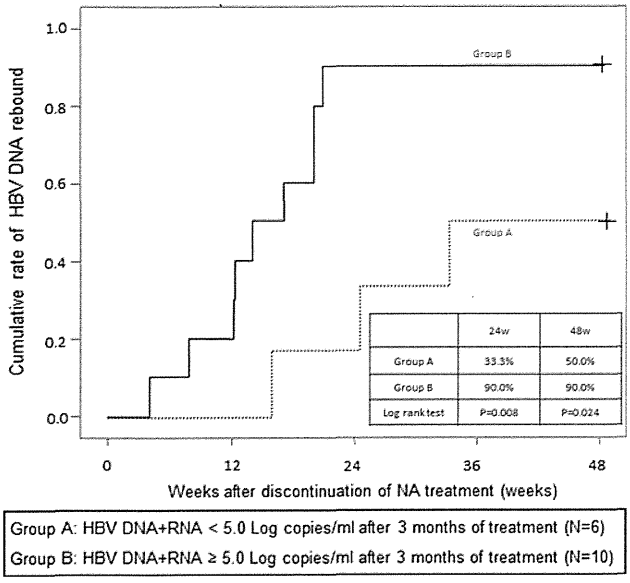

Fig. 5 Cumulative rate of ALT rebound after discontinuation of NA treatment in HBeAg-positive chronic hepatitis B patients. Six patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment were assigned to group A; the other ten patients, whose HBV DNA + RNA titers were ≥5.0 log copies/mL after 3 months of treatment, were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

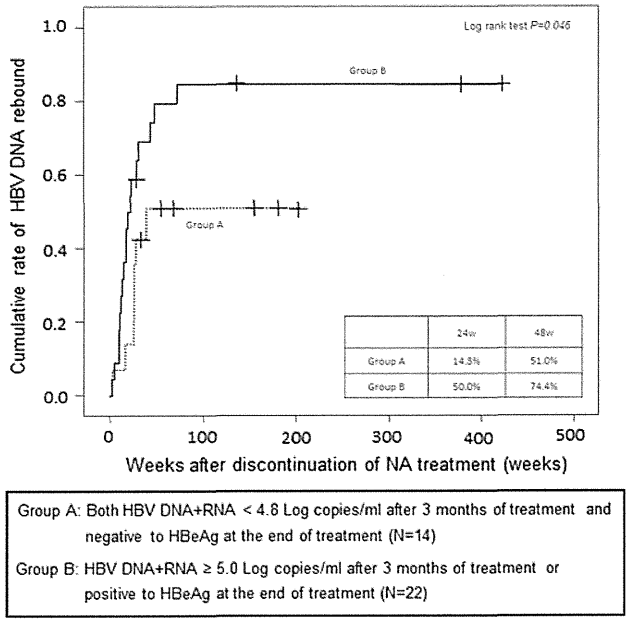

Fig. 6 Cumulative rate of ALT rebound after discontinuation of NA treatment by using combined criteria. The subjects were divided using combined criteria. Fourteen patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment and who were HBeAg negative at the end of NA treatment were assigned to group A; the other 22 patients were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

DNA + RNA titer after 3 months of treatment was found to be significantly associated with HBV DNA rebound ($P = 0.043$, OR = 9.474; Table 2). Two other factors, HBV DNA titer after 3 months of treatment and HBsAg titer at the end of treatment, were marginally associated with HBV DNA rebound ($P = 0.074$, $P = 0.070$, respectively). After 3 months of NA treatment, HBV DNA titers decreased in both the HBV DNA relapse and non-relapse groups, but HBV DNA + RNA levels in the relapse group remained high. NA therapy suppressed the production of mature HBV particles in both groups, but in the HBV DNA relapse group, high HBV replication activity was likely maintained during the treatment, and immature HBV particles associated with HBV RNA genomes were continuously produced and accumulated in hepatocytes. After discontinuation of the treatment, these accumulated immature HBV particles may have been maturated and been released from the hepatocytes. Thus, rebound of HBV DNA titers occurred rapidly after the discontinuation of NA therapy.

Although the presence of HBeAg before treatment, HBV DNA and DNA + RNA titers after 3 months of treatment, and the presence of HBeAg, HBsAg titer, and HBV DNA + RNA titer at the end of treatment were all significantly associated with ALT rebound in the univariate analysis, only the presence of HBeAg at the end of treatment was identified as an independent predictive factor for ALT rebound following multivariate analysis (Table 4). HBeAg is commonly strongly associated with the activity of HBV replication, and HBV DNA levels are high in HBeAg-positive HBV carriers. Thus, HBe seroconversion usually indicates suppression of HBV activity, and the absence of HBeAg is thought to indicate the inactivation of HBV replication.

ALT rebound following the discontinuation of NA therapy was not observed in six of the 16 patients (37.5 %) who were HBeAg-positive at the end of treatment. After examining predictive factors for ALT rebound in these HBeAg-positive patients, only the HBV DNA + RNA titer after 3 months of treatment was identified as an independent predictive factor for ALT rebound in HBeAg-positive patients (Table 6). Although the presence of HBeAg indicates high activities of HBV replication and hepatitis, it is expected to be difficult to discontinue NA therapy without ALT rebound in these patients. However, these results indicate that HBV replication activities vary greatly among individuals and suggest that it might be possible to predict future replication activity based on HBV DNA + RNA titers after 3 months of treatment.

A limitation of this study is the small sample size; as such, selection bias might have affected the internal validity of the study. As it is not common to discontinue

NA therapy in Japan, we were only able to examine 36 subjects in our study. Because HBV-related markers such as HBsAg, HBcrAg, and HBV DNA + RNA titers varied widely among individuals, HBeAg an HBV DNA + RNA titers were only marginally associated with HBV DNA or ALT rebound after the discontinuation of NA therapy. In a previous study, Matsumoto et al. [34] analyzed predictive factors for the safe discontinuation of NA therapy in 126 clinical HBeAg-negative subjects from 12 clinical centers. These authors reported that HBsAg and HBcrAg titers at the end of treatment were predictive factors for the safe discontinuation of therapy. In our study, we also found that the absence of HBeAg at the end of treatment was important for the safe discontinuation of NA therapy, but we found no association between safety and HBsAg or HBcrAg titers. However, while HBsAg and HBcrAg are known to be associated with HBV replication activity, our results involving HBeAg and HBV DNA + RNA titers as important factors for safe discontinuation appear to be consistent.

In our study, the duration of NA therapy was quite short (mean duration was 36 weeks). Similar results might be observed if the NA therapy was extended, but it might be difficult to depress the potential of infected HBV replication with long-term NA therapy. HBsAg titers represent HBV replication in human hepatocytes, and it is difficult to decrease HBsAg levels by NA therapy. Thus, HBV DNA + RNA levels might be an important factor for predicting the HBV DNA or ALT rebounds.

As it may be difficult to discontinue therapy in patients with advanced liver fibrosis, our study subjects were selected based on liver spare capacities. As shown in Fig. 1, ALT rebound is likely to occur in most patients following the discontinuation of NA therapy, and severe hepatitis could occur in some patients. Thus, if the liver spare capacity were low, NA therapy would not be discontinued; the patients in this study were selected solely based on clinical aspects, which may have influenced our interpretation of the results.

In conclusion, HBV replication activity was found to be an important predictor of safe discontinuation of NA therapy. These findings suggest that monitoring of serum HBV DNA + RNA levels would be a useful method for predicting the re-activation of chronic hepatitis B following discontinuation of NA therapy.

Acknowledgments This study was supported in part by a Grant-in-aid from the Ministry of Health, Labor and Welfare of Japan and was carried out at the Research Center for Molecular Medicine, Faculty of Medicine, Hiroshima University and the Analysis Center of Life Science, Hiroshima University. The authors thank Rie Akiyama for technical assistance and Aya Furukawa for clerical assistance.

Conflict of interest None to declare.

References

1. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350(11):1118–29.
2. Wright TL, Lau JY. Clinical aspects of hepatitis B virus infection. Lancet. 1993;342(8883):1340–4.
3. Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, et al. Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. Hepatology. 2011;53(4):1237–45.
4. Brechot C, Gozuacik D, Murakami Y, Paterlini-Brechot P. Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol. 2000;10(3):211–31.
5. Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, Brechot C, et al. Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. Gut. 2005;54(8):1162–8.
6. Nagaya T, Nakamura T, Tokino T, Tsurimoto T, Imai M, Mayumi T, et al. The mode of hepatitis B virus DNA integration in chromosomes of human hepatocellular carcinoma. Genes Dev. 1987;1(8):773–82.
7. Yaginuma K, Kobayashi H, Kobayashi M, Morishima T, Matsuyama K, Koike K. Multiple integration site of hepatitis B virus DNA in hepatocellular carcinoma and chronic active hepatitis tissues from children. J Virol. 1987;61(6):1808–13.
8. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. J Hepatol. 2003;38[Suppl 1]:S90–103.
9. Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, 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):1–7.
10. Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology. 2006;43(6):1385–91.
11. Buti M, Jardi R, Cotrina M, Rodriguez-Frias F, Esteban R, Guardia J. Transient emergence of hepatitis B variants in a patient with chronic hepatitis B resistant to lamivudine. J Hepatol. 1998;28(3):510–3.
12. Chayama K, Suzuki Y, Kobayashi M, Kobayashi M, Tsubota A, Hashimoto M, et al. Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. Hepatology. 1998;27(6):1711–6.
13. Ghany M, Liang TJ. Drug targets and molecular mechanisms of drug resistance in chronic hepatitis B. Gastroenterology. 2007;132(4):1574–85.
14. Kobayashi M, Suzuki F, Akuta N, Yatsuji H, Hosaka T, Sezaki H, et al. Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment. Hepatol Res. 2010;40(2):125–34.
15. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology. 2007;46(1):254–65.
16. Suzuki F, Tsubota A, Arase Y, Suzuki Y, Akuta N, Hosaka T, et al. Efficacy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. Intervirology. 2003;46(3):182–9.
17. Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. Antimicrob Agents Chemother. 2004;48(9):3498–507.

18. Tenney DJ, Rose RE, Baldick CJ, Levine SM, Pokornowski KA, Walsh AW, et al. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. Antimicrob Agents Chemother. 2007;51(3):902–11.
19. Yatsuji H, Hiraga N, Mori N, Hatakeyama T, Tsuge M, Imamura M, et al. Successful treatment of an entecavir-resistant hepatitis B virus variant. J Med Virol. 2007;79(12):1811–7.
20. Yatsuji H, Suzuki F, Sezaki H, Akuta N, Suzuki Y, Kawamura Y, et al. Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. J Hepatol. 2008;48(6): 923–31.
21. Zoulim F, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. Gastroenterology. 2009;137(5):1593–608. e1–2.
22. Hatakeyama T, Noguchi C, Hiraga N, Mori N, Tsuge M, Imamura M, et al. Serum HBV RNA is a predictor of early emergence of the YMDD mutant in patients treated with lamivudine. Hepatology. 2007;45(5):1179–86.
23. Huang YW, Chayama K, Tsuge M, Takahashi S, Hatakeyama T, Abe H, et al. Differential effects of interferon and lamivudine on serum HBV RNA inhibition in patients with chronic hepatitis B. Antivir Ther. 2010;15(2):177–84.
24. Su Q, Wang SF, Chang TE, Breitkreutz R, Hennig H, Takegoshi K, et al. Circulating hepatitis B virus nucleic acids in chronic infection: representation of differently polyadenylated viral transcripts during progression to nonreplicative stages. Clin Cancer Res. 2001;7(7): 2005–15
25. Zhang W, Hacker HJ, Tokus M, Bock T, Schroder CH. Patterns of circulating hepatitis B virus serum nucleic acids during lamivudine therapy. J Med Virol. 2003;71(1):24–30.
26. Pugh JC, Bassendine MF. Molecular biology of hepadnavirus replication. Br Med Bull. 1990;46(2):329–53.
27. Loguercio C, Di Pierro M, Di Marino MP, Federico A, Disalvo D, Crafa E, et al. Drinking habits of subjects with hepatitis C virus-related chronic liver disease: prevalence and effect on clinical, virological and pathological aspects. Alcohol Alcohol. 2000;35(3): 296–301.
28. Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, et al. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. J Clin Microbiol. 2002;40(2):439–45.
29. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol. 2009;81(1):27–33.
30. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med. 2006;354(10):1001–10.
31. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med. 1998;339(2): 61–8.
32. Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. Gastroenterology. 2002;123(6):1831–8.
33. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2006;354(10): 1011–20.
34. Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, et al. Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B. Hepatol Res. 2012;42(2):139–49.

J Gastroenterol
DOI 10.1007/s00535-012-0727-4

EDITORIAL

What is the benefit of computer-assisted image analysis of liver fibrosis area?

Kentaro Yoshioka

Received: 28 November 2012 / Accepted: 29 November 2012

Liver fibrosis is usually semiquantitatively assessed in liver biopsy specimens by the numerical system of Scheuer [1], the Metavir group [2], or Ishak [3]. Fibrosis is staged as F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Staging mainly depends on the degree of the architectural changes of liver structure.

Computer-assisted image analysis of the stained fibrosis area in liver biopsy specimens is a method for quantitatively measuring the amount of liver fibrosis [4]. It is not used for the clinical assessment of liver fibrosis in general, but is often used in the assessment of fibrosis in animal models. Its low popularity in clinical practice may be attributed to the complexity of the method.

The fibrosis stage as determined by the numerical systems and the relative area of fibrosis measured by computer-assisted image analysis usually correlate well to each other. However, discrepancy between the two sometimes occurs. Which of the two is more useful in clinical practice may depend on the objectives of assessing liver fibrosis.

The current study by Isgro et al. showed that collagen proportionate area (CPA) has a better relationship with liver stiffness measurement (LSM) and with hepatic venous pressure gradient (HVPG) compared with the Ishak stage. They also reported that CPA at 1-year post-transplantation in hepatitis C virus-infected patients predicts subsequent clinical decompensation more accurately than Ishak stage or HVPG [5]. They conclude that CPA should be the histological parameter with which to compare LSM and other non-invasive fibrosis markers and also be used to subclassify cirrhosis.

Nitta et al. [6] also reported the good correlation between LSM and fibrosis area measured by image analysis in the patients with chronic hepatitis C, while LSM and Metavir score yielded better correlation. Xie et al. [7] reported that fibrosis area measured by image analysis significantly correlated with model for end-stage liver disease score, serum bilirubin levels and prothrombin time in the patients with hepatitis B virus-related decompensated cirrhosis.

Arima et al. [8] reported that 42 % of chronic hepatitis C patients with pretreatment F3-4 who obtained sustained virological response by interferon (IFN) therapy had decreased fibrosis assessed by the numerical staging system, while the fibrosis area measured by image analysis decreased in 92 %. Thus the computer-assisted image analysis of liver fibrosis is more sensitive to measure the reduction of liver fibrosis after IFN treatment than the numerical system.

In conclusion, the relative fibrosis area measured by computer-assisted image analysis is suitable for the comparison with newly developing non-invasive methods for fibrosis assessment, such as LSM. It is also useful to assess the degree of severe fibrosis in cirrhosis for predicting prognosis and to assess the change of fibrosis after antiviral treatment or in natural courses. It is better to add computer-assisted image analysis to the interpretation of liver biopsy in order to obtain valuable quantitative information in the specimens. The standardization and simplification of the method is needed in order that computer-assisted image analysis of fibrosis area will be widely used.

This comment refers to the article available at doi:10.1007/s00535-012-0694-9.

K. Yoshioka (✉)
Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Toyoake, Aichi, Japan
e-mail: kyoshiok@fujita-hu.ac.jp

Published online: 15 December 2012

Conflict of interest K. Yoshioka received research grants from MSD K.K. and serves as a consultant to SANWA KAGAKU KENKYUSHO CO., LTD.

References

1. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol. 1991;13:372–4.
2. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20:15–20.
3. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–9.
4. Goodman ZD, Becker RL Jr, Pockros PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. Hepatology. 2007;45:886–94.
5. Manousou P, Dhillon AP, Isgro G, Calvaruso V, Luong TV, Tsochatzis E, et al. Digital image analysis of liver collagen predicts clinical outcome of recurrent hepatitis C virus 1 year after liver transplantation. Liver Transpl. 2011;17:178–88.
6. Nitta Y, Kawabe N, Hashimoto S, Harata M, Komura N, Kobayashi K, 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.
7. Xie SB, Ma C, Lin CS, Zhang Y, Zhu JY, Ke WM. Collagen proportionate area of liver tissue determined by digital image analysis in patients with HBV-related decompensated cirrhosis. Hepatobiliary Pancreat Dis Int. 2011;10:497–501.
8. Arima M, Terao H, Kashima K, Arita T, Nasu M, Nishizono A. Regression of liver fibrosis in cases of chronic liver disease type C: quantitative evaluation by using computed image analysis. Intern Med. 2004;43:902–10.

栄養
評価と治療

別刷

メディカルレビュー社

〒541-0046 大阪市中央区平野町3-2-8 淀屋橋MIビル TEL06-6223-1468
〒113-0034 東京都文京区湯島3-19-11 湯島ファーストビル TEL03-3835-3041

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

中野若香奈[1]／池　夏希[1]／石渡朝子[1]／伊藤広子[1]／菱田麻由佳[1]／伊藤志歩[1]
花下順子[1]／池田綾子[1]／原田雅生[2]／川部直人[2]／橋本千樹[2]／吉岡健太郎[2]
Wakana Nakano／Natsuki Ike／Asako Ishiwatari／Hiroko Ito／Mayuka Hishida／Shiho Ito／Junko Hanashita／Ayako Ikeda／Masao Harata／Naoto Kawabe／Senju Hashimoto／Kentaro Yoshioka

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。

1：藤田保健衛生大学病院食養部，2：藤田保健衛生大学肝胆膵内科

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

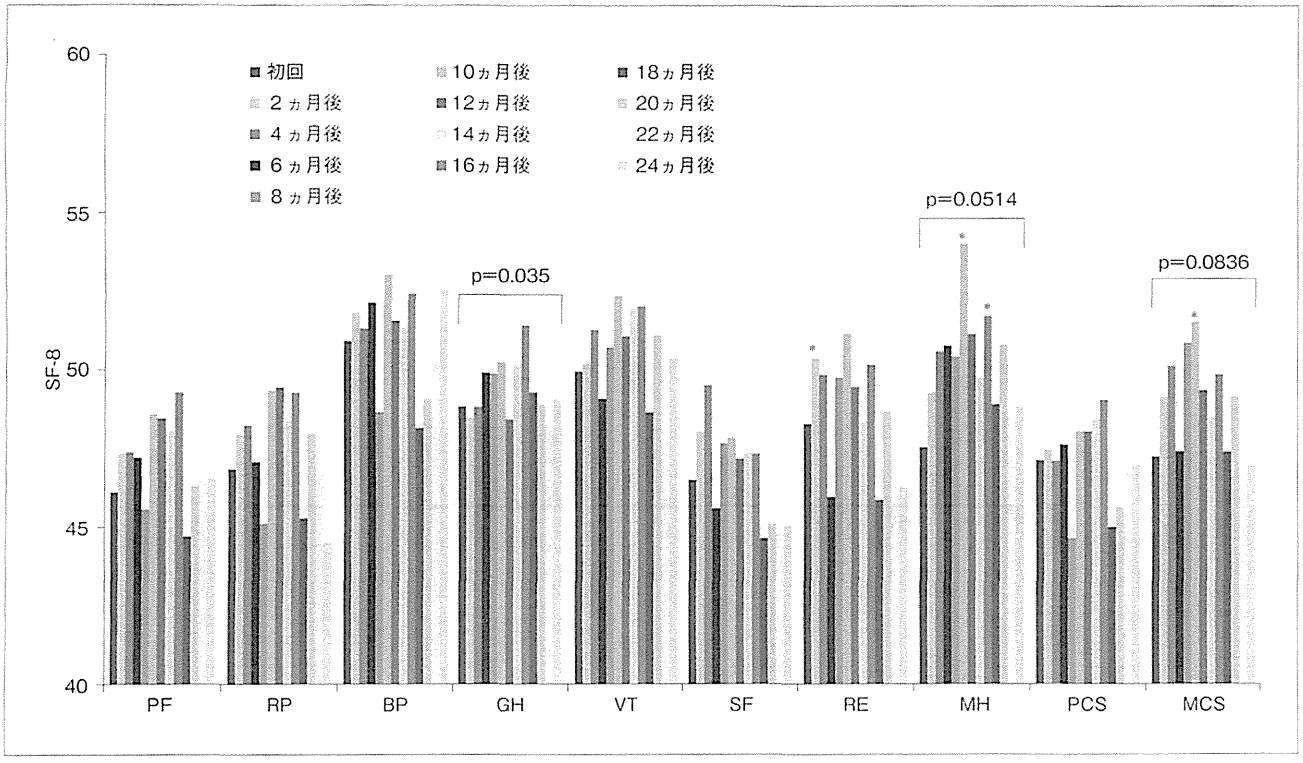

図1．全症例における栄養介入前後のSF-8の変化（6ヵ月ごとでみたANOVA分析結果）

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[11]。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

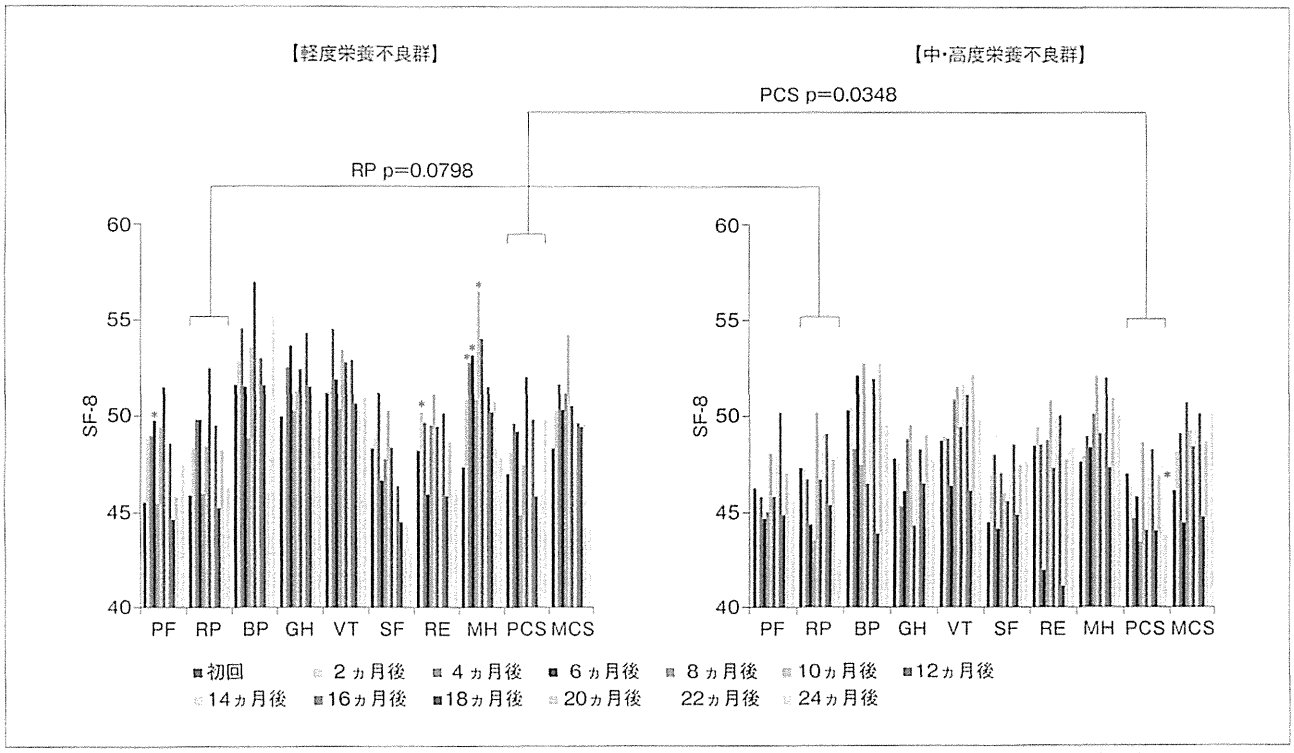

図２．MIによる軽度栄養不良群と中・高度栄養不良群の栄養介入後SF-8の比較（６ヵ月ごとでみたANOVA分析結果）

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）

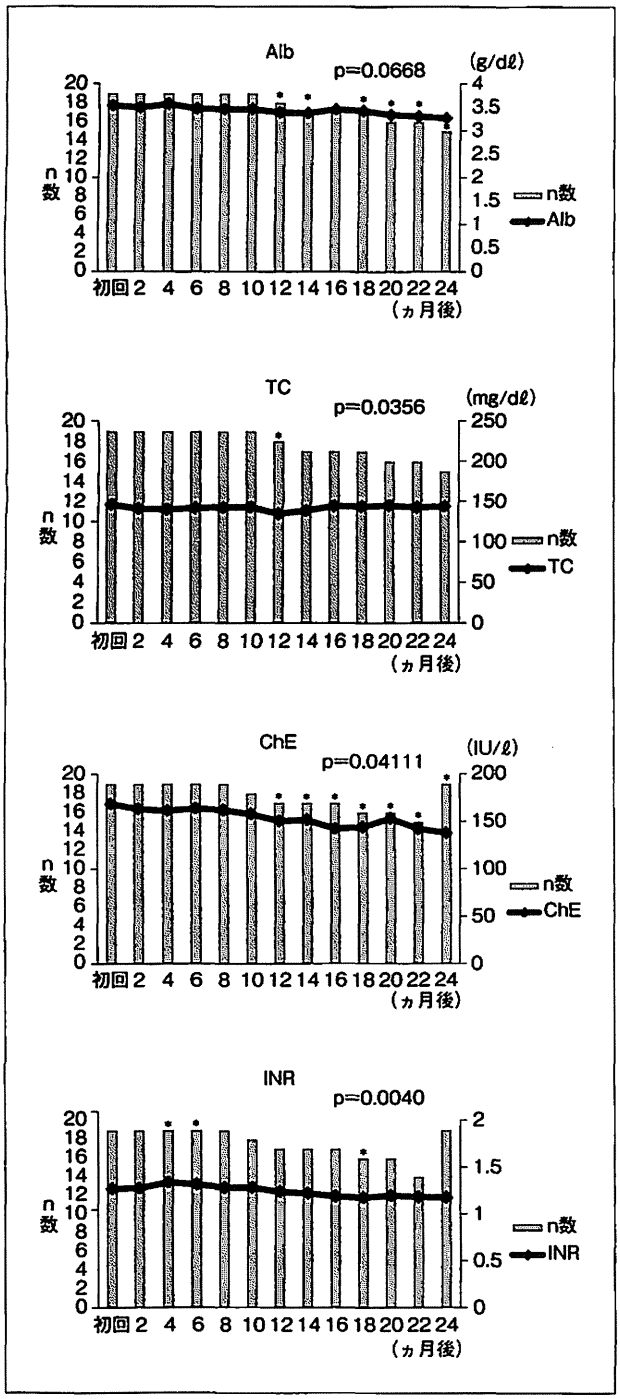

図3．摂食不良群の栄養介入前後変化（6ヵ月ごとでみたANOVA分析結果）

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

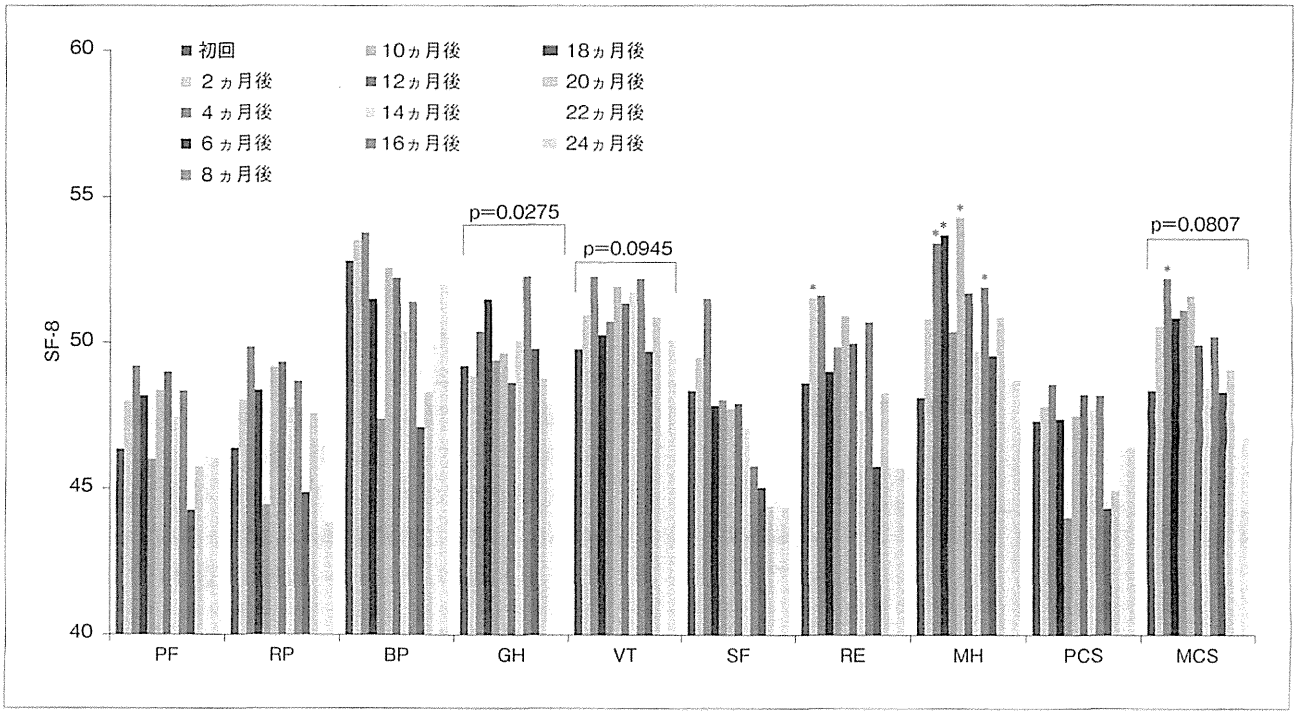

図4. 摂食不良群の栄養介入前後のSF-8の変化（6ヵ月ごとでみたANOVA分析結果）

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

Hepatology Research

Hepatology Research 2012; 42: 233–240 doi: 10.1111/j.1872-034X.2011.00928.x

Review Article

Can non-invasive assessment of liver fibrosis replace liver biopsy?

Kentaro Yoshioka and Senju Hashimoto

Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Aichi, Japan

Transient elastgraphy, acoustic radiation force impulse 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 the information on inflammatory activity, steatosis, iron deposition or other findings derived from 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. However they provide us useful clinical information that liver biopsy has been providing us, 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, steatosis and iron deposition in the liver have been developed. Thus in the near future, non-invasive methods will replace liver biopsy.

Key words: acoustic radiation force impulse, fibrosis stage, inflammatory activity, liver stiffness, real-time elastography, transient elastography

INTRODUCTION

NON-INVASIVE ASSESSMENT OF liver fibrosis has been one of major objectives in the society of hepatologists for a long time. Routine laboratory tests, serum markers of fibrosis[1–7] and apparatuses for measuring liver stiffness (LS) have been tested. The apparatuses include transient elastography (TE),[8,9] acoustic radiation force impulse (ARFI),[10] real-time elastography,[11] and magnetic resonance imaging (MRI).[12]

Liver biopsy is the gold standard for the assessment of fibrosis stage in chronic viral hepatitis. However, liver biopsy is an invasive and expensive procedure, and its accuracy is sometimes questionable because of sampling errors, inadequate specimens and the subjectivity of diagnosis.[13,14]

Infections of hepatitis B virus (HBV) and hepatitis C virus (HCV) are world-wide problems and cause the need of a great number of liver biopsies mainly for assessment of fibrosis stage and inflammatory activity, which sometimes cause serious complications. Thus the replacement of liver biopsies with non-invasive methods is an important subject to be deal with as soon as possible.

In this article, we review the manuscripts that applied non-invasive methods to estimate fibrosis stages for the five different clinical aims in the replacement of liver biopsies. These aims include the determination of 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. We will discuss whether non-invasive methods can replace liver biopsies for these aims.

We discuss the three methods that have been often reported; TE, ARFI imaging, and real-time elastography. Algorithm of serum fibrosis markers such as FibroTest[2] will be also described. There have been published a lot of manuscripts on non-invasive methods, and we selected the manuscripts that seem to us to be important in discussing whether non-invasive methods can replace liver biopsies.

Transient elastography measures LS with the use of an apparatus, FibroScan (EchoSens, Paris, France).[8] FibroScan is equipped with a probe including an

Correspondence: Dr Kentaro Yoshioka, Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi 470-1192, Japan. Email: kyoshiok@fujita-hu.ac.jp

Received 29 August 2011; revision 28 September 2011; accepted 11 October 2011.

ultrasonic transducer and a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on the body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. LS is calculated from velocity and expressed in kilopascal (kPa).

Acoustic radiation force impulse imaging is a radiation force-based imaging method that is provided with conventional B-mode ultrasonography (Siemens Acuson S2000, Siemens AG, Germany).[10] In ARFI imaging, an initial ultrasonic pulse is transmitted at diagnostic intensity levels to obtain a baseline signal for later comparison. A short-duration, high-intensity acoustic pushing pulse is transmitted from the probe, and cause shear wave in the liver. A series of diagnostic intensity pulses are used to quantitate shear wave velocity (Vs; m/s). The velocity of the shear wave depends on LS.

Real-time elastography is an imaging technique that can reveal the physical property of tissue using conventional ultrasound probes; the Hitachi EUB-8500 and EUB-900 machines (Hitachi Medical Systems, Tokyo, Japan).[11] The region of interest is divided up in to 30 000 finite elements before compression. During the compression by the probe or heart beats, the displacement of each element is measured. In hard tissue, the amount of displacement is low, whereas in soft tissue, the amount of displacement is high. The calculation of tissue elasticity distribution is performed in real time, and the results are displayed as color-coded images with the conventional B-mode image in the background. In this way, a large number of summarizing variables were obtained to characterize elastography. The final score was based on 10 summarizing variables selected from them to obtain high reproducibility. The variables selected for the final score differ among the investigators.

APPROPRIATE TIME TO START ANTIVIRAL THERAPY: DIAGNOSIS OF SIGNIFICANT FIBROSIS (F> OR =2)

IN CHRONIC VIRAL hepatitis, the presence of significant fibrosis (F> or =2) indicates the need of antiviral therapies both in chronic hepatitis B and in chronic hepatitis C.[15,16]

A meta-analysis of the performance of TE for staging of liver fibrosis demonstrated that the area under the receiver operating characteristic curve (AUROC) for significant fibrosis ranged 0.68–1.0 among different studies with a mean of 0.84 (95% confidence intervals [CI], 0.82–0.86) and an adjusted AUROC of 0.91 and that the optimal cut-off value for the significant fibrosis suggested from the summary ROC techniques was 7.65 kilopascals (kPa).[17]

We published a review article on the investigations of TE for assessment of fibrosis stages and presented the summary table.[18] Thus we do not show the table in the present article.

Friedrich-Rust *et al.* studied 134 patients with chronic liver diseases and reported that the AUROC for the diagnosis of significant fibrosis of real-time elastography, TE and FibroTest was 0.69, 0.84 and 0.85, respectively.[19]

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C[20]. The elastic ratio (ratio of the value in the intrahepatic venous small vessels divided by the value in the hepatic parenchyma) was calculated. The cut-off value and AUROC for significant fibrosis were 2.73 and 0.89, respectively.

Although real-time elastography is a hopeful noninvasive method, the calculations of elastic value differ among the investigators. Thus we think it is inappropriate to present the summary table.

Friedrich-Rust *et al.* studied 86 patients with chronic viral hepatitis and reported that the AUROC for the diagnosis of significant fibrosis of ARFI, TE, and FibroTest was 0.82, 0.84, and 0.82, respectively.[10] The cut-off values for significant fibrosis of ARFI and TE were 1.37 m/s (sensitivity 68.5%, specificity 92.6%) and 6.3 kPa (sensitivity 83.3%, specificity 74.1%), respectively.

Takahashi *et al*[21] studied 55 patients mainly consisting of people with HCV by ARFI. The AUROC and cut-off value of the Vs for significant fibrosis were 0.94 (95% CI, 0.87–0.99) and 1.34 m/s (sensitivity 91.4%, specificity 80%).

Fierbinteanu-Braticevici[22] studied 74 patients with HCV by ARFI. The AUROC and cut-off value of Vs for significant fibrosis were 0.902 (95% CI, 0.831–0.972, $P < 0.001$) and 1.215 m/s (sensitivity 100%, specificity 71%).

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

Generally the diagnostic accuracy of test with AUROC of 0.7–0.8 was considered as good, that of 0.8–0.9 as very good, and that of 0.9–1.0 as excellent. The diagnostic accuracy of TE, ARFI and real-time elastography for significant fibrosis is very good or excellent. They do not give us the estimation completely corresponding to that of liver biopsy; in our study (AUROC 0.88; sensitivity 81%;

Table 1 Summary of investigations of acoustic radiation force impulse for assessment of liver fibrosis

Author (year) reference	Disease	Number of patients	System of fibrosis staging	Fibrosis stage							
				F> or =1		F> or =2		F> or =3		F> or =4	
				Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC
Friedrich-Rust (2009)[10]	Chronic viral hepatitis	86	Metavir			1.37	0.82	1.45	0.91	1.75	0.91
Lupsor (2009)[23]	HCV	112	Metavir	1.19	0.725	1.34	0.869	1.61	0.9	2	0.936
Takahashi (2009)[21]	Chronic liver disease	55	Metavir			1.34	0.94	1.44	0.94	1.8	0.96
Fierbinteanu-Braticevici (2009)[22]	HCV	74	Metavir	1.185		1.215	0.902	1.54	0.993	1.94	0.993
Sporea (2011)[27]	Chronic liver disease	76	Metavir	1.4	0.747					1.78	0.951
Grgurevic (2011)[28]	Chronic liver disease	38	Ishak							1.86	0.99
Sporea (2010)[24]	Chronic viral hepatitis	71	Metavir			1.33	0.649			1.8	0.868
Toshima (2011)[25]	Chronic liver disease	79	Scheuer	1.45	0.81	1.52	0.81	1.69	0.85	1.79	0.87
Piscaglie (2011)[29]	Chronic liver disease	90								1.75	0.941
Ebinuma (2011)[26]	Chronic viral hepatitis	59	Metavir			1.4	0.905	1.53	0.923	1.88	0.854

AUROC, area under the receiver operating characteristic curve; HCV, hepatitis C virus.

specificity 80%), 17 of 42 patients with biopsy proven F2 (40%) had LS by TE corresponding to F0-1.[30] However, they are still useful for determining the indication of antiviral therapies, if we use them in combination with laboratory tests and other clinical data. The combination of TE and biomarkers is being studied to improve the diagnostic accuracy of significant fibrosis.[30,31]

PREDICTION OF RESPONSE TO ANTIVIRAL THERAPY

FIBROSIS STAGE IS an important predictor for response to combination therapy of pegylated interferon (PEG-IFN) and ribavirin for chronic hepatitis C. Hayashi *et al.* reported that the factors related to sustained virological response (SVR) on multivariate analysis were single nucleotide polymorphism (SNP) of interleukin 28B (IL28B) ($P = 0.0001$), fibrosis ($P = 0.0111$) and mutations in the core region70 ($P = 0.0267$) and IFN sensitivity determining region (ISDR) of HCV genome ($P = 0.0408$).[32]

Poynard *et al.* studied the predictive factors for SVR in 1459 patients with chronic hepatitis C retreated with PEG-IFN alfa-2b plus weight-based ribavirin. Uni- (UV) and multi-variable (MV) analyses were performed. Five baseline factors were associated ($P < 0.001$) with SVR in UV and MV analyses (odds ratio: UV/MV): fibrosis stage estimated using FibroTest (4.5/5.9) or biopsy (1.5/1.6), genotype 2/3 (4.5/5.1), viral load (1.5/1.3), prior relapse (1.6/1.6), previous treatment with non-PEG-IFN (2.6/2.0). Poynard *et al.* concluded that FibroTest at baseline is a possible non-invasive alternative to biopsy for the prediction of SVR, in patients with previous failures and advanced fibrosis, retreated with PEG-IFN alfa-2b and ribavirin.[33]

We have studied the predictive factors for SVR in 88 patients with chronic hepatitis C genotype 1 treated with combination of IFN and ribavirin and found that gender ($\beta = 1.6$, $P = 0.0012$) and LS by TE ($\beta = -0.1$, $P = 0.0214$) are independent predictive factors by multivariate analysis (manuscript in preparation).

Thus FibroTest and LS by TE can substitute liver biopsy for the purpose of predicting response to antiviral therapy in chronic hepatitis C.

EVALUATION OF EFFECTS OF ANTIVIRAL THERAPY

THE OUTCOME OF antiviral therapy should be assessed not only by ALT levels or viral loads but

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.