminemia was reported to depend on the prescribed daily BCAA doses (8, 12 or 16 g),[10] but the present study first showed that, at the fixed prescribed dose (three sachets or 12 g/day), serum albumin level rose sufficiently only when the patient had good adherence (Fig. 3). Thus, good treatment adherence resulted in an improved serum albumin level (Fig. 3), and, consequently brought about a higher event-free survival (Fig. 2), as a decreased serum albumin level was also an independent risk factor for the patients (Table 2).

As to possible clinical factors that affect patients' BCAA adherence, we detected male sex, younger age, distribution of etiologies of liver cirrhosis, lower incidence of previous hepatic cancer, higher platelet count and lower serum ALT activities in the poor adherence group (Table 3). Among these factors, only male sex was also a possible unfavorable outcome marker (Table 2), but other factors were rather favorable or had no significance (e.g. cause of liver cirrhosis) for patients' outcome (Table 2). Such observation suggests that particular caution should be paid for drug adherence in male cirrhotics.

The limitation of such studies on advanced liver cirrhosis is the possibility that earlier development of events shortly after the start of the study influenced treatment adherence. To address this concern, we additionally performed analysis after excluding the patients who developed any event within 6 months of the study, and the cumulative event-free survival rate was still significantly higher for the good adherence group than that for the poor adherence group (hazard ratio = 1.57, $P = 0.0043$), as was the case with the analysis on the whole analysis set.

In conclusion, higher treatment adherence for BCAA is considered to be associated with an improved serum albumin level, thereby leading to improved patient outcome. These results indicate the importance of patient instruction for the adequate use of BCAA granules.

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FORUM

肝炎をめぐる医療政策

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わが国には肝炎ウイルスキャリアが約350万人存在すると推定されており，その内訳はB型肝炎110～140万人，C型肝炎190～230万人である．まさに“ウイルス肝炎は国民病である”との認識が妥当であり，国の医療政策の原点となっている．とくに2010年1月に“肝炎対策基本法”が施行されたことにより，現行の肝炎総合対策に対してこれまで以上の俊敏さ，具体的実効性が求められている．

■肝炎対策基本法

さて，この法律の前文には「B型肝炎及びC型肝炎に係るウイルスへの感染については，国の責めに帰すべき事由によりもたらされ，又はその原因が解明されていなかったことによりもたらされたものがある．特定の血液凝固因子製剤にC型肝炎ウイルスが混入することによって不特定多数の者に感染被害を出した薬害肝炎事件では，感染被害者の方々に甚大な被害が生じ，その被害の拡大を防止し得なかったことについて国が責任を認め，集団予防接種の際の注射器の連続使用によってB型肝炎ウイルスの感染被害を出した予防接種禍事件では，最終の司法判断において国の責任が確定している(下線は著者による追加)」と明記されていることからわかるように，ウイルス肝炎蔓延の原因の一部にわが国固有の事案が存在する．誌幅の都合上，詳細は割愛するが，薬害肝炎事件は凝固因子製剤(フィブリノゲン，第Ⅸ因子)へのC型肝炎ウイルスの混入に起因し(推定患者数1万人以上)，一方，予防接種禍事件は，集団予防接種など(予防接種およびツベルクリン反応検査)の際の注射器の連続使用によってB型肝炎ウイルスの水平感染を招いたとされる事案(推定患者数40万人以上)である．前者では患者・国・製薬会社の3者間，後者では患者・国の2者間での和解が成立し，補償が進められているところである．

■都道府県肝疾患診療ネットワークの構築

国がこれまで行ってきたさまざまな肝炎対策のなかで，もっとも画期的な施策の一つが2002～2006年度の5年間全国で展開された節目検診，節目外検診である．B型肝炎ウイルス検診受診者はのべ8,704,587人で，うち100,983人(1.16%)が“陽性”と判定された．一方，C型肝炎ウイルス検診

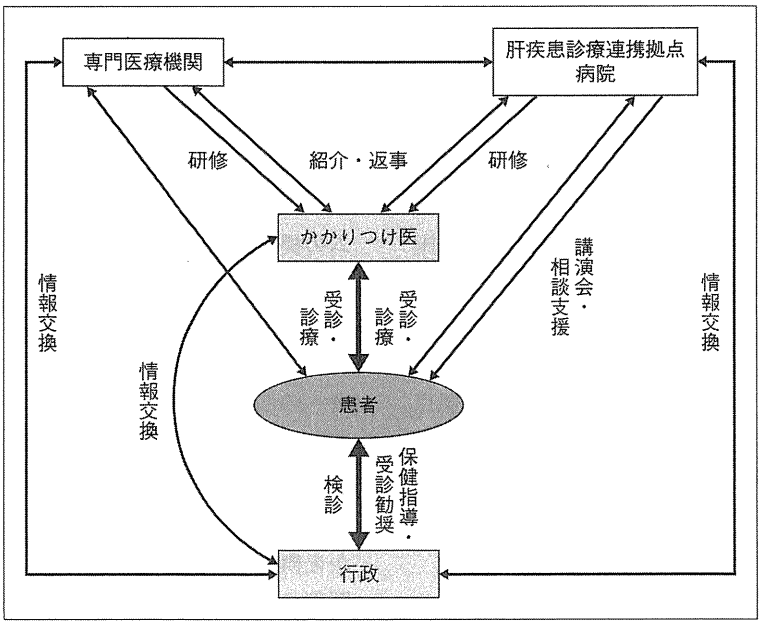

図1 都道府県における肝疾患診療ネットワーク構築(2007年1月厚生労働省)

表 1　肝疾患診療連携拠点病院，専門医療機関に必要とされる資格要件

肝疾患診療連携拠点病院
①肝疾患診療にかかわる一般的な医療情報の提供
②都道府県内の専門医療機関等に関する情報の収集や紹介
③医療従事者や地域住民を対象とした研修会や講演会の開催や肝疾患に関する相談支援
④肝疾患に関する相談医療機関と協議の場の設定
専門医療機関
①専門的な知識をもつ医師による診断と治療方針の決定が可能
②インターフェロンなどの抗ウイルス療法が可能
③肝癌の高危険群の同定と早期診断が可能

受診者はのべ 8,634,509 人に達し，うち 99,950 人(1.16%)が“現在，C 型肝炎ウイルスに感染している可能性がきわめて高い”と判定された．しかし，その結果が検診受診者に通知されたにもかかわらず，二次精検を目的とした医療機関への受診率は3〜4割程度にとどまり，インターフェロン療法などの抗ウイルス療法を受けた患者数も当初の期待に遠く及ばなかったと推定された．さらに，全国津々浦々における肝疾患診療体制がかならずしも整備されていないという状況も指摘されていた．これを改善するために，国は 2007 年 1 月に“都道府県における肝炎検査後肝疾患診療体制に関するガイドライン”を発出し，各都道府県において“かかりつけ医と患者の最小単位”を支援する診療ネットワークを行政側，医療側含めて構築することとした(図 1)．この施策に基づいて，自治体ごとに原則 1 カ所の肝疾患診療連携拠点病院，二次医療圏ごとに肝疾患専門医療機関の指定が進められてきた．これらの施設指定に必要な資格要件を表 1 に示す．2011 年 4 月 1 日現在，肝疾患診療ネットワークの要である肝疾患診療連携拠点病院の指定がようやく 47 都道府県で完了し，全国で 70 病院となっている．その内訳をみると，国立大学法人が 34 病院，公立・私立大学が 24 病院，その他(国立病院機構，県立病院，一般病院など)が 12 病院となっている．なお，肝疾患患者数が多く広域に分布しているなどの理由で，複数の拠点病院を指定している自治体もある(国立国際医療研究センター肝炎情報センターホームページ：http://www.ncgm.go.jp/center/index.html 参照)．

■肝炎情報センターの果たすべき役割

さらに，都道府県単位の活動を支援するシステムとして，国立国際医療センター(現国立国際医療研究センター)に 2008 年 11 月，肝炎情報センターが設置された(千葉県市川市)．その果たすべき役割として 3 つのミッションがある[1]．

第 1 に“インターネットなどによる最新情報提供”であり，2008 年 12 月には肝疾患医療に関する診療ガイドライン，肝炎診療をめぐる国内外の情報などを“一般向け，医療従事者向け，および肝臓専門医向け”に発信するためのホームページを立ち上げた．第 2 に“拠点病院間での情報共有を支援する”ことで，肝疾患診療連携拠点病院で構成する連絡協議会を年に 2 回開催し，拠点病院事業における問題点の解決をめざした話し合いを行っている．第 3 に，肝疾患診療連携拠点病院などに勤務する医療従事者(医師，看護師，相談員，臨床検査技師ほか)を対象とした“研修会”の企画・立案・推進を行っている．とくに，拠点病院事業のひとつである肝疾患相談センターの運営にとって必要不可欠な相談員の育成は最重要課題として位置づけており，相談員が患者からのさまざまな問い合わせに対応できるように，医療資源の活用法に関する知識の習得，患者とのコミュニケーションスキルの向上をめざした研修プログラムの提供をはかっている．

■肝疾患患者に対する医療費助成事業

国と都道府県が共同で行う施策には，肝疾患患者を取り巻く医療環境の整備のほかに，肝疾患患者への治療促進を目的とした医療費助成事業がある．その実施主体は各都道府県であり，財源負担は国：地方＝1：1 である．肝炎治療に 1 カ月分(3 割負担)としてどれくらいの薬剤費が必要であるかを概算すると，B 型肝炎に対する核酸アナログ製剤の 1 日分薬価がラミブジン，アデホビル，エンテカビルそれぞれ，622.00 円，1,252.10 円，

1,032.30円であることから，ラミブジン耐性患者でラミブジン・ヘプセラを併用すると1,874.1×28×0.3=15,742円，エンテカビル単独で1,032.3×28×0.3=8,671円となる．一方，C型慢性肝炎の標準的治療であるペグインターフェロン・リバビリン併用療法については，ペグイントロン100 μg注29,550.0円，レベトール200 mgカプセル764.60円であることから，体重65 kgとして1カ月分(3割負担)で(29,550×4+764.6×4×28)×0.3=61,151円となる．ペガシス・コペガス併用療法の場合も，ほぼ同額である．さらに，2011年9月に保険承認されたテラプレビル(テラビック®)も1錠1,422.1円と非常に高額で，1日分(9錠)が1,422.1×9×0.3=3,840円のため，12週間3剤併用+12週間2剤の24週間治療で3割負担の場合，約68.9万円(1カ月分11.5万円)に達する．したがって，抗ウイルス療法を広く普及させるためには，医療費助成がきわめて有効と考えられる．このような観点から，国と都道府県は肝炎治療特別促進事業として，2008年度からはB型・C型ウイルス性肝炎に対するインターフェロン治療，2010年度からはB型肝炎に対する核酸アナログ製剤治療への医療費助成を開始している．自己負担限度額は所得に応じて当初は1万円・3万円・5万円であったが，その後，1万円・2万円とさらなる負担軽減がはかられている．テラプレビルについても，医療費助成の対象となることが2011年11月28日付でいち早く決定された．これとは別に，身体障害者認定による重度肝硬変患者への医療費助成が2010年度から開始されている．対象者は，肝硬変の重症度分類として繁用されているChild-Pugh分類の合計点数10点以上(グレードCに該当)が3カ月以上持続していることが前提で，加えて日常生活活動の制限などに関する項目数などに応じて，もっとも障害程度の重い1級から，もっとも軽症な4級までの4等級に分類されている．肝硬変の原因として肝炎ウイルスに起因するもの以外も含まれているが，とくにアルコールに起因するものについては，6カ月以上の禁酒の確認が厳しく求められている．さらに，肝移植とこれに伴う医療も自立支援医療の対象とされており，医療費の自己負担額軽減がはかられている．とくに，移植後に抗免疫療法を必要とする期間は，これを実施しないと肝機能が廃絶する危険性があるため，障害程度1級と認定される．

■おわりに

冒頭で述べたように，現行の肝炎総合対策は“肝炎対策基本法”という法律に基づいて進められている．第一章第一条において「国，地方公共団体，医療保険者，国民及び医師等の責務を明らかにし，……」と述べられているように，国民すべてに担うべき役割があることを認識すべきである．

文献

1) 正木尚彦：肝炎情報センターの役割．肝・胆・膵，**61**(5)：721-729，2010.

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ORIGINAL ARTICLE

Estimation of two real-time RT-PCR assays for quantitation of hepatitis C virus RNA during PEG-IFN plus ribavirin therapy by HCV genotypes and IL28B genotype

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Abstract Hepatitis C virus (HCV) RNA values measured with two real-time PCR methods (Cobas Ampliprep/Cobas TaqMan, CAP/CTM, and the Abbott real-time PCR test, ART) vary among patients with genotype 1. We investigated HCV RNA values measured by two real-time PCR assays during pegylated interferon plus ribavirin (PEG-IFN/RBV) therapy. We evaluated 185 cases of chronic hepatitis C patients, among which 97 patients received the PEG-IFN/RBV therapy. HCV RNA values of CAP/CTM for genotype 1 were significantly higher than those of ART ($p < 0.05$) The difference in HCV RNA values (CAP/CTM minus ART) of genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values in genotype 1 was 100 % (55/55), which was significantly higher than the 78.6 % (33/42) of genotype 2 ($p < 0.001$). There was no difference between TT and TG/GG genotype groups in terms of difference of HCV RNA values (CAP/CTM minus ART). After PEG-IFN/RBV therapy was administered, reduction of HCV measurements was observed from day 1 for both assays regardless of genotype. The HCV value of CAP/CTM during PEG-IFN/RBV therapy was consistently higher than the value of ART, although the difference in these two values gradually became smaller during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the sustained virological response (SVR) rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment.

Keywords Abbott real-time PCR test · Cobas Ampliprep/Cobas TaqMan · Hepatitis C virus · Genotype · PEG-IFN plus ribavirin therapy · Real-time RT-PCR assay

Introduction

Approximately 80 % of patients infected by hepatitis C virus (HCV) develop chronic hepatitis [1, 2]. Currently, there are more than 100 million HCV carriers worldwide. Chronic hepatitis C could gradually progress to cirrhosis and liver cancer. The first treatment option for chronic hepatitis C is the pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy [3, 4]. Several virological predictive factors for sustained virological response (SVR) of PEG-IFN/RBV combination therapy are HCV genotype, baseline viral loads, and early virological response [5–7]. The SVR rate of PEG-IFN/RBV therapy is approximately 50 % for genotype 1 and 80 % for genotype 2. HCV RNA monitoring early in PEG-IFN/RBV therapy is an important predictive factor for SVR for either genotype 1 or genotype 2 [8, 9]. Detection of HCV RNA during PEG-IFN/RBV therapy is important in determining the length of IFN treatment [10]. Currently, Cobas Ampliprep/Cobas TaqMan (CAP/CTM) and Abbott real-time PCR test (ART) are used for HCV RNA measurement. The HCV RNA value in genotype 1 measured by CAP/CTM assay was significantly higher than values by ART assay [11]. The HCV RNA

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value in genotype 1 was measured by two real-time polymerase chain reaction (PCR) methods in this study to investigate whether there is a significant difference in HCV RNA values during PEG-IFN/RBV therapy.

Materials and methods

Of patients with chronic hepatitis C who visited Shin-Kokura hospital from April 2009 to December 2010, 185 were enrolled in this study. Of these 185 patients, 92 subjects were male and 93 were female, 96 subjects were 60 years old or older, and 89 were younger than 60 years old. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Of the 185 subjects in the study, 97 patients received the PEG-IFNa-2b plus ribavirin combination therapy: 55 patients had genotype 1, and 42 patients had genotype 2. PEG-IFNa-2b (PEG-Intron; MSD, Tokyo, Japan) was injected subcutaneously at a median dose 1.5 μg/kg (range, 1.3–1.5 μg/kg) once a week. Ribavirin (Rebetol; MSD, Tokyo, Japan) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1,000 mg). Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. The SVR rate was evaluated separately in patients with genotype 1 and genotype 2. Fifty-two of 55 cases of genotype 1 that received PEG-IFN/RBV were evaluated because the treatment was discontinued in 3 patients. Forty-one of 42 cases of genotype 2 were evaluated; treatment was discontinued in 1 patient.

Two real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays, CAP/CTM (Roche Molecular Systems, Pleasanton, CA, USA) and Abbott real-time HCV test (ART; Abbott Molecular, Abbott Park, IL, USA) were used for the quantitative measurement of HCV RNA concentrations before PEG-IFN/RBV treatment and at day 1, week 1, and week 2 during PEG-IFN/RBV treatment.

Abbott real-time HCV test

The Abbott real-time HCV test is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA (RT-PCR assay). The assay has adopted the second international WHO standard for HCV RNA (code 96/798) for calibration. HCV RNA concentration is expressed in IU/ml. The ART assay has a lower limit of detection (LOD) of 12 IU/ml with a linear quantitation range of 12×10^7 IU/ml.

Cobas Ampliprep/Cobas TaqMan assay

The Cobas Ampliprep/Cobas TaqMan assay is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA from 850 ml serum. CAP/CTM is standardized against the first WHO international standard for HCV RNA (code 96/798). HCV RNA concentration is reported in IU/ml. CAP/CTM assay has an LOD of 15 IU/ml with a linear quantitation range of $43–6.9 \times 10^7$ IU/ml.

We genotyped 115 patients for a single nucleotide polymorphism (SNP): rs8099917, an IL28B SNP previously reported to be associated with PEG-IFN/RBV therapy outcome. Samples were genotyped using the Illumina Human Hap 610-Quad Genotyping Bead Chip, with the Invader, or TaqMan assay, as described elsewhere [11–13].

Data analysis

Statistical analysis was performed using PASW Statistics, version 18 (SPSS) and R, version 2.11. Categorical data were analyzed using the chi-squared test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney U test. p values (two-tailed) <0.05 were considered statistically significant. Correlation coefficient (R) was assessed by the Spearman's correlation coefficient implemented in STATA software version 8.0 (Stata-Corp. LP, College Station, TX, USA).

Results

Figure 1 shows the correlation between the HCV RNA measurements obtained by the two real-time PCR assays: CAP/CTM versus ART in the study variables. A strong correlation was noted between the two real-time RT-PCR assays with an overall coefficient of correlation (R^2) of 0.8975 ($p < 0.0001$).

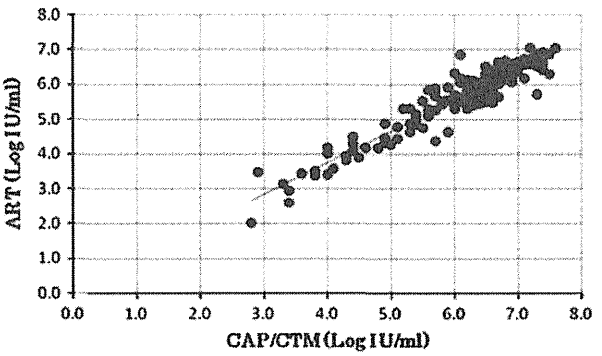

Fig. 1 Correlation between hepatitis C virus (HCV) RNA measurements obtained by two real-time RT-PCR assays: Cobas Ampliprep/Cobas TaqMan (*CAP/CTM*) versus Abbott real-time HCV test (*ART*)

Table 1 Correlation between hepatitis C (HCV) RNA measurements obtained by two real-time RT-PCR assays: CAP/CTM versus ART for study variables ($y = 0.9064x + 0.1176$, $R^2 = 0.8975$, $p < 0.0001$)

Study variables	n	CAP/CTM (log IU/ml)		ART (log IU/ml)		Correlation coefficient	
		Mean	SD	Mean	SD	R^2	p value
Gender							
Male	92	6.18	1.06	5.71	0.97	0.9009	<0.0001
Female	93	6.18	0.92	5.72	0.92	0.8972	<0.0001
Age (years)							
≥60	96	6.04	1.03	5.56	1.00	0.9142	<0.0001
<60	89	6.33	0.92	5.89	0.89	0.8709	<0.0001
Platelet counts (10^9/l)							
≥150	126	6.24	0.96	5.78	0.94	0.9106	<0.0001
<150	59	6.04	1.03	5.58	0.96	0.8705	<0.0001
IL28B							
TT	84	6.06	1.06	5.67	1.06	0.8826	<0.0001
TG/GG	31	5.91	1.18	5.45	1.07	0.9461	<0.0001
Genotype							
1	55	6.06	1.09	5.52	1.09	0.9647	<0.0001
2	42	5.94	1.21	5.65	1.15	0.9233	<0.0001

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real-time HCV test, *IL28B* interleukin 28B

Table 1 shows the correlation between the HCV RNA measurements obtained by the two real-time RT-PCR assays, CAP/CTM versus ART, in the study variables. All the coefficient of correlation (R^2) values based on the variables, such as gender, age (≥60 or >60 years), and number of platelets (≤150 × 10^9/l or >150 × 10^9/l), were more than 0.8700 ($p < 0.0001$) and were strongly correlated with the HCV RNA values obtained by the two real-time RT-PCR assays. The coefficients of correlation (R^2) for IL28B genotype (TT, TG/GG) were 0.8826 ($p < 0.000$) and 0.9461 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time RT-PCR assays. Similarly, the coefficients of correlation (R^2) for the HCV genotypes (genotypes 1, 2) were 0.9647 ($p < 0.0001$) and 0.9233 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time PCR assays.

Table 2 shows HCV RNA concentrations of study variables as measured by the two real-time RT-PCR assays. HCV RNA values measured by CAP/CTM were significantly higher than those by ART for all variables, such as gender, age (≥60 or >60 years), and the number of platelets (≥150 × 10^9/l or >150 × 10^9/l) ($p < 0.05$). The HCV RNA values of the IL28B group with TT genotype measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, no difference was observed for the TG/GG genotypes. The difference of HCV RNA values (CAP/CTM minus ART) between the TT genotype and the TG/GG genotypes was not statistically significant (Fig. 2). The positive rates of the difference of HCV RNA values in the TT genotype and the TG/GG genotypes (CAP/CTM minus ART) were 90.5 and 90.3 %, respectively, and were not statistically significant. The difference of HCV RNA values in the TG/GG genotypes were not statistically significant, which can be explained by the small number of subjects enrolled in this study. The HCV RNA values in genotype 1 measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, the difference was not statistically significant in genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) was significantly higher in genotype 1 than in genotype 2 ($p < 0.0001$) (Fig. 3). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 % (55/55), significantly higher ($p < 0.001$) compared to the positive rate of 78.6 % (33/42) in genotype 2.

Table 3 shows HCV RNA concentrations of HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by the two real-time PCR assays. The HCV RNA values decreased during PEG-IFN/RBV therapy for both genotype 1 and genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group decreased gradually. The difference was not statistically significant at day 1 of treatment. The HCV RNA values of CAT/CTM in the genotype 1 group were higher than those of ART at day 1, week 1, and week 2; however, the difference was not statistically significant.

Table 4 shows the SVR rate in patients who received PEG-IFN/RBV therapy by differences of HCV RNA values between CAT/CTM and ART before PEG-IFN/RBV

Table 2 HCV RNA concentrations for study variables as measured by two real-time RT-PCR assays: CAP/CTM and ART

Study variables	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (CAP/CTM−ART)		Quantitation difference		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Gender										
Male	92	6.18	1.06	5.71	0.97	5.95	1.00	0.47	0.33	0.0019
Female	93	6.18	0.92	5.72	0.92	5.95	0.91	0.45	0.30	0.0010
Age (years)										
≥60	96	6.04	1.03	5.56	1.00	5.80	1.00	0.48	0.30	0.0010
<60	89	6.33	0.92	5.89	0.89	6.11	0.87	0.44	0.33	0.0016
Platelet counts (10^9/l)										
≥150	126	6.24	0.96	5.78	0.94	6.01	0.94	0.46	0.29	0.0002
<150	59	6.04	1.03	5.58	0.96	5.81	0.98	0.47	0.37	0.0123
IL28B										
TT	84	6.06	1.06	5.67	1.06	5.86	1.04	0.40	0.34	0.0131
TG/GG	31	5.91	1.18	5.45	1.07	5.68	1.12	0.45	0.29	0.1199
Genotype										
1	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21†	0.0161
2	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34†	0.2734

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test, *IL28B* interleukin 28B

* CAP/CTM versus ART

† $p < 0.0001$, genotype 1 versus genotype 2

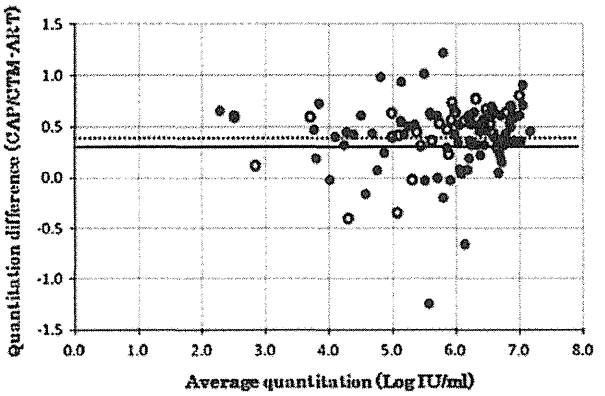

Fig. 2 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with interleukin 28B (IL28B) genotypes; TT and TG/GG, before pegylated interferon plus ribavirin (PEG-IFN/RBV) treatment [*closed circles* genotype TT, *open circles* genotype TG/GG, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TT, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TG/GG]

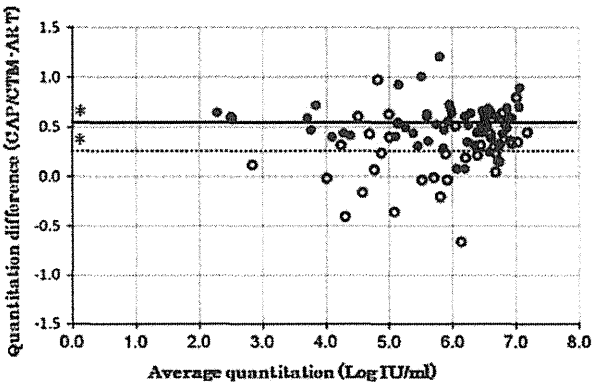

Fig. 3 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with HCV subtypes 1 and 2 before PEG-IFN/RBV treatment [*closed circles* genotype 1, *open circles* genotype 2, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 1, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 2]. *$p < 0.0001$, genotype 1 versus genotype 2

therapy. Group L comprises patients with a difference of HCV RNA values of 0.5 IU/ml or more (CAP/CTM minus ART), and group S comprises patients with a difference of HCV RNA values of less than 0.5 IU/ml (CAP/CTM minus ART). The SVR rate of genotype 1 (55.8 %) was significantly higher than that of genotype 2 (78.0 %, $p = 0.015$). For genotype 1, the SVR rate of IL28B genotype TT was significantly higher ($p = 0.016$) than that of genotype TG or GG. The SVR rates in group L and group S were not significantly different for IL28B genotypes TT, TG, or GG. The SVR rate of genotype TT was significantly higher than that of genotype TG or GG. The SVR rates in groups L and S were evaluated for the CAP/CTM HCV RNA values and ART before therapy, but no significant difference was

Table 3 HCV RNA concentrations for HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by two real-time RT-PCR assays: CAP/CTM and ART

Genotype	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (log IU/ml)		Quantitation difference (CAP/CTM−ART)		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Genotype 1										
Before treatment	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21	0.0161
Day 1	53	4.64	1.11	4.23	1.12	4.44	1.12	0.41	0.15	0.0662
Week 1	55	3.79	1.93	3.41	1.74	3.60	1.83	0.38	0.38	0.2739
Week 2	55	3.06	2.05	2.80	1.74	2.93	1.88	0.26	0.49	0.4966
Genotype 2										
Before treatment	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34	0.2734
Day 1	39	4.55	1.19	4.49	1.08	4.52	1.13	0.06	0.27	0.8618
Week 1	41	3.39	1.51	3.21	1.48	3.30	1.47	0.18	0.45	0.6946
Week 2	42	1.98	1.30	1.89	1.06	1.96	1.14	0.09	0.29	0.8014

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test

* CAP/CTM versus ART

observed. The SVR rate of patients with less than 6.0 log IU/ml of the HCV RNA values measured by both CAP/CTM and assay was higher than that with 6.0 log IU/ml or more, but this difference was not significant. For genotype 2, the SVR rate of IL28B genotype TT was higher than that of genotype TG or GG, but this difference also was not significant. There was no difference in the SVR rates between less than 6.0 and 6.0 log IU/ml or more. The SVR rates in group L and group S were not significantly different. The SVR rate of patients with genotype 2 was high regardless of HCV RNA values and IL28B genotype.

Discussion

The study revealed that the HCV RNA values measured by CAP/CTM were higher than those by ART among the subjects with genotype 1; however, no difference was observed among the patients with genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 %, which was significantly higher than the positive rate of 78.6 % in the genotype 2 group ($p < 0.001$). The differences of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group were all positive (>0), and all the viral load measurements obtained from CAP/CTM were higher than those from ART (Fig. 3). Ohnishi et al. [11] reported that the difference of the WHO standard versions used for each assay calibration might be the reason for the findings. CAP/CTM adopted the First International HCV RNA WHO standard for the assay calibration, while ART adopted the Second International HCV RNA WHO standard. Direct comparison of the two assays for measuring the WHO standard revealed a consistently higher quantitation of the WHO standard by CAP/CTM than by ART. The HCV RNA values of CAT/CTM in genotype 2 were reported to be lower those of ART [14]. Some cases in this evaluation also had lower CAP/CTM HCV RNA values (Fig. 3). No consistency was observed in genotype 2; some CAT/CTM HCV RNA values were higher than ART and some were lower. Base substitution is thought to contribute to this inconsistency [14]; this could have resulted from the differences between the two PCR methods. Also, this is consistent with a previous study in which CAP/CTM values were relatively higher for genotype 1 and lower for genotype 2 [2].

The difference of the HCV RNA values between CAP/CTM and ART was investigated in this study based on viral kinetics from the early stage of PEG-IFN/RBV treatment. After administration of PEG-IFN/RBV treatment, reduction of HCV RNA measurements obtained from both the CAP/CTM assay and the ART assay was observed from day 1 regardless of genotype (1 or 2). The HCV RNA values of CAP/CTM were consistently higher than those of ART during PEG-IFN/RBV therapy. The difference between these two values eventually became smaller because of the effect of PEG-IFN/RBV therapy, and a significant difference was no longer observed.

The IL28B genotype is one of the predictors of PEG-IFN/RBV therapy outcome before administration of treatment [15, 16]. In this study, for the genotype 1 patients, the SVR rate of IL28B genotype TT was significantly higher than the SVR rates of genotype TG or GG.

Table 4 Sustained virological response (SVR) rate in patients who received PEG-IFN/RBV therapy by difference between CAT/CTM HCV value and ART value before PEG-IFN/RBV therapy

	Group S SVR/*n* (%)	Group L SVR/*n* (%)	*p* value*	Total SVR/*n* (%)
Genotype 1				
IL28B				
TT	15/19 (79)	10/19 (53)	0.087	25/38 (66)
TG or GG	1/5 (20)	2/9 (22)	0.481	3/14 (21)
p value	0.012	0.128		0.004
CAP/CTM				
<6.0 log IU/ml	6/8 (75)	5/7 (71)	0.875	11/15 (73)
≥6.0 log IU/ml	10/16 (63)	7/21 (33)	0.077	17/37 (46)
p value	0.540	0.077		0.072
ART				
<6.0 log IU/ml	8/10 (80)	8/14 (57)	0.241	16/24 (67)
≥6.0 log IU/ml	8/14 (57)	4/14 (29)	0.126	12/28 (43)
p value	0.241	0.126		0.085
Total	16/24 (67)	12/28 (43)	0.086	28/52 (54)†
Genotype 2				
IL28B				
TT	19/22 (86)	4/6 (67)	0.264	23/28 (82)
TG or GG	5/7 (71)	4/6 (67)	0.852	9/13 (69)
p value	0.362	1.000		0.112
CAP/CTM				
<6.0 log IU/ml	11/15 (73)	3/4 (75)	0.946	14/19 (74)
≥6.0 log IU/ml	12/14 (86)	6/8 (75)	0.531	18/22 (82)
p value	0.411	1.000		0.530
ART				
<6.0 log IU/ml	14/15 (93)	4/5 (80)	0.717	18/20 (90)
≥6.0 log IU/ml	10/14 (71)	4/7 (57)	0.305	14/21 (67)
p value	0.564	0.407		0.293
Total	24/29 (83)	8/12 (67)	0.257	32/41 (78)†

Group L, ≥0.5 log IU/ml (CAP/CTM−ART); group S, <0.5 log IU/ml (CAP/CTM−ART)

* Group S versus group L

† $p = 0.015$, genotype 1 versus genotype 2

The HCV RNA values obtained from the two real-time PCR assays were analyzed based on the IL28B genotypes in this study. The HCV RNA values in the TT genotype group measured by CAP/CTM were significantly higher than those by ART; however, there was no significant difference in the TG or GG genotype groups. IL28B genotypes TT, TG, or GG were evaluated by differences of HCV RNA values between the CAP/CTM and ART: only the genotype TT group had a higher SVR rate. No SVR rate difference depending on the difference of HCV RNA values between CAP/CTM and ART was observed for genotypes 1 and 2. Clinically, a higher SVR rate was observed in the genotype TT group. It is assumed that the HCV RNA values of CAP/CTM were significantly higher than those of ART in genotype 1 patients because 73 % were in the genotype TT group. Therefore, there is assumed to be no correlation between IL28B and the difference of HCV RNA values between CAP/CTM and ART.

The data were also analyzed based on gender, age, and the number of platelets. For all variables, HCV RNA values as measured by CAP/CTM were significantly higher than those by ART; however, there was no difference in the HCV RNA values measured by CAP/CTM and ART when the measurements were compared against each variable.

The difference in HCV RNA measurements is suggested to be the result of HCV genotype. The prevalence of genotype 1 is higher in Japanese; therefore, the difference was observed in the measurements obtained from both assays.

The details of primer design and the PCR protocol for the products of both manufacturers used for this evaluation

are not disclosed. The PCR protocol of CAP/CTM method has two steps whereas the ART method has a three-step protocol. For the CAP/CTM method, elongation and probe hybridization are conducted simultaneously in the low-temperature step, and the temperature is generally 50–60 °C . In the ART method, a single-stranded linear probe is used instead of a TaqMan Probe and it has three steps, although it is also a real-time PCR method. Also, probe hybridization takes place at a lower temperature than for the CAP/CTM method, which is thought to optimize the tolerance level for HCV detection.

The newly developed ART features nucleic acid extraction using m2000 system, automated real-time PCR analysis, and high processing capacity. The assay results correlate well with the CAP/CTM assay, which suggests the wide application of the platform in clinical settings in the future. Additionally, the sample volume is 0.5 or 0.2 ml, which is highly practical for pediatric patients or when only a limited amount of patient sample is available. Also, some research has suggested that the genotype reactivity of ART is superior [17, 18].

In this study, the SVR rate was higher in genotype 2 than in genotype 1. For genotype 1, the SVR rate in IL28B genotype TT was higher than that in genotype TG or GG. For genotype 2, there was no difference of SVR rate between genotype TT and genotype TG or GG. These results were similar to the results of a previous study.

In summary, the HCV RNA values in genotype 1 obtained from the CAP/CTM assay were significantly higher compared to the values obtained from ART; however, no difference was observed in genotype 2. The HCV RNA values decreased during PEG-IFN/RBV therapy regardless of genotype. The HCV RNA value for CAP/CTM during PEG-IFN/RBV therapy was consistently higher than that for ART. However, the difference in these two values gradually became less during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the SVR rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment. Both CAP/CTM assay and ART assay were useful for PEG-IFN/RBV therapy. In this study, it was not clear which of the two HCV RNA assays was useful regarding the effects of IFN therapy. More detailed study is necessary.

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Original Article

Factors responsible for the discrepancy between *IL28B* polymorphism prediction and the viral response to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients

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Aim: *IL28B* polymorphisms serve to predict response to pegylated interferon plus ribavirin therapy (PEG IFN/RBV) in Japanese patients with chronic hepatitis C (CHC) very reliably. However, the prediction by the *IL28B* polymorphism contradicted the virological response to PEG IFN/RBV in some patients. Here, we aimed to investigate the factors responsible for the discrepancy between the *IL28B* polymorphism prediction and virological responses.

Methods: CHC patients with genotype 1b and high viral load were enrolled in this study. In a case–control study, clinical and virological factors were analyzed for 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype who were matched according to sex, age, hemoglobin level and platelet count.

Results: Higher low-density lipoprotein (LDL) cholesterol, lower γ-glutamyltransferase and the percentage of wild-type phenotype at amino acids 70 and 91 were significantly associated with the rs8099917 TT genotype. Multivariate analysis showed that rs8099917 TG or GG genotype, older age and lower LDL cholesterol were independently associated with the non-virological responder (NVR) phenotype. In patients with rs8099917 TT genotype (predicted as virological responder [VR]), multivariate analysis showed that older age was independently associated with NVR. In patients with rs8099917 TG or GG genotype (predicted as NVR), multivariate analysis showed that younger age was independently associated with VR.

Conclusion: Patient age gave rise to the discrepancy between the prediction by *IL28B* polymorphism and the virological responses, suggesting that patients should be treated at a younger age.

Key words: aging, genotype, IL28B, low-density lipoprotein cholesterol, single nucleotide polymorphism

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a global health problem with worldwide estimates of 120–130 million carriers.[1] Chronic HCV infection, the leading cause of liver transplantation, can lead to progressive liver disease, resulting in cirrhosis and complications, including decompensated liver disease and hepatocellular carcinoma.[2] The current standard-of-care treatment for suitable patients with chronic HCV infection consists of pegylated interferon-α-2a or -2b (PEG IFN) given by injection in combination with oral ribavirin (RBV) for 24 or 48 weeks, depending on HCV genotype. Large-scale treatment in the USA and Europe showed that 42–52% of patients with HCV genotype 1

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achieved a sustained virological response (SVR),[3–5] and studies conducted in Japan produced similar results. This treatment is associated with well-known side-effects (e.g. influenza-like syndrome, hematological abnormalities and neuropsychiatric events) resulting in reduced compliance and fewer patients completing treatment.[6] It is important to predict an individual's response before treatment with PEG IFN/RBV to avoid side-effects, as well as to reduce the treatment cost. The HCV genotype, in particular, is used to predict the response: patients with the HCV genotype 2/3 have a relatively high rate of SVR (70–80%) with 24 weeks of treatment, whereas those infected with genotype 1 have a much lower rate of SVR, despite 48 weeks of treatment.[5]

Our recent genome-wide association studies (GWAS) revealed that several highly correlated common single nucleotide polymorphisms (SNP) in the region of the interleukin-28B (*IL28B*) gene on chromosome 19, coding for interferon (IFN)-λ3, are implicated in the non-virological responder (NVR) to PEG IFN/RBV phenotype among patients infected by HCV genotype 1.[7] The association between response to PEG IFN/RBV and SNP associated with *IL28B* was concurrently reported by two other groups who also employed GWAS.[8,9] The *IL28B* polymorphism was highly predictive of the response to PEG IFN/RBV therapy in Japanese chronic hepatitis C (CHC) patients.[10–12] However, this was not always the case. Therefore, we attempted to determine why the *IL28B* polymorphism did not predict the response of all patients. The nature of the functional link between the *IL28B* polymorphism and HCV clearance is unknown, and this must be defined to understand how the *IL28B* polymorphism correlates with HCV clearance. Therefore, we also investigated the association between the *IL28B* polymorphism and clinical characteristics of CHC patients.

METHODS

Patients

A TOTAL OF 696 CHC patients with genotype 1b and high viral load were recruited from the National Center for Global Health and Medicine, Hokkaido University Hospital, Tokyo Medical and Dental University Hospital, Yamanashi University Hospital, Tonami General Hospital, and Shin-Kokura Hospital in Japan. In a case–control study, sex, age, hemoglobin level and platelet count were matched between patients with the rs8099917 TT genotype ($n = 130$) and patients with rs8099917 TG or GG genotypes ($n = 96$) to eliminate background biases.

Each patient was treated with PEG IFN-α-2b (1.5 μg/kg s.c. weekly) or PEG IFN-α-2a (180 μg/body s.c. weekly) plus RBV (600–1000 mg daily, depending on bodyweight). Because a reduction in the dose of PEG IFN/RBV can contribute to a lower SVR rate,[13] only patients with an adherence of more than 80% dose for both drugs during the first 12 weeks were included in this study. Those positive for hepatitis B surface antigen and/or anti-HIV were excluded from this study.

Non-virological response was defined as less than a 2 log-unit decline in the serum level of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. Virological response (VR) was defined as attaining SVR or transient virological response (TVR) in this study; SVR was defined as undetectable HCV RNA in serum 6 months after treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after the treatment was discontinued for a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. At the time of enrollment, written informed consent was obtained for the collection and storage of serum and peripheral blood. This study was conducted in accordance with provisions of the Declaration of Helsinki.

Clinical and laboratory data

The sex, age, hemoglobin (Hb) and platelet counts were matched between study groups. Other parameters determined were as follows: alkaline phosphatase (ALP), alanine transaminase (ALT), total cholesterol, fasting blood sugar (FBS), low-density lipoprotein (LDL) cholesterol, γ-glutamyl transpeptidase (γ-GTP), α-fetoprotein (AFP), HCV RNA level and the rs8099917 polymorphism near *IL28B*.

DNA extraction

Genomic DNA was extracted from the buffy coat fraction of patients' whole blood using a GENOMIX kit (Talent SRL; Trieste, Italy).

IL28B genotyping

We have reported that the rs8099917 polymorphism is the best predictor for the response of Japanese CHC patients to PEG IFN/RBV therapy than other SNP near *IL28B*.[14] Therefore, the rs8099917 polymorphism was genotyped using the InvaderPlus assay (Third Wave Japan, Tokyo, Japan), which combines polymerase