18. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104
19. Ge D, Fellay J, Thompson AJ, Simon JS, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401
20. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801
21 Ogura S, Akuta N, Hirakawa M, et al. Virological and biochemical features in elderly HCV patients with hepatocellular carcinoma: amino acid substitutions in HCV core region as predictor of mortality after first treatment. Intervirology 2009;52:179–188
22. Nakamoto S, Imazeki F, Fukai K, et al. Association between mutations in the core region of hepatitis C virus genotype 1 and hepatocellular carcinoma development. J Hepatol 2010;52:72–78
23. Kobayashi M, Akuta N, Suzuki F, et al. Influence of amino-acid polymorphism in the core protein on progression of liver disease in patients infected with hepatitis C virus genotype 1b. J Med Virol 2010;82:41–48
24. Hu Z, Muroyama R, Kowatari N, Chang J, Omata M, Kato N. Characteristic mutations in hepatitis C virus core gene related to the occurrence of hepatocellular carcinoma. Cancer Sci 2009;100:2465–2468
25. Akuta N, Suzuki F, Kawamura Y, et al. Substitution of amino acid 70 in the hepatitis C virus core region of genotype 1b is an important predictor of elevated alpha-fetoprotein in patients without hepatocellular carcinoma. J Med Virol 2008;80:1354–1362

Hepatol Int
DOI 10.1007/s12072-011-9306-7

ORIGINAL ARTICLE

Characterization of naturally occurring protease inhibitor-resistance mutations in genotype 1b hepatitis C virus patients

Hiroko Shindo · Shinya Maekawa · Kazuki Komase · Ryota Sueki · Mika Miura · Makoto Kadokura · Kuniaki Shindo · Fumitake Amemiya · Takatoshi Kitamura · Yasuhiro Nakayama · Taisuke Inoue · Minoru Sakamoto · Shun-ichi Okada · Yasuhiro Asahina · Namiki Izumi · Masao Honda · Shuichi Kaneko · Nobuyuki Enomoto

Received: 21 April 2011 / Accepted: 22 July 2011

Abstract

Background and aims Protease inhibitor (PI)-resistant hepatitis C virus (HCV) variants may be present in substantial numbers in PI-untreated patients according to recent reports. However, influence of these viruses in the clinical course of chronic hepatitis C has not been well characterized.

Methods The dominant HCV nonstructural 3 (NS3) amino acid sequences were determined in 261 HCV genotype 1b-infected Japanese patients before pegylated interferon plus ribavirin (PEG-IFN/RBV) therapy, and investigated the patients' clinical characteristics as well as treatment responses including sustained virological response (SVR) rate. HCV-NS3 sequences were also determined in 39 non-SVR patients after completion of the therapy.

Results Four single mutations (T54S, Q80K, I153V, and D168E) known to confer PI resistance were found in 35 of 261 patients (13.4%), and double mutations (I153V plus T54S/D168E) were found in 6 patients (2.3%). Responses to PEG-IFN/RBV therapy did not differ between patients with and without PI-resistance mutations (mutation group, SVR 48%; wild-type group, SVR 40%; $P = 0.38$). On the other hand, two mutations appeared in two non-SVR patients after PEG-IFN/RBV therapy (I153V and E168D, 5.1%).

Conclusions PI-resistance-associated NS3 mutations exist in a substantial proportion of untreated HCV-1b-infected patients. The impact of these mutations in the treatment of PIs is unclear, but clinicians should pay attention to avoid further development of PI resistance.

Keywords HCV · Protease inhibitor · Naturally occurring viral resistance mutations

Introduction

Hepatitis C virus (HCV) infects more than 170 million persons worldwide and thus represents a global health problem. At least 130 million infected individuals are chronic carriers of HCV and are at significant risk of developing liver cirrhosis and hepatocellular carcinoma [1]. The current standard treatment with pegylated interferon plus ribavirin (PEG-IFN/RBV) is complicated by frequent adverse reactions, and a sustained virologic response (SVR) can be achieved only in 50% of patients infected with the most prevalent genotype 1 [2]. In Japan, since 70% of patients are infected with intractable genotype 1b HCV, more effective treatments are urgently required.

A promising approach is the development of specifically targeted antiviral therapies for hepatitis C (STAT-C). HCV-specific protease inhibitors (PIs) target an essential step in HCV replication by blocking the nonstructural 3/4A (NS3/4A) protease-dependent cleavage of the HCV polyprotein

Electronic supplementary material The online version of this article (doi:10.1007/s12072-011-9306-7) contains supplementary material, which is available to authorized users.

H. Shindo · S. Maekawa (✉) · K. Komase · R. Sueki · M. Miura · M. Kadokura · K. Shindo · F. Amemiya · T. Kitamura · Y. Nakayama · T. Inoue · M. Sakamoto · S. Okada · N. Enomoto
First Department of Internal Medicine, University of Yamanashi, 1110, Shimokato, Chuo, Yamanashi 409-3898, Japan
e-mail: maekawa@yamanashi.ac.jp

Y. Asahina · N. Izumi
Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan

M. Honda · S. Kaneko
Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

Published online: 18 August 2011

[1]. Among these NS3/4A PIs, telaprevir, boceprevir, SCH446211, danoprevir (ITMN-191), nalaprevir (SCH900 518), and TMC435 are now under clinical trials [1, 3–7]. In PROVE1 and PROVE2 studies [3, 4] undertaken in North America and Europe, the SVR rate was favorable (67 and 69%, respectively) in a triple therapy regimen including telaprevir. In addition, some studies have suggested that shortening of treatment duration may be possible for patients who achieve a rapid virologic response (RVR) [8, 9].

However the sole use of STAT-C drugs, such as PIs, promotes production and selection of drug-resistant variants in patients experiencing viral rebound during treatment [3, 10, 11] as well as in HCV replicon experiments [11, 12]. Therefore, these drugs should be used in combination with the PEG-IFN/RBV to prevent the appearance of drug-resistant variants. However, Kuntzen et al. [13] demonstrated the presence of these drug-resistant variants in high frequencies (8.6–16.2%) by population-based sequencing in patients not treated with the drugs [1, 13]. Gaudieri et al. [14] have suggested that regions of NS3 protease and NS5B polymerase are likely to be under HLA immune pressure and therapeutic selection, and that drug-resistant variants may occur naturally to escape the immune system. These observations seem quite astonishing and troubling, since a substantial number of patients may not respond to the new therapies such as STAT-C drugs.

In the present study, to assess the prevalence of NS3 mutations conferring PI resistance in HCV genotype 1b-infected Japanese patients who had not been previously treated with PIs, as well as to assess the influence of those mutations in response to PEG-IFN/RBV therapy, the dominant HCV-NS3 sequences were determined in 261 HCV-1b patients before starting the PEG-IFN/RBV therapy.

Methods

Patients

Serum samples were acquired from 261 HCV genotype 1b-infected adult Japanese patients before combination therapy with PEG-IFN (PEGINTRON®, Schering-Plough, Tokyo, Japan) plus RBV (REBETOL®, Schering-Plough) between 2004 and 2008 at the University of Yamanashi, Musashino Red Cross Hospital and Kanazawa University. The therapy was administered according to the standard PEG-IFN/RBV treatment protocol established for Japanese patients by a hepatitis study group of the Ministry of Health, Labor, and Welfare, Japan. Specifically, the patients were subcutaneously administered PEG-IFNα-2b, 1.5 μg/kg body weight, once weekly and RBV 600–800 mg daily per os for 48 weeks. These patients were not infected with human immunodeficiency virus (HIV). The study was approved by the ethics committees of all participating universities and the hospital, and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Institutional Review Board at Massachusetts General Hospital. Written informed consent was obtained from each study participant.

Amplification and sequencing of full-length HCV genomes

Viral loads were determined using the Amplicor HCV RNA kit, version 2.0 (Roche Diagnostics, Tokyo, Japan) or the Cobas TaqMan test (Roche Diagnostics). HCV RNA was extracted from pretreatment serum samples by the AGPC method using Isogen (Wako, Osaka, Japan) according to the manufacturer's protocol. Complementary DNA was synthesised using Superscript II (Invitrogen, Tokyo, Japan) and random primers (Invitrogen), and then amplified by two-step nested PCR using the primers listed in Supplementary Table 1. All samples were initially denatured at 95°C for 7 min, followed by 40 cycles of amplification with denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 45 s using the BD Advantage™ 2 PCR Enzyme system (BD Biosciences Clontech, CA, USA). PCR amplicons were directly sequenced using BigDye Terminator version 3.1 (ABI, Tokyo, Japan) and universal M13 forward/reverse primers using an ABI prism 3130 sequencer (ABI).

Sequence alignment and analysis

Sequences were determined in both directions, particularly for the ambiguous stretches, were assembled using the Vector NTI software (Invitrogen), and base-calling errors were corrected following the inspection of chromatograms. If mixed bases were detected as two different chromatogram peaks at the same residue, only the dominant base was called after evaluation of all overlapping fragments. A consensus sequence was generated from the alignment on the basis of the most common amino acid at each site.

Determination of PI resistance mutations

Multiple viral NS3 mutations were observed in amino acid positions reported to confer PI resistance among 261 patients: V36, Q41, F43, T54, V55, Q80, R109, I153, R155, A156, D168, V170, and M175. NS3 amino acid mutations with proven PI resistance in previously published studies (Table 1) were designated as resistance proven mutations (e.g., V36M/A). Mutations in the PI-resistance site not known to confer drug resistance were designated resistance unproven mutations (e.g., V36I). Patients were allocated to two groups according to the presence of PI-resistance

mutations (including resistance unproven mutations), and clinical characteristics including HCV RNA levels and responses to PEG-IFN/RBV therapy were compared. To assess the influence of PEG-IFN/RBV therapy on NS3 mutational status, posttreatment HCV-NS3 sequences in 39 of 58 non-SVR patients were also examined.

Statistical analysis

Statistical differences in the data, including all available patients' demographic, biochemic, hematologic, and virologic data such as sequence variation factors, were determined among the various groups by Student's t test or Mann–Whitney U test for numerical variables and Fisher's exact probability test for categorical variables.

Results

Prevalence of dominant PI-resistance-associated nonstructural 3 mutations in untreated patients

Figure 1 shows the frequency of substitutions in 261 patients for each of 181 NS3 protease amino acid residues compared to the consensus sequence. A total of 41 resistance proven mutations were detected in 35 (13.4%) patients: T54S (14 patients, 5.4%), Q80K (1 patient, 0.4%), I153V (22 patients, 8.4%), D168E (4 patients, 1.5%), T54S plus I153V double mutation (4 patients, 1.5%), and I153V plus D168E double mutation (2 patients, 0.8%). The mutation number increased to 54 in 47 (18.0%) patients when resistance unproven mutations were included: V36I (2 patients, 0.8%), I153L (11 patients, 4.2%), and I153V plus V36I double mutation (2 patients, 1.5%). Double mutations were found in 7 patients (2.7%) (Table 1). Q80L was observed in 47 (18%) patients but these were excluded from consideration because a previous study demonstrated that this mutation does not confer resistance [15]. All mutations observed in this study would confer low- to moderate-level PI resistance according to previous studies [6, 15–19]. No mutations conferring high-level resistance such as R155 or A156 [11, 17, 19–22] were observed.

Clinical characteristics of patients with PI-resistance mutations

Table 2 presents the characteristics of patients classified according to the presence of PI-resistance mutations

Table 1 Prevalence of PI-resistance-associated NS3 mutations

Drug-resistance mutations described in the literature				Detected resistance mutations
NS3 residue	Resistance mutations	Drugs	References	Genotype 1b ($N = 261$), (%)
V36	A, M, L, G, C	Telaprevir, Boceprevir	[1, 3, 4, 10, 11, 19, 31, 37]	I × 2 (0.8)
Q41	R	ITMN-191, Boceprevir	[19]	
F43	S, C	ITMN-191, Boceprevir, Telaprevir, TMC435	[15, 19]	
T54	A, S	Telaprevir, Boceprevir, SCH900518	[1, 3, 10, 11, 19, 20, 31, 38]	S × 14 (5.4)
V55	A	Boceprevir	[1]	
Q80	R, K	TMC435	[6, 15]	K × 1 (0.4)
R109	K	SCH446211	[17]	
I153	V	SCH446211	[17]	V × 22 (8.4), L ×11 (4.2)
R155	K, T, I, M, G, L, S, Q	Telaprevir, Boceprevir, ITMN-191, BILN2061, TMC435	[1, 3, 4, 6, 10, 11, 15, 19, 20]	
A156	S, T, V, I, G	Telaprevir, Boceprevir, ITMN-191, BILN2061, SCH446211, TMC435, SCH900518	[1, 3, 4, 10, 11, 15, 17, 19, 20, 38]	
D168	A, V, E, N, T, H	BILN2061, ITMN-191, TMC435	[6, 15, 20]	E × 4 (1.5)
V170	A	Telaprevir, Boceprevir	[1, 19, 20]	
M175	L	Boceprevir	[39]	
Total number (%) of patients with resistance proven mutations				35 (13.4)
Total number (%) of patients with resistance proven and unproven mutations				47 (18.0)

Amino acid mutations conferring PI resistance in the literatures and those observed in PI-treatment-naive patients in this study are indicated. Bold indicates resistance proven mutations, and the others indicate resistance unproven mutations

Double mutations found were as follows: V36I and I153V × 1, T54S and I153V × 4, I153V and D168E × 2

(including resistance unproven mutations). Age, sex ratio, body mass index, alanine aminotransferase (ALT) levels, serum albumin, platelet count, and fibrosis stage did not differ between the NS3 mutation and wild-type groups. No significant difference was observed between the two groups in the parameters of PEG-IFN/RBV treatment response, HCV sequence variations in interferon sensitivity determining region (ISDR), Core 70, interferon plus ribavirin resistance-determining region (IRRDR), or interleukin 28B (IL28B) single nucleotide polymorphism (SNP) (rs8099917; T/G and G/G vs. T/T) [23–30]. These clinical variables were also compared between the mutation group defined as resistance proven mutations and the wild-type group, but no notable differences were observed.

Unimpaired in vivo fitness of viral strains with resistance mutations

Because most PI-resistance mutations described till date have been associated with reduced replicative capacity of varying degrees [1, 10, 11, 13, 17, 20–22, 31, 32], we examined viral replication levels in patients with drug-resistance mutations (Fig. 2). The estimated P value indicated no significant difference between the mutation (median 1,500 KIU/ml) and wild-type (median 1,800 KIU/ml) groups ($P = 0.69$). The results indicate that drug-resistant HCVs were not necessarily impaired in their ability to replicate in vivo. However, patients with double mutations ($N = 7$) tended to have low viral loads (median 1,200 KIU/ml) ($P = 0.09$).

Resistance mutations and virologic response to PEG-IFN/RBV therapy

To determine the difference in virologic response to PEG-IFN/RBV therapy according to the PI mutation, frequency of HCV RNA levels below detection at 4 weeks (rapid viral response, RVR) and 12 weeks (complete early viral response, cEVR), and SVR rate (%) were investigated in each group. The frequency of HCV RNA levels below detection at 4 and 12 weeks was 14 and 50%, respectively, in the mutation group, and was 11 and 46%, respectively, in the wild-type group. The SVR rate was 48 and 40% in the mutation and wild-type groups, respectively ($P = 0.38$). No significant difference was observed between the two groups in any of the indexes investigated (Table 2). The time-dependent viral clearance rate during PEG-IFN/RBV therapy was estimated in 133 patients including 25 patients (19%) with PI-resistance mutations available for the analysis. Kaplan–Meier analysis demonstrated that HCV clearance did not differ between the two groups with and without resistance mutations (log-rank test, $P = 0.30$) (Fig. 3).

Changes in nonstructural 3 amino acid sequence diversity during PEG-IFN/RBV therapy

Full-length NS3 protease sequences were determined in 39 non-SVR patients after PEG-IFN/RBV therapy. A single amino acid change at resistance-associated sites in two patients was observed. In one patient, isoleucine (Ile) at position 153 changed to valine (Val), and glutamic acid (Glu) changed to aspartic acid (Asp) at position 168 in the second (Fig. 4). At the nucleotide level, ATC (Ile) changed to GTC (Val) in I153V, and GAA (Glu) changed to GAC (Asp) in E168D. Both mutations were caused by one nucleotide exchange. No other changes were observed in the other 37 patients.

Discussion

Here we report that in 18% (47/261) HCV genotype 1b-infected patients who had not been previously treated with NS3 PIs, the viral genome contained dominant amino acid mutations within the NS3 PI-resistance sites. Even after confining the data to established PI-resistance mutations, the mutation rate was still significant in 13.4% (35/261). No clinical differences were observed between patients

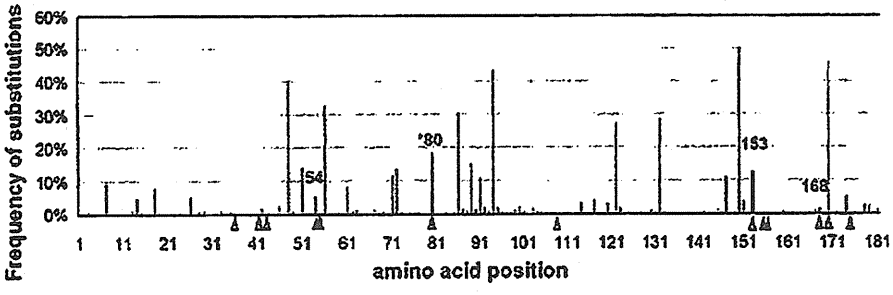


Fig. 1 Frequency of polymorphic mutations for each of the 181 NS3 protease amino acid residues in 261 patients. *Arrowheads* indicate the sites reported to confer PI resistance. *Dark bars* denote the amino acid variations at the resistant sites in this study. **80*, we detected one resistant mutation (Q80K) and 47 (18%) non-resistant variations (Q80L) at the 80th residue

Table 2 Characteristics of patients with or without HCV genomes harboring drug-resistance mutations

Characteristics	Mutation type ($N = 47$)	Wild-type ($N = 214$)	P value
Patients' characteristics			
Age, median (range)	59 (46–72)	57 (19–77)	0.17
Male, no. (%)	26 (55)	112 (52)	0.70
BMI, median (range)	23.2 (15.5–31.9)	22.8 (16.1–31.9)	0.41
ALT IU/ml	81.3 ± 72.6[a]	74.8 ± 51.9	0.93
Serum albumin g/dl	4.00 ± 0.37	4.01 ± 0.36	0.81
Platelet count × 10^4/μl	15.8 ± 4.3	14.5 ± 4.8	0.18
HCV RNA KIU/ml, median (range)	1,500 (58–6,310)	1800 (28–15,849)	0.69
Fibrosis, no. (%)			0.97
F0	0 (0)	7 (3)	
F1	23 (50)	89 (42)	
F2	9 (20)	52 (24)	
F3	9 (20)	40 (19)	
F4	5 (11)	26 (12)	
IFN pre-treatment no. (%)	15/40 (38)[b]	66/172 (38)	1.00
IL28B (rs8099917) T/G or G/G no. (%)	6/20 (30)	19/67 (28)	1.00
Response to PEG-IFN/RBV therapy			
SVR total cases no. (%)	22/46 (48)	83/210 (40)	0.38
RVR in total cases no. (%)	6/44 (14)	22/195 (11)	0.83
cEVR in total cases no.(%)	22/44 (50)	92/200 (46)	0.75
SVR 48w treatment no. (%)	16/29 (55)	55/130 (42)	0.29
End of treatment response no. (%)	26/41 (63)	123/202 (61)	0.91
HCV genome sequence variation			
ISDR mutation ≤1 no. (%)	32/46 (70)	167/210 (80)	0.21
Core70 R no. (%)	26/44 (59)	136/210 (65)	0.56
IRRDR mutation >3 no. (%)	25/38 (66)	107/190 (56)	0.34

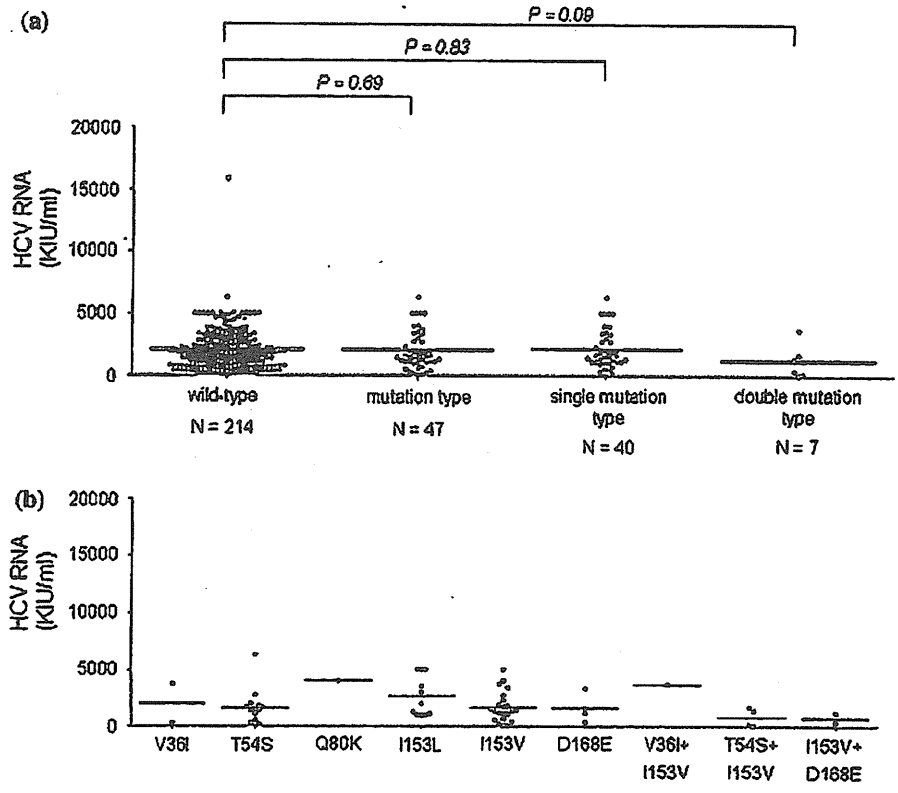
[a] Mean ± SD

[b] Number/total number (%)

harboring viruses with and without these mutations. Moreover, no differences were observed in the responses of either group to PEG-IFN/RBV therapy.

Recent studies reported that significant number of patients who were never treated with PI possess viral sequences with PI-resistance-associated NS3 mutations. In these studies, the prevalence of PI-resistance mutations was determined to be 8.6–16.2% [13, 14], in HCV genotype 1- and 3-infected patients in European–American populations. These patients were often coinfected with HIV. Analysis of the public HCV databases (EuHCVdb and Los Alamos) also reported the presence of naturally occurring PI-resistance-associated NS3 mutations in worldwide isolates [33]. However, in vivo and in vitro studies demonstrated that most of the mutations observed conferred only low- to moderate-level PI resistance [7, 13, 14, 34, 35]. Regarding viral fitness, PI-resistant HCVs show lower fitness at varying degrees as revealed by in vitro studies [1, 10, 11, 17, 20–22, 31, 32], but HCV RNA levels in a clinical study did not differ significantly. The response to PEG-IFN/RBV therapy was almost comparable to that in HCV-infected patients without PI-resistance mutations either in HCV replicon experiments or in a clinical study of small number of treated patients [34].

The prevalence of 13.4% for PI-resistance-proven patients observed in the present study was almost comparable to the results of previous studies. Although HIV is known to increase HCV replication in coinfection with HCV [36], and HIV patients are often treated with the HIV-specific PIs, the HIV infection might not affect the natural occurrence of HCV-specific PI-resistance mutations since our studied patients were all proven to be free from coinfection with HIV infection. As shown in Table 1 and Fig. 1, I153 V (22/261, 8.4%), T54S (14/261, 5.4%), and D168E (4/261, 1.5%) were among the most prevalent PI-resistance-proven mutations in the present study. The most frequent mutation detected in our study I153V was reported to appear secondarily to the occurrence of R109K mutations in a HCV replicon system [17]. Although the role of this mutation is not understood, the I153V mutation on its own conferred SCH446211 resistance to the HCV replicon to a lesser degree [17]. Interestingly, I153V was often found in double mutations in our study, as shown in Fig. 2. This suggests analogy between in vitro and in vivo data. T54S and D168E, the other frequent mutations, have been also reported to occur as single dominant mutations in previous in vitro or in vivo studies in HCV genotype 1



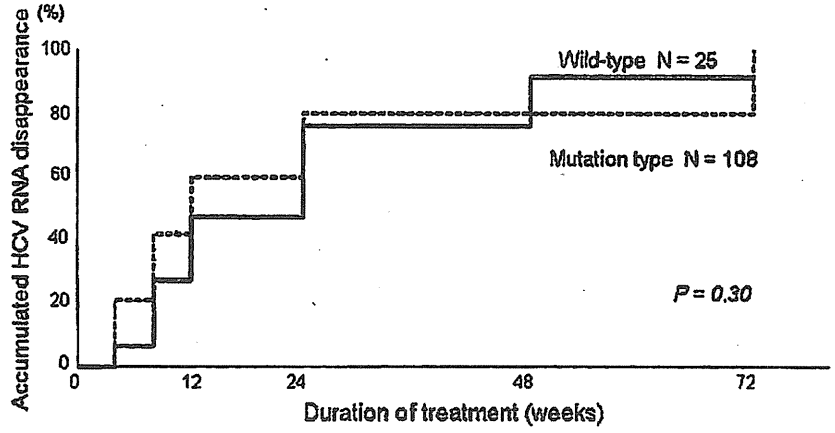


Fig. 2 In vivo fitness of HCV with PI-resistance-associated NS3 mutations. HCV RNA levels were compared between patients with and without NS3 PI-resistance-associated mutations (a) and between patients with each resistance mutation (b). The estimated *P* value (Mann–Whitney *U* test) indicates no significant difference between the wild-type and other groups (wild-type vs. mutation type, wild-type vs. single mutation type, and wild-type vs. double mutation type). (Wild-type, $N = 214$; mutation type, $N = 47$; single mutation type, $N = 40$; double mutation type, $N = 7$; V36I, $N = 2$; T54S, $N = 14$; Q80K, $N = 1$; I153L, $N = 11$; I153V, $N = 22$; D168E, $N = 4$; E176A, $N = 1$; V36I + I153V, $N = 1$; T54S + I153V, $N = 4$, and I153V + D168E, $N = 2$)

Fig. 3 Comparison of virologic response to PEG-IFN/RBV therapy between HCV-infected patients with and without PI-resistance-associated NS3 mutations. Time-dependent HCV clearance rate analysis was based on serum HCV RNA positivity during PEG-IFN/RBV therapy for HCV isolates with resistance mutations or wild-type sequences. A total of 133 patients for whom the limit of viral genome detection could be determined were analyzed. Among this group, NS3 mutations were detected in 25 patients (19%). The estimated *P* value (log-rank test) shows no significant difference between the two groups ($P = 0.30$)

infections showing moderate degrees of resistance [16, 18, 19].

Most PI-resistance mutations described to date have been associated with varying degrees of reduced replicative capacity [10, 11, 17, 20–22, 31, 32]. In the present study, HCV RNA levels of those patients with low- to moderate-level resistance mutations were similar to those in patients in the wild-type groups, suggesting that in vitro viral fitness

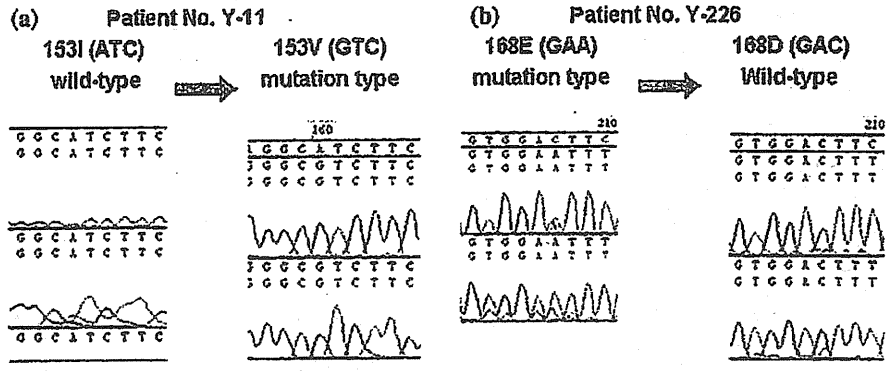


Fig. 4 Appearance of PI-resistance-associated NS3 mutations during the PEG-IFN/RBV therapy. Chromatograms show part of the HCV NS3 sequence demonstrating PI-resistance mutations in two patients receiving therapy. a Site 153 isoleucine (Ile) (ATC) changed to valine (Val) (GTC), b Site 168 glutamic acid (Glu) (GAA) changed to aspartic acid (Asp) (GAC)

does not necessarily reflect in vivo viral fitness. This, however, does not rule out the possibility that some unknown compensatory viral mutations might have resulted in upregulation of reduced viral fitness. Interestingly, although the replicative capacity conferred by a single mutation seemed to be the same, the HCV RNA levels of double mutations were frequently low, suggesting that double mutations might weaken viral fitness.

In previous studies, clinical characteristics representing the state of liver disease other than HCV RNA levels were not studied in patients with PI-resistance mutations. In this study, we show that those clinical characteristics did not differ according to the presence of viral NS3 mutations. As shown in Table 2, age, sex ratio, fibrosis stage, ALT levels, serum albumin, platelet count, and past history of IFN pretreatment did not differ according to the presence of NS3 mutations. These results suggest that NS3 mutations occur independently of disease progression. Moreover, no evident differences were observed between viral and host factors known to affect IFN-based treatment responses. However, viral amino acid variations in the core and NS5A or the allelic frequency of IL28B SNPs, which were recently reported for the close relationship of responses to PEG-IFN/RBV therapy, did not differ between the two groups.

A significant outcome of the present study is the demonstration that PI-resistance mutations might not affect responses to PEG-IFN/RBV therapy. Previous in vitro studies demonstrated that HCV replicons harboring PI-resistance mutations were also sensitive to IFN treatment [31]. In addition, recent clinical studies also indicated that PI-resistance mutations were sensitive to the PEG-IFN/RBV [10, 34]. However, our analysis was more comprehensive because viral and host factors that contribute to treatment responses were simultaneously analyzed. A unique aspect of the present study is that we investigated the influence of the PEG-IFN/RBV treatment on the occurrence of new PI mutations by direct nucleotide sequencing, and were able to show that the PEG-IFN/RBV might not induce amino acid mutations.

Will the pre-existence of naturally occurring PI-resistance mutations have an influence on future treatment of HCV infections? Since new PIs are on the verge of clinical use, all clinicians should bear in mind the substantial numbers of HCV-infected patients with PI-resistance mutations. Although the degree of resistance is considered to be low or moderate in untreated patients, weak resistance might progress to more potent resistance with additional mutations, when PIs become widely used. Therefore, all clinicians need to be sufficiently prepared for the possibility of later onset of PI-resistance mutations that confer greater drug resistance and concomitant poorer responses to therapy. In SPRINT-1 study, the lead-in therapy was associated with a modestly lower rate of breakthrough than with no lead in [7]. Considering that PEG-IFN/RBV was equally effective for PI-resistant viruses, sufficient "lead-in" therapy before the administration of PIs could be an option in the forthcoming triple therapy modality.

In conclusion, we demonstrate here that PI-resistance-associated NS3 mutations exist in a substantial proportion of untreated HCV-1b-infected patients. Although the degree of resistance might not be strong, clinicians will need to consider this upon the introduction of triple therapy.

Acknowledgements This work was supported in part by a grant-in-aid scientific research fund from the Ministry of Education, Science, Sports, and Culture [grant number 20390206] and in part by a grant-in-aid from the Ministry of Health, Labor, and Welfare of Japan [grant number H19-kanen-002].

References

1. Susser S, Welsch C, Wang Y, et al. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. Hepatology 2009;50:1709–1718
2. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355

3. Hezode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850
4. McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827–1838
5. McHutchison JG, Manns MP, Muir AJ, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010; 362:1292–1303
6. Reesink HW, Fanning GC, Farha KA, et al. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. Gastroenterology 2010;138: 913–921
7. Kwo PY, Lawitz EJ, McCone J, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 2010;376:705–716
8. Forestier N, Reesink HW, Weegink CJ, et al. Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. Hepatology 2007;46:640–648
9. Suzuki F, Akuta N, Suzuki Y, et al. Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks. Hepatol Res 2009;39:1056–1063
10. Kieffer TL, Sarrazin C, Miller JS, et al. Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. Hepatology 2007;46: 631–639
11. Sarrazin C, Kieffer TL, Bartels D, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007;132: 1767–1777
12. Lin C, Lin K, Luong YP, et al. In vitro resistance studies of hepatitis C virus serine protease inhibitors, VX-950 and BILN 2061: structural analysis indicates different resistance mechanisms. J Biol Chem 2004;279:17508–17514
13. Kuntzen T, Timm J, Berical A, et al. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. Hepatology 2008;48:1769–1778
14. Gaudieri S, Rauch A, Pfafferott K, et al. Hepatitis C virus drug resistance and immune-driven adaptations: relevance to new antiviral therapy. Hepatology 2009;49:1069–1082
15. Lenz O, Verbinnen T, Lin TI, et al. (2010) In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 54:1878–1887
16. Kieffer TL, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). J Antimicrob Chemother 2010;65:202–212
17. Yi M, Tong X, Skelton A, et al. Mutations conferring resistance to SCH6, a novel hepatitis C virus NS3/4A protease inhibitor. Reduced RNA replication fitness and partial rescue by second-site mutations. J Biol Chem 2006;281:8205–8215
18. Welsch C, Domingues FS, Susser S, et al. Molecular basis of telaprevir resistance due to V36 and T54 mutations in the NS3-4A protease of the hepatitis C virus. Genome Biol 2008;9:R16
19. Tong X, Bogen S, Chase R, et al. Characterization of resistance mutations against HCV ketoamide protease inhibitors. Antiviral Res 2008;77:177–185
20. He Y, King MS, Kempf DJ, et al. Relative replication capacity and selective advantage profiles of protease inhibitor-resistant hepatitis C virus (HCV) NS3 protease mutants in the HCV genotype 1b replicon system. Antimicrob Agents Chemother 2008;52:1101–1110
21. Tong-X, Chase R, Skelton A, Chen T, Wright-Minogue J, Malcolm BA. Identification and analysis of fitness of resistance mutations against the HCV protease inhibitor SCH 503034. Antiviral Res 2006;70:28–38
22. Lu L, Pilot-Matias TJ, Stewart KD, et al. Mutations conferring resistance to a potent hepatitis C virus serine protease inhibitor in vitro. Antimicrob Agents Chemother 2004;48:2260–2266
23. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitutions in the hepatitis C virus core region of genotype 1b affect very early viral dynamics during treatment with telaprevir, peginterferon, and ribavirin. J Med Virol 2010;82:575–582
24. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401
25. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41: 1105–1109
26. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104
27. El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 2008;48:38–47
28. Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77–81
29. Akuta N, Suzuki F, Kawamura Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410
30. Akuta N, Suzuki F, Sezaki H, et al. Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 2006;78:83–90
31. Zhou Y, Bartels DJ, Hanzelka BL, et al. Phenotypic characterization of resistant Val36 variants of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother 2008;52: 110–120
32. Mo H, Lu L, Pilot-Matias T, et al. Mutations conferring resistance to a hepatitis C virus (HCV) RNA-dependent RNA polymerase inhibitor alone or in combination with an HCV serine protease inhibitor in vitro. Antimicrob Agents Chemother 2005; 49:4305–4314
33. Lopez-Labrador FX, Moya A, Gonzalez-Candelas F. Mapping natural polymorphisms of hepatitis C virus NS3/4A protease and antiviral resistance to inhibitors in worldwide isolates. Antivir Ther 2008;13:481–494
34. Bartels DJ, Zhou Y, Zhang EZ, et al. Natural prevalence of hepatitis C virus variants with decreased sensitivity to NS3.4A protease inhibitors in treatment-naive subjects. J Infect Dis 2008;198:800–807
35. Cubero M, Esteban JI, Otero T, et al. Naturally occurring NS3-protease-inhibitor resistant mutant A156T in the liver of an untreated chronic hepatitis C patient. Virology 2008;370:237–245
36. Soriano V, Puoti M, Sulkowski M, et al. Care of patients with hepatitis C and HIV co-infection. Aids 2004;18:1–12
37. Barbotte L, Ahmed-Belkacem A, Chevallez S, et al. Characterization of V36c, a novel amino acid substitution conferring hepatitis C virus (HCV) resistance to telaprevir, a potent peptidomimetic inhibitor of HCV protease. Antimicrob Agents Chemother 2010;54:2681–2683

38. Tong X, Arasappan A, Bennett F, et al. Preclinical characterization of the antiviral activity of SCH 900518 (Narlaprevir), a novel mechanism-based inhibitor of hepatitis C virus NS3 protease. Antimicrob Agents Chemother 2010;54:2365–2370

39. Chase R, Skelton A, Xia E, et al. A novel HCV NS3 protease mutation selected by combination treatment of the protease inhibitor boceprevir and NS5B polymerase inhibitors. Antiviral Res 2009;84:178–184

Hepatology Research

Hepatology Research 2011

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
Crgurevic (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.

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.

Hepatology Research

Hepatology Research 2011; 41: 423–429 doi: 10.1111/j.1872-034X.2011.00797.x

Original Article

Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase

Masao Harata,[1] Senju Hashimoto,[1] Naoto Kawabe,[1] Yoshifumi Nitta,[1] Michihito Murao,[1] Takuji Nakano,[1] Yuko Arima,[1] Hiroaki Shimazaki,[1] Tetsuya Ishikawa,[4] Akihiko Okumura,[5] Naohiro Ichino,[2] Keisuke Osakabe,[2] Toru Nishikawa[3] and Kentaro Yoshioka[1]

[1]*Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University,* [2]*Faculty of Medical Technology, School of Health Sciences,* [3]*Department of Clinical Laboratory, Fujita Health University Hospital, Toyoake,* [4]*Department of Medical Technology, Nagoya University School of Health Sciences, Nagoya, and* [5]*Department of Gastroenterology, Kainan Hospital, Yatomi, Aichi, Japan*

Aim: Transient elastography is a non-invasive tool to measure liver stiffness (LS), which has been reported to correlate with stage of liver fibrosis. Extrahepatic cholestasis was reported to cause elevated LS, which is considered to be attributed to the increased hydrostatic pressure in the liver. In the present study, the correlation of LS with laboratory data was investigated in extrahepatic cholestasis. The change of LS after biliary drainage was also assessed.

Methods: LS was measured in 29 patients with extrahepatic cholestasis due to carcinomas in 12 and non-neoplastic diseases of biliary tract or pancreas in 17.

Results: In 15 patients, LS was 11.4 kPa or higher which suggested liver cirrhosis in chronic infection of hepatitis C virus. LS significantly correlated positively with serum bilirubin levels ($r = 0.726$, $P < 0.0001$) and negatively with serum aspartate aminotransferase (AST) levels ($r = -0.481$, $P = 0.0082$) and alanine aminotransferase (ALT) levels ($r = -0.631$, $P = 0.0002$). Biliary drainage led to a reduction of bilirubin by 13.5 to 0.9 mg/dL which was significantly correlated with a reduction of LS by 14.3 to 0.5 kPa ($r = 0.524$, $P = 0.0257$).

Conclusion: In extrahepatic cholestasis, the elevation of LS which is probably attributed to the increased hydrostatic pressure in the liver, correlates positively with the accumulation of bilirubin but negatively with damage of hepatocytes indicated by ALT levels. Further studies on the mechanism underlying the elevation of LS should be helpful to elucidate the pathogenesis of extrahepatic cholestasis.

Key words: biliary drainage, Fibroscan, intrahepatic pressure, transient elastography

INTRODUCTION

TRANSIENT ELASTOGRAPHY (TE) is a rapid, non-invasive and reproducible method for measuring liver stiffness (LS) that has been reported to correlate with stages of liver fibrosis in various liver diseases.[1–7] Cut-off values of 6.9–8.8 and 11.4–14.6 kPa were considered to be optimal for discrimination of fibrosis stage 2 (F2) and liver cirrhosis (F4) in patients with chronic hepatitis C virus (HCV) infection, respectively.

However several reports suggested that LS is also affected by inflammatory activity.[8] LS increases during alanine aminotransferase (ALT) flares in patients with chronic viral hepatitis and acute hepatitis.[9–11] ALT levels and grade of inflammatory activity have been reported to correlate with LS in patients with chronic hepatitis C.[6]

So far, only Millonig *et al.* have reported the increase of LS in extrahepatic cholestasis.[12] LS almost always decreased after successful bile duct drainage. They showed that the experimental bile duct ligation of pigs led to elevation of LS to the values suggesting F3 fibrosis, and considered that increased LS is attributed to the increased hepatic hydrostatic pressure. They reported

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 5 December 2010; revision 19 January 2011; accepted 13 February 2011.

the correlation of decrease of LS after bile duct drainage with decrease of bilirubin, while the correlation of LS with laboratory data was not fully elucidated. Extrahepatic cholestasis is caused by benign diseases or carcinomas of the biliary tract or pancreas. The increase of LS in extrahepatic cholestasis may differ between benign diseases and carcinomas, while the differences of increase of LS among the causes of obstruction have not been studied.

In the present study, LS was measured by a Fibroscan in the patients with extrahepatic cholestasis caused by benign diseases and carcinomas of the biliary tract or pancreas. The correlation of increased LS with laboratory data and the causes of bile duct obstruction were assessed. The changes of LS after bile duct drainage were also assessed. This is the first report describing the correlations of LS with bilirubin and ALT levels.

METHODS

Patients

TWENTY-NINE PATIENTS who were admitted with extrahepatic cholestasis to Fujita Health University Hospital from March 2008 to July 2009 were analyzed (Table 1). The underlying diseases were established according to standard criteria using laboratory tests, ultrasound, endoscopic ultrasound, computed tomography (CT) imaging, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC). Twelve patients had carcinomas which were originated from the pancreas in four patients, gallbladder in one, duodenal papilla in one and bile duct in six. Seventeen patients had benign diseases in the biliary tract or pancreas, stones in the common bile duct (CBD) in 13 patients, Mirizzi's syndrome in one, autoimmune pancreatitis in two and retroperitoneal fibrosis in one. With the exception of a patient with carcinoma of the gallbladder, all patients had no apparent hepatic invasion. LS was measured immediately before biliary drainage.

In 18 patients, LS was measured 5–45 days after biliary drainage by stone extraction or stent implementation by endoscopic methods in 16 patients or percutaneous transhepatic cholangio-drainage (PTCD) in two patients.

The study was performed in accordance with the principles of good clinical practice, the principles of the 1975 Declaration of Helsinki and its appendices, and local and national laws. Informed consent in writing was obtained from each patient.

LS measurement

Liver stiffness measurement was performed with a Fibroscan (EchoSens, Paris, France). Ten validated measurements were made on each patient. The results were expressed in kPa. Only procedures with 10 validated measurements and a success rate of at least 60% (ratio of the number of successful acquisitions over the total number of acquisitions) were considered reliable. The median value was considered representative of the liver elastic modulus.

Table 1 Characteristics of the patients with extrahepatic cholestasis

	All	Causes of cholestasis		*P*-value in comparison between benign diseases and carcinomas
		Benign diseases	Carcinomas	
Sex (female/male)	12/17	5/12	7/5	NS
Age (year)	72 ± 11	69 ± 11	75 ± 10	NS
Total bilirubin (mg/dL)	8.7 ± 4.6	6.4 ± 3.7	11.9 ± 3.9	$P = 0.0008$
Direct bilirubin (mg/dL)	6.2 ± 4.0	4.2 ± 3.3	9.0 ± 3.0	$P = 0.0004$
AST (IU/L)	153 ± 104	168 ± 107	131 ± 101	NS
ALT (IU/L)	218 ± 169	237 ± 174	190 ± 167	NS
ALP (IU/L)	1175 ± 664	1003 ± 541	1419 ± 765	NS
γ-GTP (IU/L)	695 ± 604	851 ± 718	474 ± 301	$P = 0.0705$
WBC (/μL)	6966 ± 3243	7024 ± 3265	6883 ± 3354	NS
CRP (mg/dL)	5.1 ± 5.8	6.6 ± 6.4	3.0 ± 4.2	$P = 0.0851$
Liver stiffness (kPa)	11.8 ± 6.5	9.5 ± 4.8	15 ± 7.5	$P = 0.0401$
Diameter of common bile duct (mm)	12.6 ± 4.9	12.2 ± 5.6	13.1 ± 3.8	NS

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.