also by the alleviation of fibrosis stage both in chronic hepatitis B and in chronic hepatitis C.

Ogawa *et al.* studied 145 HCV infected patients treated with PEG-IFN plus ribavirin by TE[34]. LS were significantly decreased in SVR patients (the mean rate of change; −16.2%, −32.2% and −43.5%) in comparison with non-SVR patients (−7.2%, −2.1% and +17.3%) at the end of treatment (EOT) ($P = 0.0127$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT. Among non-SVR patients, LS were significantly decreased in patients with biochemical response (BR) (−17.9%, −30.0% and −27.1%) in comparison with non-BR (−4.1%, +6.4% and +30.6%) at EOT ($P = 0.0270$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT.

Arima *et al.* measured LS by TE before treatment, at EOT, one year and 2 years after EOT in 145 patients with chronic hepatitis C treated by IFNs with or without ribavirin.[35] In 93 patients with SVR and 28 relapsers, LS significantly decreased at EOT (median, 5.4 [interquartile range, 4.0–8.6] kPa, $P < 0.0001$ and 6.8 [4.5–8.9] kPa, $P = 0.0023$) and one year after EOT (5.3 [4.2–7.0] kPa, $P < 0.0001$ and 6.8 [4.5–9.3] kPa, $P = 0.0204$) compared with baseline (8.0 [5.0–11.9] kPa and 10.6 [7.0–16.6] kPa). In SVR patients, LS significantly decreased 2 years after EOT (5.3 [4.1–6.3] kPa) compared with baseline ($P < 0.0001$) and LS at EOT ($P = 0.0034$). In 24 patients with non virological response (NVR), LS at EOT, one year after EOT, and 2 years after EOT did not significantly differ from pretreatment values.

Arima *et al.* proposed the use of deduced fibrosis stage from LS based on cut-off values for fibrosis stage. The use of deduced fibrosis stage enables evaluation of the degrees of changes of LS. 2-point or greater reduction of deduced stage was observed in 78% (29/37) of SVR patients, 59% (10/17) of relapsers and 15% (2/13) of NVR patients. A 2-point or greater decrease of deduced fibrosis stage were associated with milder baseline fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher ALT levels.

Thus, we can assess not only the alleviation of fibrosis but also the factors that affect the alleviation of fibrosis by measuring LS in chronic hepatitis C.

Wang *et al.* studied LS by TE in 144 patients receiving IFN-based therapy, including 95 SVR patients and 49 non-SVR patients.[36] There was a significant decrease of LS among SVR patients (median, 0.6; $P < 0.001$). non-SVR patients showed an increase of LS (median, 0.8; $P = 0.557$). For SVR patients, a high initial LS was the predictive factor of a rapid reduction of LS values. However, advanced fibrosis stage before therapy, higher body mass index (BMI) and longer time remission were predictive factors for slow reduction of LS values.

Osakabe *et al.* measured LS by TE in 29 HBV-infected patients treated with nucleotide or nucleoside analogs and assessed the changes of LS.[37] By antiviral therapy, LS significantly reduced from 12.9 (6.2–17.9) kPa to 6.6 (4.4–10.3) kPa in the interval of 512 (366–728) days ($P < 0.0001$). Eleven of 19 (58%) patients with baseline fibrosis stages of F3-4 deduced from LS had 2-point or greater reduction of deduced stage at last LS measurement. The change ratio of hyaluronic acid ($P = 0.0390$) was associated with a 2-point or greater reduction.

Enomoto *et al.* studied LS by TE in 50 patients with chronic hepatitis B virus infection.[38] LS of the patients with entecavir significantly decreased from 11.2 kPa (7.0–15.2) to 7.8 kPa (5.1–11.9; $P = 0.0090$) during 12 months of treatment.

It is difficult to repeat liver biopsies after or during antiviral therapy to assess its effect. Since there is the heterogeneity of the effect of treatment, it is important to know who is a good responder or not and investigate the factors affecting the effect of therapy. Non-invasive measurement of LS can be done repeatedly and provide the information of effect of antiviral therapy.

The results of TE were not confirmed by the results of liver biopsies in the articles reviewed. The absence of comparison with biopsies is the limitations of these studies.

ASSESSMENT OF NATURAL COURSE OF VIRAL HEPATITIS

ARIMA *ET AL.* STUDIED 35 patients with chronic HCV infection without IFN treatment and reported that LS at 2nd measurement (12.2 [6.3–16.8] kPa) did not differ significantly from LS at 1st measurement (10.5 [5.8–15.3] kPa) in the interval of 656 (360–922) days.[35]

Osakabe *et al.* reported that, in 52 HBV-infected patients without antiviral therapy, LS tended to increase from 6.1 (3.9–8.5) kPa to 6.3 (4.4–9.7) kPa in the interval of 422 (358–709) days ($P = 0.0682$).[37] Without antiviral therapy, 11 of 50 (22%) patients with deduced fibrosis stages of F0-3 at 1st measurement had an increase of deduced stage, while 8 of 20 (40%) patients with deduced fibrosis stages of F2-4 at 1st measurement had a reduction of deduced stage. The factor associated with an increase of deduced fibrosis stage was lower baseline albumin levels ($P = 0.0092$).

The reason why the significant increase of LS was not detected in the natural course in these reports is

probably attributed to the fact that the subjects of the studies are the patients who had mild disease and needed no antiviral therapy. TE would be a useful tool to detect the patients with progressive fibrosis for the physicians in the follow-up of the patients with chronic viral hepatitis.

The results of TE were not confirmed by the results of liver biopsies in the articles reviewed. The absence of comparison with biopsies is the limitations of these studies.

ESTIMATION OF PROGNOSIS OF HEPATITIS

THE RISK OF hepatocellular carcinoma (HCC) or bleeding from esophageal varices is high in patients with advanced fibrosis.[39,40] Thus it is important to detect advanced fibrosis early and start the search for HCC and varices in order to treat them in early stage or before bleeding.

A meta-analysis of performance of TE for fibrosis staging demonstrated that the mean AUROC for cirrhosis was 0.94 (95% CI, 0.93–0.95) and an adjusted AUROC of 0.99 and that the optimal cut-off value for cirrhosis suggested from the summary ROC techniques was 13.01 kPa.[17]

Piscaglia *et al.* studied 90 patients with chronic liver disease with ARFI.[29] The AUROC for the diagnosis of cirrhosis was 0.941 with 1.75 m/s as the optimal cut-off (sensitivity 93.0%; specificity 85.1%).

Lupsor *et al.* studied 112 patients with chronic hepatitis C with ARFI.[23] The AUROC for the diagnosis of cirrhosis was 0.936 with 2 m/s as the optimal cut-off (sensitivity 80.0%; specificity 95.45%).

Sporea *et al.* studied 71 patients with chronic liver diseases with ARFI.[24] The AUROC for the diagnosis of cirrhosis was 0.868 with 1.8 m/s as the optimal cut-off (sensitivity 100%; specificity 77%).

Toshima *et al.* studied 79 patients with chronic liver diseases with ARFI.[25] The AUROC for the diagnosis of cirrhosis was 0.87 with 1.79 m/s as the optimal cut-off (sensitivity 86%; specificity 79%).

Ebinuma *et al.* studied 59 patients with chronic viral hepatitis with ARFI.[26] The AUROC for the diagnosis of cirrhosis was 0.854 with 1.88 m/s as the optimal cut-off (likelihood ratio 4.55).

The summary of investigations of ARFI for assessment of cirrhosis is shown in Table 1.[10,21–29]

Friedrich-Rust *et al.* studied 79 patients with chronic viral hepatitis with real-time elastography.[11] The cut-off value of elastic ratio and AUROC for cirrhosis was 111.75 and 0.69, respectively (sensitivity 29.2%; specificity 90.7%).

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C[20]. The cut-off value of elastic ratio and AUROC for cirrhosis were 3.93 and 0.95, respectively (sensitivity 90.9%; specificity 91.5%).

Stefanescu et al. compared the performance of common serum fibrosis scores and TE in diagnosing esophageal varices in 231 cirrhosis patients.[41] The Lok Score[42] was the best among all the serum scores for diagnosing the varices; cut-off value for large varices is 0.8 (positive predictive value 45.5%, negative predictive value 86.4% and diagnostic accuracy 67.72%). The cut-off value of LS for large varices is 30.8 kPa (positive predictive value 47.3%, negative predictive value 81% and diagnostic accuracy 68.32%). Using both tests simultaneously, the presence of large varices was predicted with a diagnostic accuracy of 78.12%, obtaining an increment in negative predictive value and negative likelihood ratio up to 93.67% and 0.21, respectively.

Jung *et al.* investigated the usefulness of LS by TE as a predictor of HCC development in 1130 patients with chronic HBV infection.[43] During the follow-up period (median, 30.7 months; range, 24.0–50.9 months), HCC developed in 57 patients (2.0% per 1 person-year). The 1-, 2-, and 3-year cumulative incidence rates of HCC were 0.80%, 3.26%, and 5.98%, respectively. On multivariate analysis, together with old age, male sex, heavy alcohol consumption (>80 g/day), serum albumin, and hepatitis B e antigen positivity, patients with a higher LS (>8 kPa) were at a significantly greater risk of HCC development, with the following hazard ratios: 3.07 (95% confidence interval [CI], 1.01–9.31; $P = 0.047$) for LS 8.1–13 kPa; 4.68 (95% CI, 1.40–15.64; $P = 0.012$) for LS 13.1–18 kPa; 5.55 (95% CI, 1.53–20.04; $P = 0.009$) for LS 18.1–23 kPa; and 6.60 (95% CI, 1.83–23.84; $P = 0.004$) for LS > 23 kPa.

Masuzaki *et al.* investigated the relationship between LS and HCC presence in the cross-sectional study.[44] LS was measured in chronic hepatitis C patients (85 with HCC and 180 without) by TE. Multivariate analysis showed that HCC presence was significantly associated with LS ($P < 0.0001$) along with age, male, and α-fetoprotein concentration. AUROC was 0.805, 0.741, 0.714, 0.673, 0.670, and 0.654 for LS, α-fetoprotein, albumin, prothrombin activity, aspartate aminotransferase (AST)-platelet ratio index, and platelet count, respectively. Stratum-specific likelihood ratio for HCC presence by LS was 0.22 (95% CI: 0.11-0.42) in

<10 kPa, 0.73 (0.39 to 1.39) in 10.1 to 15 kPa, 1.30 (0.80 to 2.12) in 15.1 to 25 kPa, and 5.0 (2.96 to 8.47) in >25 kPa.

Masuzaki *et al.* investigated the relationship between baseline LS and HCC development prospectively among 866 patients with chronic hepatitis C.[45] During the follow-up period (mean, 3.0 years), HCC developed in 77 patients (2.9% per 1 person-year). The cumulative incidence rates of HCC at 1, 2, and 3 years were 2.4%, 6.0%, and 8.9%, respectively. Adjusting for other significant factors for HCC development, patients with higher LS were revealed to be at a significantly higher risk, with a hazard ratio, as compared to LS< or =10 kPa, of 16.7 (95% CI, 3.71–75.2; $P < 0.001$) when LS 10.1–15 kPa, 20.9 (95% CI, 4.43–98.8; $P < 0.001$) when LS 15.1–20 kPa, 25.6 (95% CI, 5.21–126.1; $P < 0.001$) when LS 20.1–25 kPa, and 45.5 (95% CI, 9.75–212.3; $P < 0.001$) when LS > 25 kPa.

Thus TE, real-time elastography and ARFI are useful for diagnosis of cirrhosis and prediction of development of varices or HCC.

CAN LIVER STIFFNESS REPLACE LIVER BIOPSY?

TRANSIENT ELASTOGRAPHY, ARFI and real-time elastography are the methods with very good or excellent diagnostic accuracy for the assessment of liver fibrosis stage. They do not provide information on inflammatory activity, steatosis, iron deposition or other findings in liver biopsy. Even on account of fibrosis stage, these non-invasive methods do not give us the estimation completely corresponding to that of liver biopsy. In addition, the values of LS might be affected by factors other than fibrosis stage, for example, inflammatory activity[9,18] and intrahepatic pressure.[46] However they provide us useful clinical information, which liver biopsy has been providing us as described in the present article, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. Recently non-invasive methods for assessment of inflammatory activity,[47] steatosis[48,49] and iron deposition[50] in the liver have been developed. Such as ActiTest,[47] SteatoTest,[49] and MR imaging for quantification of fat[48] and iron contents[50] in liver provide the information other than fibrosis derived from liver biopsy. Thus in the near future, non-invasive methods will replace liver biopsy.

REFERENCES

1 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986–92.
2 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
3 Murawaki Y, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; **36**: 399–406.
4 Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. *J Viral Hepat* 1997; **4**: 199–208.
5 Rosenberg WM, Voelker M, Thiel R *et al.* Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704–13.
6 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
7 Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297–306.
8 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705–13.
9 Nitta Y, Kawabe N, Hashimoto S *et al.* Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. *Hepatol Res* 2009; **39**: 675–84.
10 Friedrich-Rust M, Wunder K, Kriener S *et al.* Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; **252**: 595–604.
11 Friedrich-Rust M, Ong MF, Herrmann E *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* 2007; **188**: 758–64.
12 Yin M, Talwalkar JA, Glaser KJ *et al.* Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207–13. e2.
13 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449–57.
14 Regev A, Berho M, Jeffers LJ *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614–18.

15 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 8–13.
16 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 1–7.
17 Friedrich-Rust M, Ong MF, Martens S *et al.* Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960–74.
18 Yoshioka K, Kawabe N, Hashimoto S. Transient elastography: applications and limitations. *Hepatol Res* 2008; **38**: 1063–8.
19 Friedrich-Rust M, Schwarz A, Ong M *et al.* Real-time tissue elastography versus FibroScan for noninvasive assessment of liver fibrosis in chronic liver disease. *Ultraschall Med* 2009; **30**: 478–84.
20 Koizumi Y, Hirooka M, Kisaka Y *et al.* Liver fibrosis in patients with chronic hepatitis C: noninvasive diagnosis by means of real-time tissue elastography – establishment of the method for measurement. *Radiology* 2011; **258**: 610–17.
21 Takahashi H, Ono N, Eguchi Y *et al.* Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. *Liver Int* 2009; **30**: 538–45.
22 Fierbinteanu-Braticevici C, Andronescu D, Usvat R, Cretoiu D, Baicus C, Marinoschi G. Acoustic radiation force imaging sonoelastography for noninvasive staging of liver fibrosis. *World J Gastroenterol* 2009; **15**: 5525–32.
23 Lupsor M, Badea R, Stefanescu H *et al.* Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. *J Gastrointestin Liver Dis* 2009; **18**: 303–10.
24 Sporea I, Sirli R, Popescu A, Danila M. Acoustic Radiation Force Impulse (ARFI) – a new modality for the evaluation of liver fibrosis. *Med Ultrasound* 2010; **12**: 26–31.
25 Toshima T, Shirabe K, Takeishi K *et al.* New method for assessing liver fibrosis based on acoustic radiation force impulse: a special reference to the difference between right and left liver. *J Gastroenterol* 2011; **46**: 705–11.
26 Ebinuma H, Saito H, Komuta M *et al.* Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: comparison with Fibroscan((R)). *J Gastroenterol* 2011; **46**: 1238–48.
27 Sporea I, Sirli RL, Deleanu A *et al.* Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. *Ultraschall Med* 2011; **32** (Suppl 1): S46–52.
28 Grgurevic I, Cikara I, Horvat J *et al.* Noninvasive assessment of liver fibrosis with acoustic radiation force impulse imaging: increased liver and splenic stiffness in patients with liver fibrosis and cirrhosis. *Ultraschall Med* 2011; **32**: 160–6.
29 Piscaglia F, Salvatore V, Di Donato R *et al.* Accuracy of VirtualTouch Acoustic Radiation Force Impulse (ARFI) imaging for the diagnosis of cirrhosis during liver ultrasonography. *Ultraschall Med* 2011; **32**: 167–75.
30 Ichino N, Osakabe K, Nishikawa T *et al.* A new index for non-invasive assessment of liver fibrosis. *World J Gastroenterol* 2010; **16**: 4809–16.
31 Boursier J, Vergniol J, Sawadogo A *et al.* The combination of a blood test and Fibroscan improves the non-invasive diagnosis of liver fibrosis. *Liver Int* 2009; **29**: 1507–15.
32 Hayashi K, Katano Y, Honda T *et al.* Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. *Liver Int* 2011 **31**: 1359–65.
33 Poynard T, Munteanu M, Colombo M *et al.* FibroTest is an independent predictor of virologic response in chronic hepatitis C patients retreated with pegylated interferon alfa-2b and ribavirin in the EPIC(3) program. *J Hepatol* 2011; **54**: 227–35.
34 Ogawa E, Furusyo N, Toyoda K, Takeoka H, Maeda S, Hayashi J. The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin. *Antiviral Res* 2009; **83**: 127–34.
35 Arima Y, Kawabe N, Hashimoto S *et al.* Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C. *Hepatol Res* 2010; **40**: 383–92.
36 Wang JH, Changchien CS, Hung CH *et al.* Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: longitudinal study using FibroScan. *J Gastroenterol Hepatol* 2010; **25**: 964–9.
37 Osakabe K, Ichino N, Nishikawa T *et al.* Reduction of liver stiffness by antiviral therapy in chronic hepatitis B. *J Gastroenterol* 2011; (in press).
38 Enomoto M, Mori M, Ogawa T *et al.* Usefulness of transient elastography for assessment of liver fibrosis in chronic hepatitis B: regression of liver stiffness during entecavir therapy. *Hepatol Res* 2010; **40**: 853–61.
39 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; **28**: 930–8.
40 Zaman A, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liver disease. *Am J Gastroenterol* 1999; **94**: 3292–6.
41 Stefanescu H, Grigorescu M, Lupsor M *et al.* A new and simple algorithm for the noninvasive assessment of esophageal varices in cirrhotic patients using serum fibrosis markers and transient elastography. *J Gastrointestin Liver Dis* 2011; **20**: 57–64.

42 Lok AS, Ghany MG, Goodman ZD *et al.* Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; **42**: 282–92.
43 Jung KS, Kim SU, Ahn SH *et al.* Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011; **53**: 885–94.
44 Masuzaki R, Tateishi R, Yoshida H *et al.* Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. *J Clin Gastroenterol* 2008; **42**: 839–43.
45 Masuzaki R, Tateishi R, Yoshida H *et al.* Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954–61.
46 Harata M, Hashimoto S, Kawabe N *et al.* Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase. *Hepatol Res* 2011; **41**: 423–9.
47 Poynard T, Munteanu M, Ngo Y *et al.* ActiTest accuracy for the assessment of histological activity grades in patients with chronic hepatitis C, an overview using Obuchowski measure. *Gastroenterol Clin Biol* 2010; **34**: 388–96.
48 Hatta T, Fujinaga Y, Kadoya M *et al.* Accurate and simple method for quantification of hepatic fat content using magnetic resonance imaging: a prospective study in biopsy-proven nonalcoholic fatty liver disease. *J Gastroenterol* 2010; **45**: 1263–71.
49 Poynard T, Ratziu V, Naveau S *et al.* The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10.
50 Sirlin CB, Reeder SB. Magnetic resonance imaging quantification of liver iron. *Magn Reson Imaging Clin N Am* 2010; **18**: 359–81. ix.

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Prevalence of Hepatitis C Virus Genotype 1a in Japan and Correlation of Mutations in the NS5A Region and Single-Nucleotide Polymorphism of Interleukin-28B With the Response to Combination Therapy With Pegylated-Interferon-Alpha 2b and Ribavirin

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Hepatitis C virus (HCV) genotype 1a is rare in Japanese patients and the clinical characteristics of this genotype remain unclear. The interferon (IFN) sensitivity-determining region (ISDR) and single-nucleotide polymorphisms (SNPs) of interleukin-28B (IL28B) among patients with HCV genotype 1b are associated with IFN response, but associations among patients with genotype 1a are largely unknown. This study investigated the clinical characteristics of genotype 1a and examined whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affects response to combination therapy with pegylated-IFN-α2b and ribavirin. Subjects comprised 977 patients infected with HCV genotype 1, including 574 men and 412 women (mean age, 55.2 ± 10.6 years). HCV was genotyped by direct sequencing of the 5′-untranslated region and/or core regions and confirmed by direct sequencing of the NS5A region. HCV genotypes 1a (n = 32) and 1b (n = 945) were detected. Twenty-three (71.9%) of the 32 patients with genotype 1a were patients with hemophilia who had received imported clotting factors. Prevalence of genotype 1a after excluding patients with hemophilia was thus 0.9%. Of the 23 patients with genotype 1a who completed IFN therapy, 11 (47.8%) were defined as achieving sustained virological response. Factors related to sustained virological response by univariate analysis were IL28B and ISDR. In conclusion, HCV genotype 1a is rare in Japan. The presence of IL28B genotype TT, and more than two mutations, in the ISDR are associated with a good response to IFN therapy in patients with HCV genotype 1a. ***J. Med. Virol. 84:438–444, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: hepatitis C virus; genotype 1a; NS5A; IL 28B; interferon

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma [Seeff, 2002]. HCV infection is a significant global health problem, affecting 170 million individuals worldwide. HCV can be divided into six genotypes and several subtypes according to genomic heterogeneity [Simmonds et al., 2005]. Each genotype shows a unique distribution and clinical characteristics such

All authors have nothing to disclose.

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as interferon (IFN) responsiveness [Ghany et al., 2009]. HCV genotypes 1b, 2a, and 2b are the major types encountered in Japan [Enomoto et al., 1990; Hayashi et al., 2003]. Genotype 1a is common worldwide, but is rare in Japan except among individuals with hemophilia who have received imported clotting factors [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. The prevalence and clinical characteristics, including IFN responsiveness, of Japanese patients with HCV genotype 1a are unclear. HCV NS5A protein reportedly includes a domain associated with IFN response. This domain, located in the NS5A region of HCV genotype 1b, is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996]. IFN acts to inhibit viral replication by inducing double-stranded RNA-dependent protein kinase (PKR). The ISDR is located at the 5′ end of the PKR-binding domain and is inhibited by PKR in vitro [Gale et al., 1998]. ISDR heterogeneity of genotype 1b is thus an important factor that may affect response to IFN [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. Several studies have reported a relationship between ISDR and IFN responsiveness among patients with HCV genotype 1a [Hofgärtner et al., 1997; Zeuzem et al., 1997; Kumthip et al., 2011; Yahoo et al., 2011]. However, this remains controversial for genotype 1a, and the utility of ISDR sequences for predicting IFN responsiveness has not been investigated for HCV genotype 1a in Japan due to the rarity of this genotype. Both genetic heterogeneity of the HCV genome and host genetics contribute to IFN responsiveness. Several genome-wide association studies have thus been performed to clarify host factors associated with IFN responsiveness, revealing that interleukin-28B (IL28B) polymorphisms are strongly associated with response to IFN therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009]. Combined use of the single-nucleotide polymorphisms (SNPs) of IL28B and amino acid substitutions in the core region and ISDR could thus improve the prediction of response to IFN in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. However, the effects of a combined evaluation of the SNPs of IL28B and amino acid substitutions in the ISDR in patients with HCV genotype 1a on IFN response are unclear. The aim of the present study was to determine whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affect response to combination therapy with pegylated-IFN-α2b and ribavirin.

PATIENTS AND METHODS

A total of 977 patients (569 men, 408 women) with chronic hepatitis C genotype 1 and high viral load (<100 KIU/ml) who were treated at Nagoya University Hospital and affiliated hospitals were enrolled in this study. Mean age of patients was 55.1 ± 12.2 years (range: 18–75 years). None of the patients had a history of chronic alcohol abuse, autoimmune disease, or metabolic disease. Patients with active intravenous drug use and immigrants were excluded from this study. The core region (aa 30–110) and ISDR (aa 2,209–2,248) of HCV were examined by direct sequencing. SNPs of IL28B (rs8099917) were identified using a real-time polymerase chain reaction (PCR) system. Patients received subcutaneous injections of pegylated-IFN-α2b (1.5 μg/kg) once each week along with oral ribavirin (600 mg/day for patients <60 kg, 800 mg/day for 60–80 kg, 1,000 mg/day for >80 kg) for 48 weeks. Patients who became negative for HCV-RNA between 16 and 36 weeks after initiating IFN treatment had the IFN treatment extended to 72 weeks, in accordance with Japanese guidelines [Kumada et al., 2010]. HCV-RNA levels in serum samples were examined at 12 weeks, at the end of IFN therapy, and at 6 months after the end of treatment. Serum was stored at −80°C for virological examination at pretreatment. Early virological response was defined as HCV-negative status at 12 weeks. Patients who were persistently negative for serum HCV-RNA at 24 weeks after withdrawal of IFN treatment were considered to show sustained virological response. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological Analysis

HCV-RNA quantitative viremia load was determined by PCR. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or core regions as described previously and confirmed by direct sequencing of the NS5A region [Otagiri et al., 2002; Dal Pero et al., 2007; Hayashi et al., 2011a]. Genotypes were classified according to the nomenclature proposed by Simmonds et al. [2005]. Direct sequencing of the core and NS5A-ISDR regions was performed as reported previously [Dal Pero et al., 2007; Hayashi et al., 2011a]. In brief, RNA was extracted from 140 μl of serum using a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligos and random hexamer primers with a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA). The HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50-μl PCR reaction mixture contained 100 nM of each primer, 1 ng of template cDNA, 5 μl of GeneAmp 10× PCR buffer, 2 μl of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primers for the core region were: sense, 5′-GGGAGGTCTCGTAGACCGTGCACCATG-3′ and antisense, 5′-GAGMGGKATRTACCCCATGAGRTCGGC-3′. Primers for the NS5A-ISDR were: sense, 5′-GCCTGGAGCCCTTGTAGTC-3′ and

TABLE I. Clinical Characteristic of Patients With HCV Genotype 1a

	N = 32
Age (y.o.)	36.4 ± 2.2
Sex: male/female	28/4
AST (IU/L)	48.8 ± 33.6
ALT (IU/L)	64.6 ± 57.8
Platelet (10^4/μl)	18.8 ± 6.0
HCV RNA level (KIU/ml)	2607.4 ± 3072.2
Source (clotting factor/BTF/unknown)	23/2/7

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

antisense, 5′-CTGCGTGAAGTGGTGGAATAC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was performed using the same reaction buffer with the first-round PCR product as template, and the following sets of primers: for the core region, sense primer 5′-AGACCGTGCACCATGAGCAC-3′ and antisense 5′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-TGTTTCCCCCACGCACTAC-3′ and antisense 5′-TGATGGGCAGTTTTTGTTCTTC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers using a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genotyping Analysis

Detection of SNPs for IL28B (rs8099917) was conducted using a real-time PCR system. In brief, genomic DNA was extracted from 150 μl of whole blood with a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μl of diethylpyrocarbonate-treated water. DNA (10 ng) was used for PCR and genotyping of IL28B SNP (rs8099917) was performed by TaqMan allelic discrimination (ABI-Prism 7300 SDS software; Applied Biosystems) with TaqMan SNP Genotyping Assays provided by Applied Biosystems (C__11710096_10).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The paired *t*-test was used to analyze differences in variables. A value of $P < 0.05$ was considered statistically significant. Statview 5.0 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Thirty-two of the 977 patients (3.3%) were infected by genotype 1a. Clinical characteristics of patients with genotype 1a are summarized in Table I. Twenty-three cases involved patients with hemophilia who had received imported clotting factors. The prevalence of genotype 1a after excluding patients with hemophilia was 0.9%. A comparison of clinical characteristics according to hemophilia status is shown in Table II. No significant differences were apparent among the two groups. Differences in clinical characteristics between genotypes 1a and 1b are shown in Table III. Males were more frequent among patients with genotype 1a (87.5%) than among those with genotype 1b (57.2%), as the majority of patients with genotype 1a were young male patients with hemophilia. Sequence alignments of the core region at codons 71 and 90 showed arginine and cysteine, respectively, in all patients. The HCV core region of genotype 1a was thus well-conserved, with no significant mutations at codons 71 or 90. This is not similar to previous findings for genotype 1b [Akuta et al., 2005, 2011; Hayashi et al., 2011a,b; Kurosaki et al., 2011]. Alignment of the amino acid sequence for NS5A-ISDR is shown in Figure 1. The sequence of the HCV-1 strain was defined as the consensus sequence of genotype 1a, and the number of mutations to the chosen consensus sequence in ISDR was used to analyze the ISDR system. Sequences of the HCV-1 strain and HCV-1 strain with only one amino acid substitution were defined as wild-type, while ISDR sequences with more than two amino acid substitutions were defined as mutant-type. Twenty-seven strains were defined as wild-type and 5 strains were defined as mutant-type. IL28B genotypes could be obtained for 25 patients, and IL28B alleles were TT (n = 14) and TG (n = 11). Twenty-three patients received pegylated-IFN-α2b plus ribavirin therapy. Twenty patients were treated for 48 weeks, and 1 patient was treated for 72 weeks. Two patients were withdrawn at 24 weeks due to a

TABLE II. Clinical Characteristic According to Hemophilia

	Patients with hemophilia (N = 23)	Patients without hemophilia (N = 9)	*P*-value
Age (y.o.)	37.1 ± 9.2	37.1 ± 16.3	0.9966
Sex: male/female	22/1	6/3	0.0572
AST (IU/L)	51.2 ± 34.8	41.9 ± 30.9	0.5072
ALT (IU/L)	68.2 ± 55.8	54.0 ± 66.1	0.5566
Platelet (10^4/μl)	18.4 ± 6.8	19.8 ± 3.0	0.5602
HCV levels (KIU/ml)	2599.6 ± 3108.0	2630.0 ± 3176.5	0.9812

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

TABLE III. Clinical Characteristic According to Genotypes

	Genotype 1a (N = 32)	Genotype 1b (N = 945)	*P*-value
Age (y.o.)	36.4 ± 2.2	55.9 ± 11.6	0.0001
Sex: male/female	28/4	546/408	0.0004
Patients with hemophilia	23	4	0.0001
AST (IU/L)	48.8 ± 33.6	59.9 ± 45.0	0.1745
ALT (IU/L)	64.6 ± 57.8	64.6 ± 57.8	0.9894
Platelet ($10^4/\mu l$)	18.8 ± 6.0	17.2 ± 6.0	0.0918
HCV levels (KIU/ml)	2607.4 ± 3072.2	2011.5 ± 1453.8	0.0642

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus.

lack of response to IFN therapy. Frequency of early virological response, characterized by undetectable HCV at 12 weeks, was 30.4% (7/23). Virological response rate at the end of treatment was 47.8% (11/23). Finally, 11 of 23 patients (47.8%) achieved sustained virological response. Clinical characteristics were compared between patients who achieved sustained virological response and patients who did not (Table IV), revealing significant differences in two factors on univariate analysis: IL28B and ISDR.

DISCUSSION

The present study investigated 977 patients with genotype 1 using direct sequencing of core and NS5A regions, revealing that genotype 1a is rare (3.3%) in Japan. Of the 33 patients with genotype 1a, 23 (71.9%) were patients with hemophilia, confirming that the majority of cases with genotype 1a involve patients with hemophilia who have received imported clotting factors, as previously reported [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. Analysis after excluding patients with hemophilia revealed the prevalence of genotype 1a in Japan was 0.9% (9/954). Recently, the distributions of HBV genotypes have been changing in Japan due to international exchange [Hayashi et al., 2007; Matsuura et al., 2009]. However, prevalences of HCV genotypes have remained stable because of the different modes of infection involved. The present study revealed that 11 (47.8%) of 23 patients achieved sustained virological response. The IFN responsiveness of HCV genotype 1a in Japanese patients was reported in 1999 from Okinawa, a far southern island in Japan [Sakugawa et al., 1997]. That study reported that the rate of sustained virological response tended to be higher in patients with genotype 1a than in those with genotype 1b, but no significant differences were identified because of the small number of patients with genotype 1a. Low virological response rates in both genotypes 1a and 1b were confirmed in the present Japanese patients, as in Caucasian patients [Manns et al., 2001; McHutchison et al., 2009]. No significant differences in sustained virological response rate were seen between genotypes 1a and 1b. Discriminating between genotypes 1a and 1b thus seems to have little clinical relevance in terms of IFN responsiveness. Viral factors associated with sustained virological response, including HCV genotype, have been studied most frequently studied and mutations in the core and NS5A regions of HCV genotype 1b have been associated with response to IFN therapy [Akuta et al., 2005, 2010, 2011; Okanoue et al., 2009; Nakagawa et al., 2010; Toyoda et al., 2010; Hayashi et al., 2011a; Hayes et al., 2011; Kumthip et al., 2011; Kurosaki et al., 2011]. These viral factors could improve prediction of sustained virological response for genotype 1a, as in 1b. Amino acid substitutions at positions 70 and 91 of the HCV core region in genotype 1b have been related to IFN responsiveness, liver steatosis, hepatic oxidative stress, insulin resistance, and carcinogenesis [Akuta et al., 2005, 2007, 2009; Tachi et al., 2010]. These substitutions may have substantial impacts on

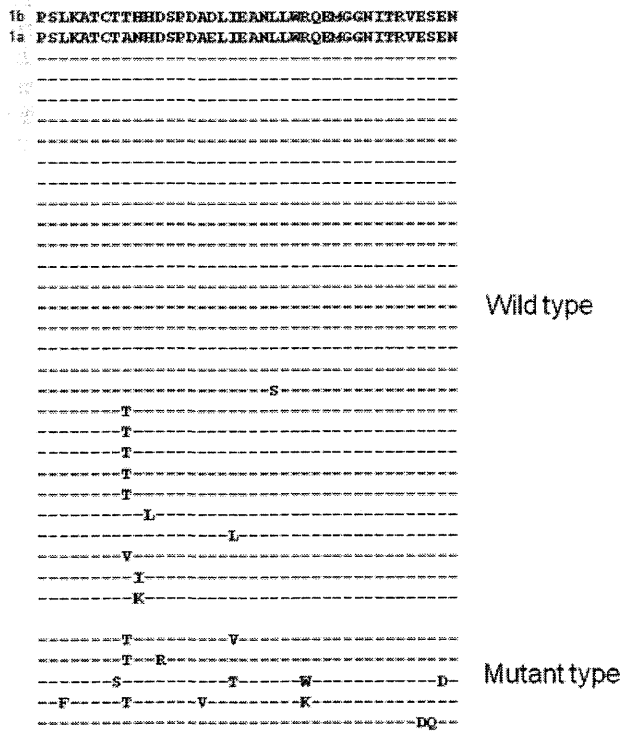

Fig. 1. Alignment of the amino acid sequence for the NS5A-ISDR. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCV1. Sequences of the HCV1 strain and HCV1 strains with one-nucleotide substitutions were defined as wild-type ISDR, and all other strains were defined as mutant-type ISDR. ISDR, interferon sensitivity-determining region.

TABLE IV. Univariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	Sustained virologic response (n = 11)	Non-sustained virologic response (n = 12)	*P*-value
Age (y.o.)	37.9 ± 10.9	39.8 ± 11.3	0.6958
Gender: male/female	10/1	10/2	0.9999
ALT (IU/L)	78.2 ± 50.8	62.6 ± 68.1	0.5435
AST (IU/L)	51.4.4 ± 29.2	48.8 ± 40.4	0.8616
PLT ($\times 10^4/mm^3$)	19.0 ± 5.4	19.3 ± 5.7	0.8870
HCV RNA level (KIU/ml)	1323.1 ± 1077.3	2567.0 ± 2940.8	0.2481
ISDR: wild/mutant	7/4	12/0	0.0373
IL28B:TT/TG	9/1	4/8	0.0115

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; IL28B, interleukin 28B.

the pathogenesis of HCV genotype 1a infection. However, the HCV core region of genotype 1a is well-conserved and no significant mutations were seen in the core region, which is associated with IFN responsiveness. Several reports have also found that the HCV core region, including positions 70 and 91, of HCV genotype 1a is highly conserved [Alestig et al., 2011; Kumthip et al., 2011]. Mutations in the core region of genotype 1a would be rare, so this region might be unsuitable for routine clinical use, unlike in genotype 1b. However, the number of patients in this study was small, and large studies including from other countries are needed to clarify these issues. The ISDR in the NS5A region of HCV genotype 1b is closely associated with response to IFN therapy. ISDR mutations of genotype 1b are well known to be more important in predicting sustained virological response in Japanese patients than European patients [Hofgärtner et al., 1997; Zeuzem et al., 1997; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. European studies have failed to detect the specific amino acid substitutions in ISDR of genotype 1a associated with IFN responsiveness [Hofgärtner et al., 1997; Zeuzem et al., 1997]. In this study, sustained virological response was achieved in 36.8% of patients with wild-type ISDR and 100% of patients with mutant-type ($P = 0.0373$). The present analysis showed a close relationship between ISDR of genotype 1a and sustained virological response, as in genotype 1b. Recent investigations in Thailand and Iran have failed to identify the usefulness of ISDR for HCV genotype 1a in predicting sustained virological response [Kumthip et al., 2011; Yahoo et al., 2011]. The high virological response rate and low prevalence of patients with mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness [Herion and Hoofnagle, 1997; Yokozaki et al., 2011]. Rates of sustained virological response among these studies were much higher than those in the present study (68.4% and 75% vs. 47.8%). The mean number of mutations in patients who achieved sustained virological response in the studies by Kumthip et al. [2011] and Yahoo et al. [2011], and the present group were 1.4, 1.4, and 1.6, respectively. Differences in sustained virological response and the number of mutations to the ISDR might underpin this discrepancy in the evaluation of ISDR. Although the sample size in the present study was small, the results indicate that ISDR represents a strong indicator of progression to sustained virological response for patients with HCV genotype 1a. Amino acid substitutions in the ISDR of genotype 1a thus also play an important role in predicting sustained virological response in Japanese patients compared to patients from other countries. IL28B polymorphisms such as host genetics, as well as mutations in the HCV genome, contribute to IFN treatment outcomes. Rates of sustained virological response in patients in this study with TT and TG were 69.2% and 11.1%, respectively. The TG allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P = 0.0115$). SNPs of IL28B would regulate the expression of IFN-stimulated genes and affect IFN responsiveness. IL28B and ISDR thus exert independent effects on IFN responsiveness and both host and viral factors impacting IFN responsiveness would improve the prediction of sustained virological response. Several studies have thus reported that both the SNP of IL28B and mutations in the ISDR were associated with sustained virological response in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. In the present study of HCV genotype 1a, among the 9 patients who had simultaneously the TG allele for IL28B and wild-type ISDR, only 1 achieved sustained virological response (11.1%). The best-sustained virological response was achieved in patients with mutant-type ISDR and the T allele (100%). The combination of SNPs for IL28B and mutations in ISDR may thus predict response to IFN therapy in patients with HCV genotype 1a as well as genotype 1b. Given the small sample size in this investigation, larger cohorts are needed to confirm the present results. Furthermore, infection with genotype 1a in Japanese patients is rare, making large-scale studies difficult to perform.

In conclusion, the prevalence of HCV genotype 1a is rare in Japan and the majority of cases involve patients with hemophilia. The TG genotype of IL28B is associated with poor response, while mutant-type ISDR is associated with good response to combination therapy with pegylated-IFN-α2b and ribavirin in patients with HCV genotype 1a. Combined use of both IL28B and ISDR could improve the prediction of IFN response.

REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2009. Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. J Med Virol 81:1032–1039.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2010. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52:421–429.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2011. Amino acid substitution in HCV core/NS5A region and genetic variation near IL28B gene affect treatment efficacy to interferon plus ribavirin combination therapy. Intervirology (in press).

Alestig E, Arnholm B, Eilard A, Lagging M, Nilsson S, Norkrans G, Wahlberg T, Wejstål R, Westin J, Lindh M. 2011. Core mutations, IL28B polymorphisms and response to peginterferon/ribavirin treatment in Swedish patients with hepatitis C virus genotype 1 infection. BMC Infect Dis 12:124.

Dal Pero F, Tang KH, Gerotto M, Bortoletto G, Paulon E, Herrmann E, Zeuzem S, Alberti A, Naoumov NV. 2007. Impact of NS5A sequences of hepatitis C virus genotype 1a on early viral kinetics during treatment with peginterferon-alpha 2a plus ribavirin. J Infect Dis 196:998–1005.

Enomoto N, Takada A, Nakao T, Date T. 1990. There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun 170:1021–1025.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Fujimura Y, Ishimoto S, Shimoyama T, Narita N, Kuze Y, Yoshioka A, Fukui H, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Genotypes and multiple infections with hepatitis C virus in patients with haemophilia A in Japan. J Viral Hepat 3:79–84.

Gale M, Jr., Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, Korth MJ, Polyak SJ, Gretch DR, Katze MG. 1998. Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: Molecular mechanisms of kinase regulation. Mol Cell Biol 18:5208–5218.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. 2009. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 49: 1335–1374.

Hayashi K, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayakawa T, Morita K, Nishimura D, Kato K, Urano F, Takamatsu J. 2003. Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. Hepatol Res 25:409–414.

Hayashi K, Katano Y, Takeda Y, Honda T, Ishigami M, Itoh A, Hirooka Y, Nakano I, Yano M, Goto H, Yoshioka K, Toyoda H, Kumada T. 2007. Comparison of hepatitis B virus subgenotypes in patients with acute and chronic hepatitis B and absence of lamivudine-resistant strains in acute hepatitis B in Japan. J Med Virol 79:366–373.

Hayashi K, Katano Y, Ishigami M, Itoh A, Hirooka Y, Nakano I, Urano F, Yoshioka K, Toyoda H, Kumada T, Goto H. 2011a. Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. J Viral Hepat 18:280–286.

Hayashi K, Katano Y, Honda T, Ishigami M, Itoh A, Hirooka Y, Ishikawa T, Nakano I, Yoshioka K, Toyoda H, Kumada T, Goto H. 2011b. Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. Liver Int 9:1359–1365.

Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K. 2011. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 60:261–267.

Herion D, Hoofnagle JH. 1997. The interferon sensitivity determining region: All hepatitis C virus isolates are not the same. Hepatolgy 25:769–770.

Hofgärtner WT, Polyak SJ, Sullivan DG, Carithers RL, Jr., Gretch DR. 1997. Mutations in the NS5A gene of hepatitis C virus in North American patients infected with HCV genotype 1a or 1b. J Med Virol 53:118–126.

Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, Chayama K, Sakisaka S, Takehara T, Oketani M, Suzuki F, Toyota J, Nomura H, Yoshioka K, Seike M, Yotsuyanagi H, Ueno Y, The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour and Welfare of Japan. 2010. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res 40: 8–13.

Kumthip K, Pantip C, Chusri P, Thongsawat S, O'Brien A, Nelson KE, Maneekarn N. 2011. Correlation between mutations in the core and NS5A genes of hepatitis C virus genotypes 1a, 1b, 3a, 3b, 6f and the response to pegylated interferon and ribavirin combination therapy. J Viral Hepat 18:e117–e125.

Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, Sugiyama M, Matsuura K, Sugauchi F, Asahina Y, Nakagawa M, Watanabe M, Sakamoto M, Maekawa S, Sakai A, Kaneko S, Ito K, Masaki N, Tokunaga K, Izumi N, Mizokami M. 2011. Pretreatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. J Hepatol 54:439–448.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, Kuramitsu T, Kawata S, Tanaka E, Izumi N, Okuse C, Kakumu S, Okanoue T, Hino K, Hiasa Y, Sata M, Maeshiro T, Sugauchi F, Nojiri S, Joh T, Miyakawa Y, Mizokami M. 2009. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol 47:1476–1483.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS, IDEAL Study Team. 2009. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 361:580–593.

Nakagawa M, Sakamoto N, Ueyama M, Mogushi K, Nagaie S, Itsui Y, Azuma S, Kakinuma S, Tanaka H, Enomoto N, Watanabe M. 2010. Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. J Gastroenterol 45:656–665.

Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. 1999. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 30:1014–1022.

Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H. 2009. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for

hepatitis C: A Japanese multi-center study. J Gastroenterol 44: 952–963.

Otagiri H, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayashi K, Hayakawa T, Fukuda Y, Kinoshita M, Takamatsu J. 2002. Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. J Virol Methods 103:137–143.

Pascu M, Martus P, Höhne M, Wiedenmann B, Hopf U, Schreier E, Berg T. 2004. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: A meta-analysis focused on geographical differences. Gut 53:1345–1351.

Sakugawa H, Nakasone H, Kinjo F, Saito A, Keida Y, Kikuchi K, Oyadomari Y, Ishihara M, Nakasone K, Yogi S, Kinjo Y, Taira M. 1997. Clinical features of patients with chronic liver disease associated with hepatitis C virus genotype 1a/I in Okinawa, Japan. J Gastroenterol Hepatol 12:176–181.

Seeff LB. 2002. Natural history of chronic hepatitis C. Hepatology 36:S35–S46.

Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42:962–973.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Tachi Y, Katano Y, Honda T, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, Samejima Y, Goto H. 2010. Impact of amino acid substitutions in the hepatitis C virus genotype 1b core region on liver steatosis and hepatic oxidative stress in patients with chronic hepatitis C. Liver Int 30:554–559.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461:798–801.

Toyoda H, Kumada T, Tada T, Arakawa T, Hayashi K, Honda T, Katano Y, Goto H. 2010. Association between HCV amino acid substitutions and outcome of peginterferon and ribavirin combination therapy in HCV genotype 1b and high viral load. J Gastroenterol Hepatol 25:1072–1078.

Yahoo N, Sabahi F, Shahzamani K, Malboobi MA, Jabbari H, Sharifi H, Mousavi-Fard SH, Merat S. 2011. Mutations in the E2 and NS5A regions in patients infected with hepatitis C virus genotype 1a and their correlation with response to treatment. J Med Virol 83:1332–1337.

Yokozaki S, Katano Y, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, Goto H. 2011. Mutations in two PKR-binding domains in chronic hepatitis C of genotype 3a and correlation with viral loads and interferon responsiveness. J Med Virol 83:1727–1732.

Zeuzem S, Lee JH, Roth WK. 1997. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 25:740–744.

栄養
評価と治療

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ORIGINAL

原著

C型肝硬変患者に対する分岐鎖アミノ酸製剤によるlate evening snackを含む栄養管理の長期効果

Long-term effect of nutrition management including late evening snack with branched-chain amino acids to cirrhosis patient related to hepatitis C virus

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Summary

Maastricht indexにより栄養不良とされたC型肝硬変症例に対して，肝不全用経口栄養剤アミノレバン®ENの就寝前軽食（LES）による栄養介入を2年間施行し，栄養状態の改善を検討した。全症例の検討では生活の質（QOL）が一部改善した。軽度栄養不良例，内服良好例ではINRが改善したが，中・高度栄養不良例，内服不良例では改善しなかった。食事摂取状況調査では，83%の患者で食事摂取量が不足しており，これらの患者では総コレステロール（TC），コリンエステラーゼ（ChE）の低下，INRの上昇がみられた。肝不全用経口栄養剤はLESとして長期投与可能であり，INRとQOLが一部改善したが，効果を得るには早期に投与を始め，服薬コンプライアンスを高くする必要があった。また食事摂取量を確保することが重要と思われた。

Key Words

■ Maastricht index　■ 食事摂取量
■ 生活の質（QOL）　■ 服薬コンプライアンス

I．緒　言

肝硬変患者の栄養状態はアルブミン（Alb），総コレステロール（TC），コリンエステラーゼ（ChE）などを指標として評価され，栄養状態不良例に対して肝不全用経口栄養剤投与，就寝前軽食（late evening snack：LES）などが行われている[1]。しかし，これらの評価法によって栄養状態が正確に把握できているかどうかは明らかになっていない。われわれは以前の研究で，栄養状態の評価のために一般的に使用されている6つの方法，上腕三頭筋皮下脂肪厚（triceps skinfold thickness：TSF），上腕筋囲（arm-muscle circumference：AMC），主観的包括的栄養評価（subjective global assessment：SGA），nutritional risk index（NRI），Maastricht index（MI），instant nutritional assessment（INA）を使ってC型肝硬変患者の栄養状態を評価し，それらの指標が有用であるかを検討した。その結果，TSF，AMC，SGAの栄養不良検出率は5～18%と低率であり，NRI，MI，INAの栄養不良検出率は63～78%と高率となったことより，C型肝硬変患者の栄養不良を検出するには，NRI，MI，INAの総合評価または最も誤診率の低かったMIが有用であると思われた[2)-5)]。

肝硬変患者ではたんぱく質エネルギー栄養障害（protein-energy malnutrition：PEM）が多くみられ，その頻度は代償性肝硬変で20%以上，非代償性肝硬変では60%以上といわれている[6)7)]。低蛋白状態の特徴は内臓蛋白と筋蛋白の両者が低下することであり，分岐鎖アミノ酸（branched chain amino acid：BCAA）の低下が主な成因とみなされている。また，エネルギー代謝異常の特徴は糖質利用の低下と脂質利用の上昇である。この燃焼パターンは飢餓状態のそれに類似しており，対策としては分割食（夜食）がよいとされている[8)-10)]。そのためBCAA補充と分割食としてのLESを活用した栄養介入が必要であると考えられる[11)-13)]。

そこで本研究では，MIにより栄養不良と判定されたC型肝硬変患者29例に，最長24ヵ月間においてBCAA製剤を1日2包（朝食後，眠前）投与し，栄養状態と生活の質（QOL）の評価を行った。

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Ⅱ．対象と方法

MIにより栄養不良と判定されたC型肝硬変患者29例（MI栄養評価：軽度栄養不良13例，中等度14例，高度1例）を対象に，2ヵ月ごとの栄養指導と肝不全用経口栄養剤アミノレバン®EN 1日2包（朝食後，眠前）の投与を最長24ヵ月間行った。ただし，長期の経過フォローのため，途中で死亡および他院転院などの理由により，24ヵ月まで継続観察できた症例は16例であった（表1）。栄養状態とQOLの変動を検討するにおいては，栄養状態はMIおよび各種血液検査値にて評価し，QOLの評価にはSF-8質問表を用いた。

指示栄養量は標準体重×30～35kcal，たんぱく質量は標準体重×1.3～1.5gとし，肝不全用経口栄養剤2包分の栄養量（2包あたりエネルギー量420kcal，たんぱく質量26.6g）を引いた栄養価で栄養指導を行った。2ヵ月ごとに15～30分の栄養指導を実施し，計算したエネルギー摂取量やたんぱく質摂取量の指示栄養量に対する過不足を確認し，過剰な場合は減らすように指導を行った。

次に，MIにより軽度栄養不良群と中・高度栄養不良群に分けて，栄養状態とQOLの変動を比較検討した。

表1．対象：C型肝硬変29例

性別（M/F）	14/15
年齢（歳）	67.0±7.7
Alb（g/dℓ）	3.5±0.3
TC（mg/dℓ）	149±22
ChE（IU/ℓ）	162.8±64
T-Bill（mg/dℓ）	1.2±0.6
Cr（mg/dℓ）	0.7±0.15
プレアルブミン（mg/dℓ）	12±6
BTR	3.2±0.81
PT（%）	74.5±10.5
INR	1.25±0.2
アンモニア（μg/dℓ）	53.3±31.7
白血球（μℓ）	3,741±1,383
血小板（μℓ）	8.1±3.8×10⁴
リンパ球数（μℓ）	1,392±667
BMI	23.0±2.8
TSF（mm）	13.4±5.5
AMC（cm）	234.0±22.4
Child-Pugh分類（A/B/C）	18/10/0
肝癌（あり/なし）	4/25
糖尿病（あり/なし）	14/15
MI（軽度/中等度/高度）	13/14/1

T-Bill：総ビリルビン，Cr：クレアチニン，PT：プロトロンビン活性

29例の対象者のなかで，肝不全用経口栄養剤を1日2包服薬できていた症例を「内服良好群」，服薬できていなかった症例を「内服不良群」とし，薬剤の服薬状況を評価した。そのうち，服薬継続が可能であった23例においてエネルギー量，たんぱく質量の指示栄養量の充足率を確認するため，食事摂取状況を調査した。規定の3日間食事記録用紙に自宅での食事記録を患者に記入させ，管理栄養士が『五訂日本食品成分表』によりエネルギー摂取量とたんぱく質摂取量を算出した。エネルギー量，たんぱく質量のいずれかが足りていない場合（エネルギー量30kcal/kg/日未満，たんぱく質量1.3g/kg/日未満）を「摂食不良」とし，栄養状態とQOLの変動を検討した。

解析方法は対応のないt検定，対応のあるt検定，Wilcoxonの符号付き順位検定，ANOVA分析で行った。

Ⅲ．結　果

1．全症例の栄養状態とQOLの変動

肝不全用経口栄養剤投与開始後，各種検査項目に関して6ヵ月ごとでみたANOVA分析ではINRが有意に上昇し（開始前と比較して4ヵ月後，6ヵ月後に有意差あり；t検定），TCは低下傾向にあった（PT：$p=0.0018$，INR：$p=0.0006$，TC：$p=0.0991$）。Alb，プレアルブミンは有意な変化がなかった。AMCも変化はなく，筋蛋白は維持された。総合栄養評価であるMI判定においては，開始前と比べて研究期間中での有意な変化は認められなかった。

SF-8についての6ヵ月ごとのANOVA分析では，全体的健康感（general health；GH）についてのみ有意な改善（2ヵ月ごとのt検定では有意差は検出できず）がみられた（$p=0.035$）。また，心の健康（mental health；MH）と精神的サマリスコア（mental component summary；MCS）では改善する傾向がみられた（MH：$p=0.0514$，MCS：$p=0.0836$）（図1）。

肝不全用経口栄養剤の服薬に関しては，1日2包服薬できている例を内服良好としており，コンプライアンスは65%が内服良好であった。残りの35%は1包を服薬継続および間欠的に服薬していた。肝不全用経口栄養剤の服薬コンプライアンスに関しては，通常の錠剤やカプセル剤などの内服薬と違い，特有の匂いや味があることや調整が必要な点など，コンプライアンスに影響を及ぼす

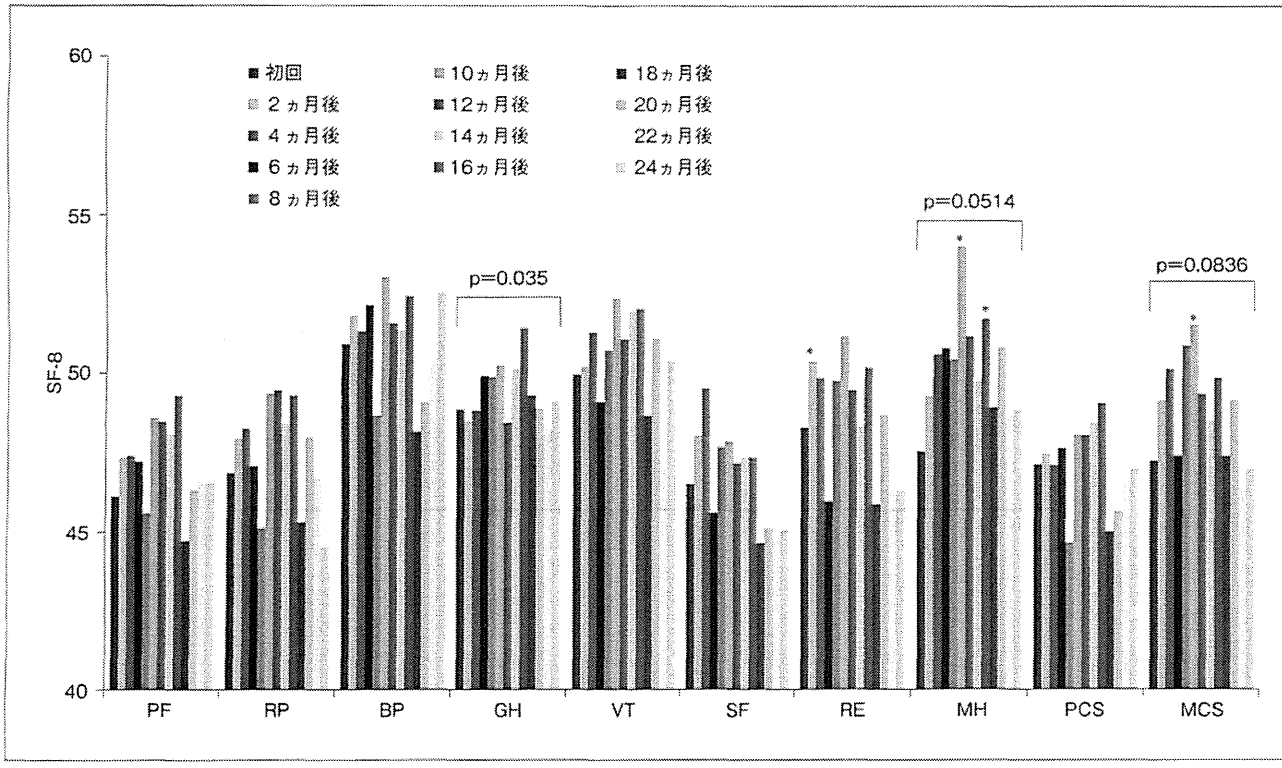

図1．全症例における栄養介入前後のSF-8の変化（6ヵ月ごとでみたANOVA分析結果）

ANOVA分析，GHは有意に改善し，MHとMCSが改善する傾向にあった。

＊：開始時からの2ヵ月ごとのt-検定結果有意差月（RE：2ヵ月後，MH：10，16ヵ月後，MCS：10ヵ月後）

点がいくつか考えられる。実際に肝不全用経口栄養剤の服薬コンプライアンスを調べた報告はほとんどないが，大谷らの調査によると，処方どおり服薬しているという患者は57.9%であった[14]。今回，指示どおり2包服薬できた患者をコンプライアンス良好としているが，65%が指示どおり服薬できたことは，前述の報告と合わせると実臨床では十分なコンプライアンスが得られていると考える。

2．MIによる軽度栄養不良群と中・高度栄養不良群の栄養状態とQOLの変動の比較

MIによる軽度栄養不良群と中・高度栄養不良群で，肝不全用経口栄養剤投与開始前のQOLに有意な差はなく，Albのみが軽度栄養不良群で有意に高値だった（表2）。

軽度栄養不良群と中・高度栄養不良群の栄養介入後の変化をみた各種検査項目に関する6ヵ月ごとのANOVA分析では，軽度栄養不良群でINRの有意な低下（開始前と比較し18ヵ月後：t検定）がみられ，中・高度栄養不良群ではアンモニア（NH_3）の上昇傾向がみられた（PT：$p=0.0031$，INR：$p=0.0120$，NH_3：$p=0.0677$）。QOLについては軽度栄養不良群，中・高度栄養不良群ともに有意差はなく，ANOVA分析において，軽度栄養不良群は中・高度栄養不良群と比較すると，身体的サマリスコア（physical component summary；PCS）が有意に改善し（6ヵ月後のt検定で有意差あり），日常役割機能（身体的）（role physical：RP）も改善する傾向がみられた（PCS：$p=0.0348$，RP：$p=0.0798$）（図2）。

3．服薬継続中23例の食事摂取状況の調査

服薬継続中23例の食事摂取状況を調べた結果，23例中19例（83%）が摂食不良であった。摂食不良例における食事調査の結果は，肝不全用経口栄養剤の飲用分を差し

表2．　LES介入前の比較（MI栄養評価による比較）

QOL(SF-8)	軽度栄養不良群	中・高度栄養不良群	p値
PF	45.56±5.86	46.11±5.86	NS
RP	45.95±6.81	47.20±4.26	NS
BP	51.67±8.78	50.19±10.58	NS
GH	49.99±5.25	47.66±7.46	NS
VT	51.22±5.06	48.59±4.99	NS
SF	48.33±8.65	44.36±8.00	NS
RE	47.73±7.21	48.32±4.07	NS
MH	47.39±5.07	47.50±6.74	NS
PCS	47.05±6.89	46.85±5.92	NS
MCS	48.34±6.69	45.99±5.20	NS
検査値	軽度栄養不良群	中・高度栄養不良群	p値
Alb	3.69±0.25	3.39±0.32	$p<0.05$*
BTR	3.47±0.78	2.86±0.62	NS
BMI	24.01±1.44	22.28±2.85	NS
INR	1.27±0.29	1.23±0.08	NS
NH_3	55.5±43.6	50.8±17.6	NS

＊：Wilcoxon符号付き順位検定

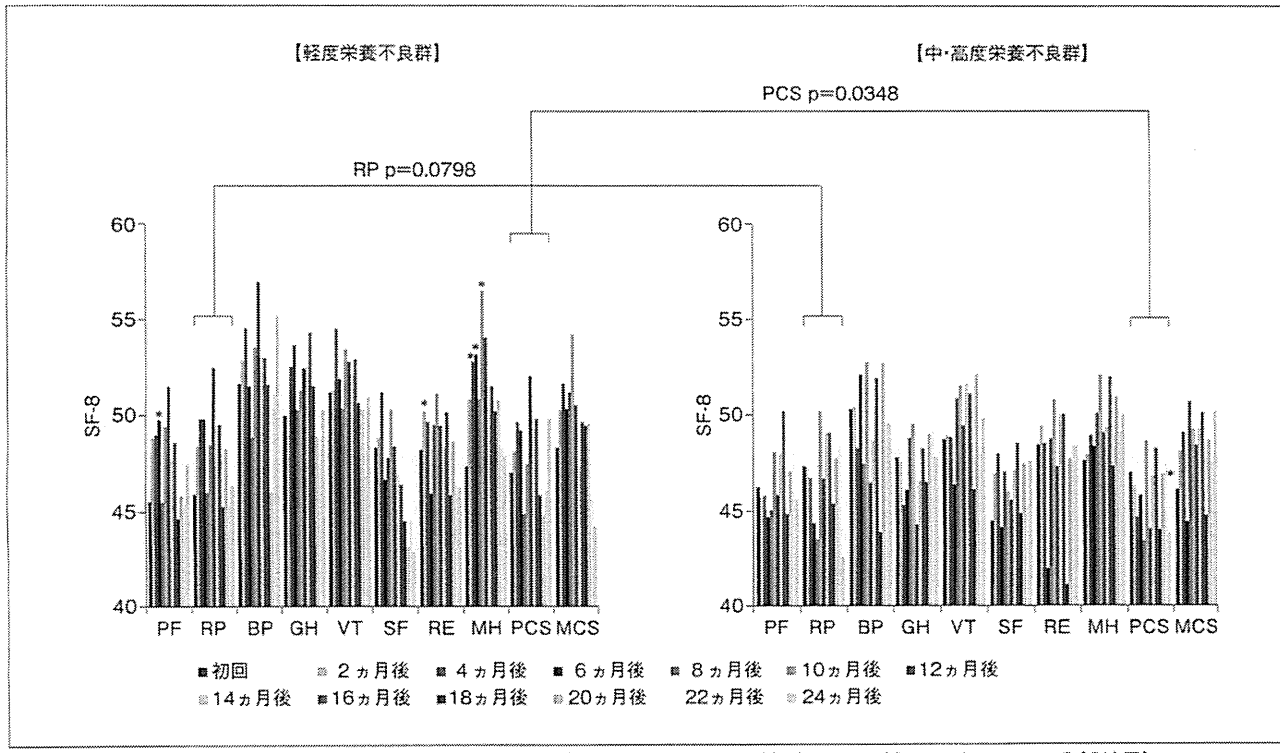

図2．MIによる軽度栄養不良群と中・高度栄養不良群の栄養介入後SF-8の比較（6ヵ月ごとでみたANOVA分析結果）

ANOVA分析．軽度栄養不良群は，中・高度栄養不良群と比較するとPCSに有意な改善がみられ，RPは改善傾向がみられた。

＊：開始時からの2ヵ月ごとのt-検定結果有意差月。軽度栄養不良群（RE：2ヵ月後，MH：4，6，10ヵ月後），中・高度栄養不良群（PCS：24ヵ月後）

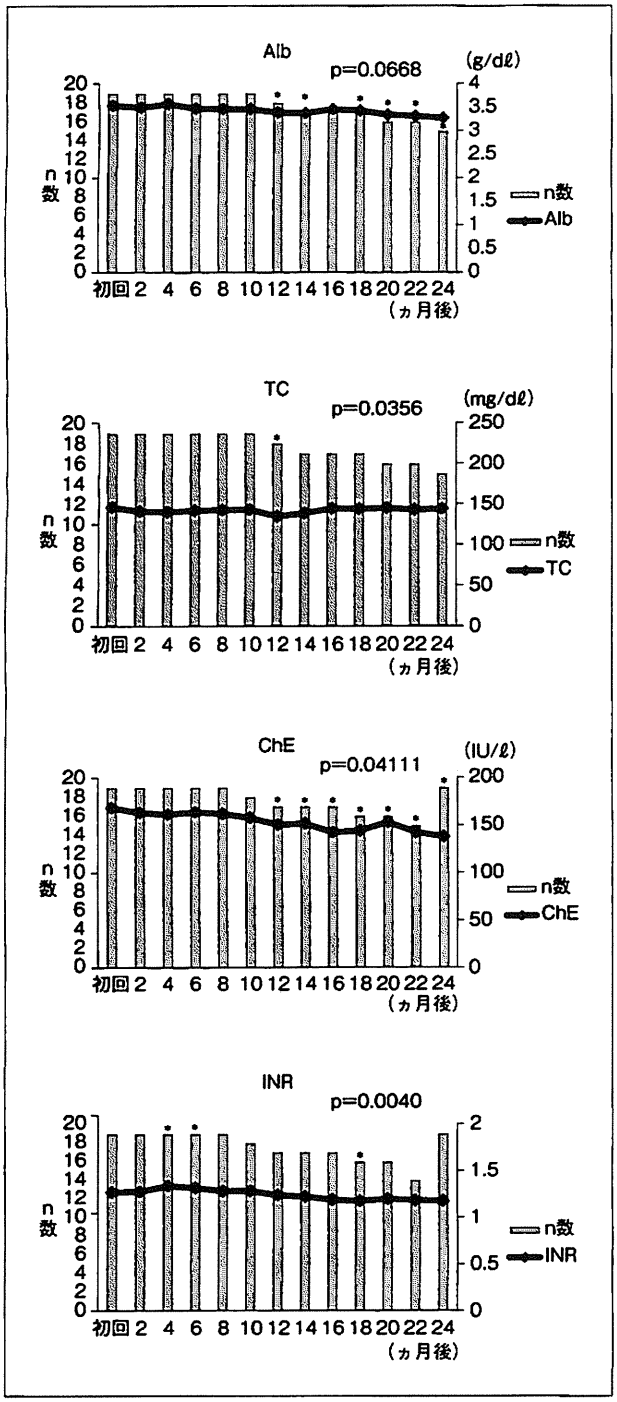

図3．摂食不良群の栄養介入前後変化（6ヵ月ごとでみたANOVA分析結果）

ANOVA分析，TC，ChEに有意な低下がみられ，Albは低下傾向がみられた。INRは有意に上昇した。

＊：開始時からの2ヵ月ごとのt-検定有意差月（Alb：12，14，18，20，22，24ヵ月後，TC：12ヵ月後，ChE：12，14，16，18，20，22，24ヵ月後，INR：4，6，18ヵ月後）

引いたエネルギー量が平均1,158kcal，たんぱく質量が37.6gであった。特に，指示栄養量のたんぱく質摂取における摂食不良が目立っていた。

食事摂取状況と服薬状況の比較をすると，内服良好群18例中14例が摂食不良であり，内服不良群5例はすべて摂食不良であった。すなわち内服良好，不良にかかわらず，摂食不良例が多いことが明らかとなった。

摂食不良群での各種検査項目に関しては，6ヵ月ごとでみたANOVA分析において，TC，ChEで有意な低下がみられ，Albでは低下傾向がみられた。また，INRは有意に上昇した（TC：$p=0.0356$，ChE：$p=0.0411$，Alb：$p=0.0668$，INR：$p=0.0040$）（**図3**）。

QOLではGHの有意な上昇がみられ，MCS，活力（vitality；VT）に上昇傾向がみられた（GH：$p=0.0275$，MCS：$p=0.0807$，VT：$p=0.0945$）（**図4**）。

Ⅳ．考　察

全症例の各種検査項目を6ヵ月ごとにみたANOVA分析では，INRの有意な上昇，TCの低下の傾向がみられたが，Alb，プレアルブミン，AMCには改善がみられなかった。栄養状態，筋肉量への直接的な結果が得られなかったのは，摂食不良が原因の1つではないかと考えられる。INRの改善についてはt検定により4ヵ月後，6ヵ月後のみに有意な上昇がみられ，肝不全用経口栄養剤服薬によるBCAAの直接効果が早期に現われた結果ではないかと推察される。実際，対象者のエネルギー摂取量とたんぱく質摂取量を算出することで，食事摂取状況を調査し栄養面からの評価を行った結果，23例中19例（83%）が摂食不良であり，特にたんぱく質摂取量が体重1kgあたり1.3～1.5g/日を下回っていた。この摂食不良例ではTC，ChEが有意に低下し，Albは低下の傾向を示し，INRは有意に上昇していた。摂食良好例は症例数が少なく解析できなかったが，全症例の検討で各種検査項目の改善がみられなかったのは，摂食不良例が多かったためと思われる。対象者は67.0歳と高齢であり，摂食不良例が多かったことの背景には高齢者への栄養管理の難しさがあると考える。高齢者は食生活パターンが確立されていることもあり，食事療法を的確に実施することは困難である。対象者への栄養指導の介入による肝硬変治療には，より多くの時間とコミュニケーションスキルが必要

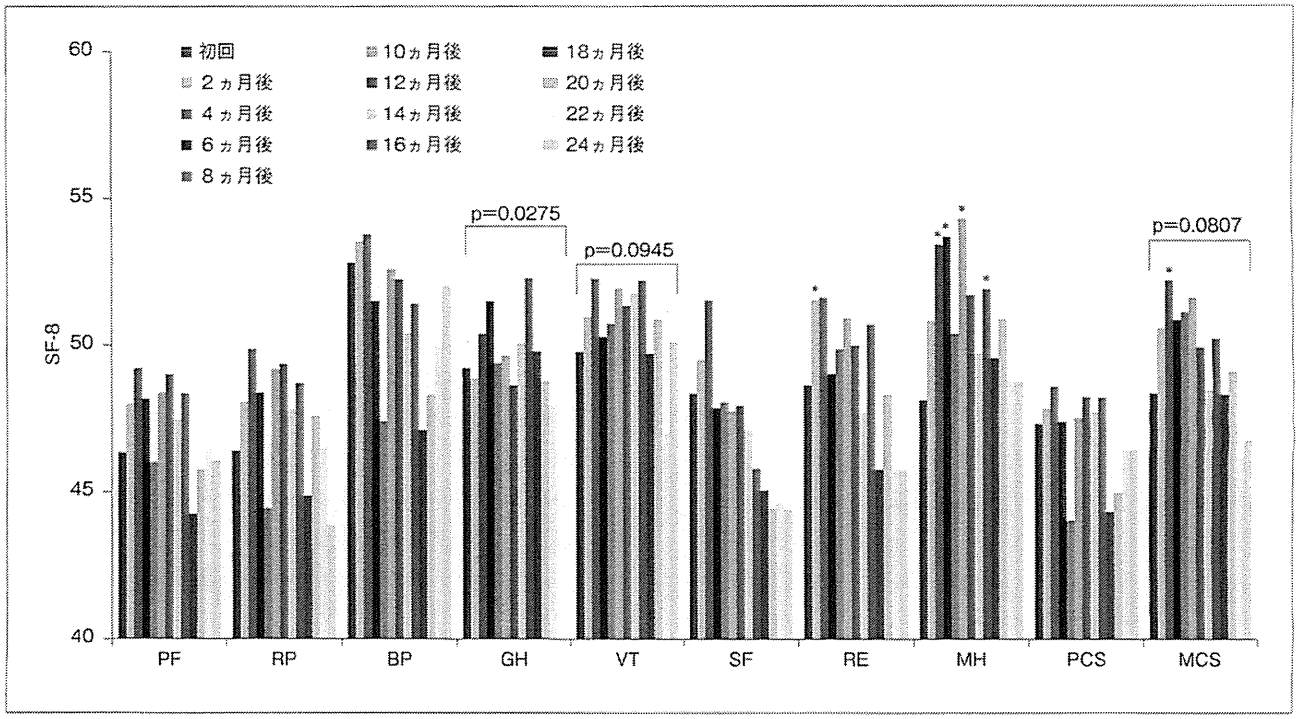

図4. 摂食不良群の栄養介入前後のSF-8の変化（6ヵ月ごとでみたANOVA分析結果）

ANOVA分析，GHに有意な改善がみられ，MCS，VTに改善する傾向がみられた。

＊：開始時からの2ヵ月ごとのt-検定結果有意差月（RE：2ヵ月後，MH：4，6，10，16ヵ月後，MCS：4ヵ月後）

であると思われる。摂食不良例のなかでは，糖質，脂質と合わせて適正エネルギー量を確保できていても，たんぱく質摂取量が不十分な症例が多かった。食事摂取状況に関しては，加藤らの報告でも対象症例の30%に摂取不足がみられ[15]，基本となる日常の食事摂取が不十分ではBCAA補充療法やLESの効果にも影響を与えると考えられ，栄養士による積極的な食事摂取状況調査や指導が必要と思われる。

また，全症例の検討で各種検査項目の改善はみられなかったが，SF-8では6ヵ月ごとにみたANOVA分析でGHに有意な改善がみられ，MHとMCSに改善の傾向がみられた。このことから，摂食不良などにより検査値には改善がみられなくても，LES投与の継続によりQOLの改善効果が得られることが示唆された。

そこでSF-8を軽度栄養不良群と中・高度栄養不良群で比較すると，ANOVA分析にて軽度栄養不良群にPCSで有意な改善がみられ，RPには改善傾向がみられた。肝不全用経口栄養剤のLESによる効果については，こむら返りや不眠，倦怠感などの自覚症状の改善も報告されている[11) 16) 17)]。今回は自覚症状の確認は実施していないが，QOLの身体的スコアの改善には少なからず自覚症状に対する効果が影響したのではないかと考えられる。軽度栄養不良群においてINRとQOLの有意な改善がみられ，中・高度栄養不良群ではNH_3の上昇傾向がみられたことより，栄養不良を早期に発見し，BCAA製剤によるLESを含む栄養管理を始めることで，血液検査値とQOLを改善させることができると思われる。

内服良好群と内服不良群を比較すると，内服良好群でBCAA/チロシン比（BTR）が上昇する傾向を示し，Albが維持された。BTRは，Albやインドシアニングリーン負荷15分値（ICGR15）と有意な相関を認め，肝障害程度を反映する。したがって，BTRを上昇させることは肝障害の改善に効果があると考えられる[18)]。一方，内服不良群では症例数が少なくANOVA分析を行えなかったが，BTRの上昇はなく，Albは低下していた。内服良好群と内服不良群においてQOLを比較すると，内服良好群にお

いてSF-8の数値はおおむね高く，特に22ヵ月後においてはすべての項目で有意に良好であった。介入前の背景において，REのみ内服良好群で有意であった以外は差がなかったことを考えると，やはり長期にコンプライアンスを維持することはQOLの低下を抑制できる可能性も示唆された。また内服良好群において，SF-8のMHがANOVA分析により改善の傾向にあった。このことは，BCAA製剤によるLESを含む栄養介入が十分に行われれば，精神面の改善も期待できることを示していると思われる。これについてはYamanaka-Okumuraらが行ったLESの肝硬変症例においても，LESを行わなかった症例に比べて6ヵ月後，12ヵ月後のSF-36においてMHが高いことを報告している[10]。また本研究において，内服不良群は内服良好群と比較して，栄養介入前よりSF-8のREが低かった。このことは介入開始前のQOLの低下が服薬コンプライアンスに影響する可能性を示唆しており，今後，服薬指導において注意が必要と思われた。

本研究は，肝不全用経口栄養剤（アミノレバン®EN）によるLESを含む栄養介入は服薬面からも長期持続が可能であり，QOLの一部が改善することを示した。軽度栄養不良例や内服良好例ではINRの改善も認められたことから，効果を得るには早期に投与を開始して確実に服薬コンプライアンスを高める必要があり，食事摂取量の確保，すなわち高蛋白食かつ適正エネルギー摂取量を確保していくことが重要だと思われる。そのため肝不全用経口栄養剤の服薬状況と食事摂取量を確認したうえでの継続的な栄養指導が必要と考える。

■文　献

1）森脇久隆：肝臓栄養治療に関する意識調査．栄評治 23：79-81，2006
2）Kawabe N, Hashimoto S, Harata M, et al：Assessment of nutritional status of patients with hepatitis C virus-related liver cirrhosis. Hepatol Res 38：484-90, 2008
3）de Jong PC, Wesdorp RI, Volovics A, et al：The value of objective measurements to select patients who are malnourished. Clin Nutr 4：61-66, 1985
4）Seltzer MH, Bastidas JA, Cooper DM, et al：Instant nutritional assessment. JPEN J Parenter Enteral Nutr 3：157-159, 1979
5）Detsky AS, McLaughlin JR, Baker JP, et al：What is subjective global assessment of nutritional status? JPEN J Parenter Enteral Nutr 11：8-13, 1987
6）Lautz HU, Selberg O, Körber J, et al：Protein-calorie malnutrition in liver cirrhosis. Clin Investig 70：478-486, 1992
7）Plauth M, Merli M, Kondrup J, et al：ESPEN guidelines for nutrition in liver disease and transplantation. Clin Nutr 16：43-55, 1997
8）森脇久隆：肝硬変に伴うエネルギー代謝異常の病態と対策．日病態栄会誌 3：18-25，2000
9）Chang WK, Chao YC, Tang HS, et al：Effects of extra-carbohydrate supplementation in the late evening on energy expenditure and substrate oxidation in patients with liver cirrhosis. JPEN J Parenter Enteral Nutr 21：96-99, 1997
10）Yamanaka-Okumura H, Nakamura T, Miyake H, et al：Effect of long-term late-evening snack on health-related quality of life in cirrhotic patients. Hepatol Res 40：470-476, 2010
11）Nakaya Y, Okita K, Suzuki K, et al：Hepatic Nutritional Therapy (HNT) Study Group：BCAA-enriched snack improves nutritional state of cirrhosis. Nutrition 23：113-120, 2007
12）Yamauchi M, Takeda K, Sakamoto K, et al：Effect of oral branched chain amino acid supplementation in the late evening on the nutritional state of patients with liver cirrhosis. Hepatol Res 21：199-204, 2001
13）Aoyama K, Tsuchiya M, Mori K, et al：Effect of a late evening snack on outpatients with liver cirrhosis. Hepatol Res 37：608-614, 2007
14）大谷　綾，木下香奈，鍋島　静，他：肝不全用経口アミノ酸製剤の服薬状況およびコンプライアンスに影響を及ぼす要因．日病薬誌会誌 42：633－636，2006
15）加藤章信：肝硬変の栄養摂取状況とQOL．臨消内科 23：709-714，2008
16）奥田博明，白鳥敬子，立松栄次：肝硬変患者に対する長期の夜間就寝前栄養（LES）による栄養アセスメントとQOLについて．静脈経口栄養 21：71-77，2006
17）Sako K, Imamura Y, Nishimata H, et al：Branched-chain amino acids supplements in the late evening decrease the frequency of muscle cramp with advanced hepatic cirrhosis. Hepatol Res 26：327-329, 2003
18）土師誠二：肝切除における経口栄養管理．栄評治 22：303-306，2005