combination therapy in patients who failed to respond to previous standard IFN therapy with or without RBV.[105–111] The SVR rate varies among these trials ranging 6–45%, and was lower among non-responders to previous IFN therapy compared with relapsers. In a study using PEG-IFN -2b and RBV at two different doses (1.5 g/kg per week of PEG-IFN -2b together with 800 mg/day of RBV or 1.0 g/kg per week of PEG-IFN together with 1000–1200 mg/day of RBV), the SVR rate was low at 10% and 6% in non-responders to previous treatment, but was high at 50% and 32% in relapsers, respectively.[109] In a phase III clinical trial in Japan, the SVR rate was also low in non-responders but sufficiently high in relapsers.[77] Accordingly, PEG-IFN and RBV combination therapy is well indicated for patients who relapse after standard IFN therapy with or without RBV.

Data on retreatment of patients who failed to respond to previous PEG-IFN plus RBV therapy have been evaluated in two trials.[112,113] In a randomized controlled trial that used two different doses of PEG-IFN- -2a (360 or 180 g/week) with two different durations of therapy (72- or 48-week),[112] an SVR was achieved in 7–14% of patients. It should be noted, however, that the SVR was favorable at 52% in patients who achieved HCV RNA clearance from serum by week 12 in the 72-week treatment arm.[112] In the other trial that used PEG-IFN- -2b and RBV in 2333 patients who failed to respond to previous PEG or standard IFN together with RBV, an SVR was achieved in 56% of patients whose HCV RNA was cleared from serum by week 12 and in 48% of those with genotype 1.[113] Accordingly, it is reasonable to propose that SVR could be obtained by retreatment with PEG-IFN and RBV in patients who achieve HCV RNA clearance by week 12 of retreatment, even if they failed to respond to previous PEG-IFN and RBV combination therapy.[112,113] In contrast, in the AASLD practice guideline, retreatment with PEG-IFN and RBV is not recommended for patients who did not achieve an SVR after a prior full course of PEG-IFN and RBV. Because it is still unclear who is more likely to respond to retreatment with PEG-IFN and RBV, and new drugs such as protease inhibitors may be indicated in the near future for patients who failed to respond to previous PEG-IFN and RBV therapy, data with retreatment of PEG-IFN and RBV should be accumulated to enable a conclusive recommendation.

Recommendation 16: Retreatment with PEG-IFN and RBV can be considered for non-responders and relapsers who were treated previously with IFN-based therapy with or without RBV. An SVR could be obtained in these patients whose HCV RNA is cleared from serum by week 12 of retreatment with PEG-IFN and RBV. (Level 2b, Grade B.)

MONOTHERAPY WITH IFN OR PEG-IFN

IN JAPAN, IFN monotherapy has been used to treat HCV infection since 1992. Today, IFN monotherapy is used only in patients with specific characteristics because combination therapy with PEG-IFN and RBV has achieved a high rate of SVR. Recently, a large randomized control trial (RCT) of maintenance therapy with a low dose of PEG-IFN was reported.[114] There were no differences in progression of liver disease between a PEG-IFN group and a control group. However, Japanese studies of elderly patients or patients who received maintenance therapy for longer periods showed that IFN can improve outcomes in advanced hepatic fibrosis.

Naïve patients with low viral loads

Previous studies showed that 3 MIU of IFN monotherapy achieved SVR rates of 15–45% in patients with fewer than 2 10^6 copies of HCV.[115–118] Monotherapy with 180 g/week of PEG-IFN- -2a or 1.5 g/kg per week of PEG-IFN- -2b produced SVR rates of 16–46% in patients with fewer than 2 10^6 copies.[119–121] In Japanese patients with fewer than 1 10^5 copies of HCV, 6 MIU of IFN treatment for 24 weeks achieved an SVR rate of 86% (127/148).[122] PEG-IFN monotherapy for 48 weeks similarly achieved an SVR rate of 86% (106/123). A recent RCT showed that PEG-IFN monotherapy for 24 weeks produced the same SVR rate as similar treatment for 48 weeks in patients with fewer than 1 10^5 copies of HCV. On the basis of these results, monotherapy with IFN or PEG-IFN is considered to be an effective treatment for naïve patients with fewer than 5.0 log copies/mL of HCV.[123]

Recommendation 17: Monotherapy with IFN or PEG-IFN can be considered for naïve patients with low viral loads (<5.0 log copies/mL). (Level 2a, Grade B.)

Patients with chronic kidney disease

Patients with chronic kidney disease (CKD) who undergo hemodialysis have a high prevalence of HCV infection. In Japan, one study reported that HCV RNA was detected in 117 (22%) of 543 patients who underwent maintenance hemodialysis.[124] Hemodialysis patients infected with HCV have a higher mortality rate than uninfected hemodialysis patients.[125] This higher

mortality is attributed to the frequent progression to cirrhosis and/or HCC in HCV-infected patients who receive hemodialysis.

Because RBV is excreted renally, it is currently contraindicated in patients with CKD who have a creatinine clearance of less than 50 mL/min. In addition, pharmacokinetic studies have shown that the clearance of IFN is lower in patients who undergo hemodialysis than in patients who have normal renal function.[126]

Studies of antiviral therapy in patients who undergo hemodialysis suggest that IFN monotherapy is generally well tolerated and that SVR rates are higher than those in patients with normal renal function.[127] The overall SVR rate was reported to be 33–37% in hemodialysis patients.[128] However, the number of subjects in these trials was too low to support confident conclusions. Adverse events are common in this population, and many patients discontinue therapy prematurely because of such events. A recent RCT showed in EASL 2008 that 135 g/week of PEG-IFN- -2a for 48 weeks achieved an SVR rate of 39% (23/38), whereas a dose of 90 g/week produced an SVR rate of 35% (16/43). In 74% of the patients, treatment was completed as scheduled.

Another important point is when to initiate antiviral therapy in hemodialysis patients. IFN might induce allograft rejection and renal failure.[129] Therefore, IFN therapy should be considered before renal transplantation. The next issue to be resolved is the efficacy and safety of low-dose RBV combination therapy in hemodialysis patients.

In 2008, KDIGO proposed guidelines for the treatment of patients with CKD.[130] In Japan, a committee including hepatologists and specialists for CKD is planning a clinical trial for HCV-infected patients with CKD.

Recommendation 18: 3 MIU of IFN thrice weekly or 90 or 135 g of PEG-IFN- -2a weekly is recommended for patients with CKD. (Level 2a, Grade B.)

Patients with acute HCV infection

Acute HCV infection progresses to chronic infection in approximately 70% of patients.[131] Antiviral treatment should therefore be considered for this group of patients. On the other hand, it is difficult to identify patients with self-limited disease not requiring therapy. The results of previous studies indicate that anti-HCV treatment should be initiated if HCV RNA is detected continuously for more than 12–16 weeks. If treatment is initiated within this period, monotherapy with IFN or PEG-IFN achieves an SVR rate of more than 80% in patients with acute HCV infection.[132] Reliable evidence showing that additional treatment with RBV improves the SVR rate in such patients is not available.

Recommendation 19: Patients with acute HCV infection should be considered as candidates for antiviral therapy. If HCV RNA is detected continuously for 12 or 16 weeks from the onset, treatment with 6 MIU of IFN or 180 g of PEG-IFN monotherapy should be initiated. (Level 2a, Grade B.)

Patients who receive curative treatment for HCC

Hepatocellular carcinoma frequently recurs in HCV-infected patients, even after curative therapy for HCC. Prevention of the recurrence of HCC is essential in such patients. Several RCT showed that the incidence of HCC was low in an IFN-treated group, compared to a control group (Table 4).[133–134] For example, Kubo *et al.* reported that 3 MIU IFN monotherapy thrice weekly for 96 weeks inhibited the recurrence of HCC in patients who had undergone a curative resection.[134] Furthermore, Shiratori *et al.* performed an RCT in 74 patients who had received curative percutaneous ethanol injection therapy for HCC. They reported that second and third recurrences of HCC were less frequent in patients who received IFN.[135] In an Italian study of 150 patients who had undergone curative resection, the recurrence rate of HCC 2 years after operation was significantly lower among patients who received IFN.[136]

Japanese studies showed that the survival rate was also improved by IFN treatment owing to the suppression of HCC and/or the progression of hepatic failure.[137,138]

Recommendation 20: IFN therapy should be considered for patients after curative treatment for HCC. (Level 1, Grade A.)

Maintenance therapy for patients with advanced hepatic fibrosis

Previous studies of patients with advanced hepatic fibrosis, defined as a fibrosis score 3 or 4, showed that IFN monotherapy inhibited the occurrence of HCC, compared to patients who did not receive IFN.[74,139,140] In Japanese studies, IFN was effective not only in SVR patients, but also in non-SVR patients.[139,141] On the other hand, an Italian study showed that the incidence of HCC decreased only in cirrhotic patients in whom HCV was eradicated by IFN therapy.[75]

Case–control studies in patients older than 60 years showed that a low dose of IFN reduced ALT and AFP levels and decreased the incidence of HCC, compared to a control group.[142,143] RCT for IFN monotherapy non-

Table 4 Interferon monotherapy for patients after curative treatment for hepatocellular carcinoma

Author	Study design	No. of patients (IFN group vs non-IFN group)	Age (IFN group vs non-IFN group)	Interferon	Sustained virological response	Follow-up duration (months)	HCC recurrence (IFN group vs non-IFN group)	Survival (IFN group vs non-IFN group)
Ikeda *et al.*	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo *et al.*	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Sueu *et al.*	Pilot study	18 vs 22	61 vs 62	alpha	6 (33%)	60	28% vs 82% $P < 0.001$	100% vs 73% $P < 0.05$
Shiratori *et al.*	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin *et al.*	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong *et al.*	Prospective case–control study	16 vs 16	69 vs 68	alpha	2 (13%)	36	69% vs 80% $P = 0.157$	100% vs 88% $P = 0.45$
Sakaguchi *et al.*	Case–control study	24 vs 33	69 vs 67	alpha	1 (4%)	36	14% vs 73% $P = 0.011$	100% vs 94% $P = 0.25$
Mazzaferro *et al.*	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu *et al.*	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo *et al.*	Case–control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% s 71% $P = 0.04$	86% vs 56% $P = 0.004$

IFN, interferon; HCC, hepatocellular carcinoma; RCT, randomized control study.

responders showed that histological fibrosis and activity was improved in the assigned IFN-treated group. In contrast, in the untreated group, the fibrosis score did not decline.[144] In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.[145–147] In the USA, an RCT of 53 patients in whom a histological response, but not a viral response was induced by 6 MIU of IFN showed that 3 MIU of IFN for 24 months improved the degree of hepatic fibrosis.

However, the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial found no difference in the progression of liver disease between a low-dose PEG-IFN group and a control group.[114] The large discrepancy in the effectiveness of IFN maintenance therapy between the HALT-C trial and Japanese trials might be attributed to several factors. First, the study designs differed. One of the most important differences was related to the patients' clinical characteristics. For example, patients enrolled in Japanese studies were older than those in the HALT-C trial. Elderly patients have a higher incidence of HCC than younger patients. It is suggested that the tumor-suppressive effect of IFN maintenance therapy might be more clearly demonstrated in a high-risk group, including elderly patients.[148]

Until more data become available, the decision to perform IFN maintenance therapy should be made on an individual basis.

Recommendation 21: IFN maintenance therapy is a treatment option that can inhibit the progression of liver disease in patients with advanced hepatic fibrosis, especially in those who are elderly. However, the effect of monotherapy with IFN or PEG-IFN remains uncertain in non-responders to combination therapy with PEG-IFN plus RBV. (Level 2a, Grade C.)

CONSENSUS ON THERAPEUTIC STRATEGY FOR CH-C

Indication of antiviral therapy

IKEDA *ET AL.* elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.[132] At 5 and 10 years, hepatocarcinogenesis rates in the intermediate (100 140 10^3/L) and low platelet (<100 10^3/L) groups were 10.9% and 21.6% in the IFN group (n = 217) and 19.5% and 43.0% in the untreated group (n = 459), respectively (P = 0.0005). IFN independently decreased the risk of carcinogenesis risk with a hazard ratio of 0.56 (P = 0.035). On the other hand, in the high platelet (≥150 10^3/L) group, no significant difference was found in 5- and 10-year carcinogenesis rates between the IFN-treated group (n = 228) and the untreated group (n = 585) (P = 0.69). Furthermore, IFN treatment significantly increased cumulative survival in the lower platelet subgroup (P = 0.0001) but did not affect the higher platelet subgroup (P = 0.08). Thus, the necessities of antiviral therapy are shown to be greater in elderly patients with advanced fibrosis, although adverse effects of IFN are reported to be more frequent and the efficacy of IFN to be lower in such patients.[146–149]

Therefore, the indication of antiviral therapy should be considered in the following order: the necessity of treatment, first; safety of treatment, second; and efficacy of treatment for a patient, last. Antiviral therapy should not be given up because the expected SVR rate is low.

Recommendation 22: Antiviral therapy should be offered even to CH-C patients whose SVR rates are expected to be low if type C chronic liver disease is the prognostic determinant (prognosis is improved by HCV elimination) for the individual patient, and the expected adverse effects are tolerable to the patients. (Level 6, Grade B/C.)

Effect of drug adherence of PEG-IFN and RBV on virological response

The relationship between drug exposure and antiviral effect of PEG-IFN plus RBV combination therapy has been reported in several papers.[101,151–155] McHutchison *et al.* revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN--2b and RBV for 80% or more of the scheduled duration of therapy was significantly higher than that of patients who received less than 80% of one or both drugs (51% vs 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment.[152]

Recently, Oze *et al.* evaluated how reducing drug doses affects complete early virological response (c-EVR) defined as HCV RNA negativity at week 12, using 984 patients with CH-C genotype 1.[156] As a result, the mean dose of PEG-IFN- -2b, and not RBV, during the first 12 weeks was the independent factor for c-EVR (P = 0.02), not RBV.

Hiramatsu *et al.* reported on whether dose reduction of RBV (or PEG-IFN) has an effect on virological relapse in PEG-IFN plus RBV treatment for patients with CH-C genotype 1.[157] In the analysis of 472 patients responding to PEG-IFN- -2b plus RBV, stepwise reduction of the

RBV dose was associated with a stepwise increase in relapse rate from 11% to 60% (Fig. 3).

Improving the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* examined low-dose PEG-IFN- -2a (135 g/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.[158] Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.[159]

Recommendation 23: In genotype 1 patients, PEG-IFN is dose-dependently correlated with c-EVR, independent of RBV dose. The administration over 80% of the scheduled dose of PEG-IFN- -2a or over 1.2 g/kg per week of PEG-IFN- -2b should be chosen as a starting dose: a marked dose reduction of PEG-IFN should not be risked at the start even for patients with disadvantage (e.g. aged patients). (Level 2b/3, Grade B.)

Recommendation 24: In genotype 1 patients, RBV shows a dose-dependent correlation with the relapse after treatment. Maintaining the RBV dose over 80% of the scheduled dose or over 10 mg/kg per day (12 mg/kg per day, if possible) during the complete treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to PEG-IFN- -2b plus RBV, especially in c-EVR patients. (Level 2b/3, Grade B.)

Recommendation 25: In genotype 2/3 patients, reducing drug doses of PEG-IFN and RBV (down to 400 mg/day) has no significant effect on virological responses. (Level 2a, Grade B.)

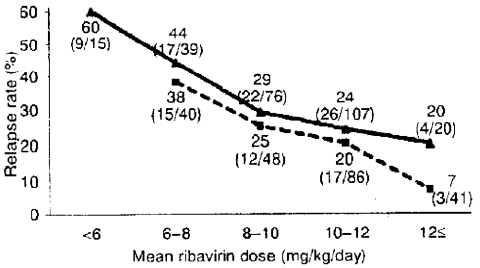

Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)- -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses (—▲—). Group with the mean PEG-IFN dose <1.4 g/kg/week (■). Group with the mean PEG-IFN dose ≥1.4 g/kg/week. There was no significant difference between the two PEG-IFN- -2b-dose groups ($P = 0.17$)

Treatment for patients without elimination of HCV

Tarao *et al.* showed the rate of HCC appearance was significantly higher in HCV-related cirrhotic patients with a high ALT value (≥80 IU/mL) than in those with a lower ALT value (<80 IU/mL).[97] This suggested that suppression of inflammation in the liver with HCV infection is very important to prevent the hepatocarcinogenesis in patients with HCV-related cirrhosis.

Omata *et al.* assessed the effects of oral ursodeoxycholic acid (UDCA) on serum biomarkers. CH-C patients with elevated ALT were assigned randomly to 150 ($n = 199$), 600 ($n = 200$) or 900 mg/day ($n = 197$) UDCA intake for 24 weeks. As a result, the median changes in serum ALT at the end of treatment were shown to be 15.3, 29.2 and 36.2%, respectively, although serum HCV RNA did not change in any group.[160]

A glycyrrhizin product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical, Tokyo, Japan), is used widely in Japan and has been reported to improve ALT levels and liver inflammation.[161,162] Furthermore, Ikeda *et al.* reported liver carcinogenesis was suppressed by long-term administration of glycyrrhizin, using a cohort of 1249 patients, and its favorable effect on hepatocellular carcinogenesis in those patients with IFN-resistant CH-C.[163,164]

Repeated phlebotomy has been shown to be effective for the improvement of serum ALT as well as progression of fibrosis,[32] however, it remains controversial whether the effects of IFN improve with extensive phlebotomy.[165–167]

In Japan, Yano *et al.* showed the iron removal by repeated phlebotomy improved serum ALT levels in patients with CH-C.[170]

Recommendation 26: Patients whose HCV RNA was not eradicated by PEG-IFN plus RBV and whose ALT and/or AFP levels were not improved by IFN monotherapy or those without indication for IFN therapy should be treated with the liver-supporting therapy (SNMC, UDCA), and if the effect of this medication is inadequate, phlebotomy can be used in combination. (Level 3/6, Grade B/C.)

Treatment of patients with decompensated cirrhosis

The compensated patients who failed to eradicate HCV by antiviral therapy and decompensated patients should be referred for consideration of liver transplantation and liver supporting therapy should be performed. Long-

term nutritional supplementation with oral branched-chain amino acid (BCAA) has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers.[151,152] Early interventional with oral BCAA was shown to prolong the liver transplant waiting period by preserving hepatic reserve in cirrhosis.

Recommendation 27: Patients with compensated cirrhosis for the prevention of hepatocellular carcinogenesis, should be treated by not only IFN but also with liver supporting therapy (SNMC, UDCA) and/or phlebotomy and/or BCAA in order to improve the liver inflammation and AFP levels. (Level 3, Grade C.)

Novel antiviral drugs

Telaprevir, a protease inhibitor specific to the HCV non-structural 3/4A serine protease, reduced HCV RNA levels rapidly in early studies. McHuthison *et al.* reported the improved SVR rate with triple therapy for 12 weeks followed by PEG-IFN- -2a and RBV for 12 weeks.

Thus, the treatment for CH-C is progressing. Therefore, as a treatment strategy, PEG-IFN plus RBV combination therapy should be performed early for aged patients and the patients with the advanced fibrosis. However, the novel antiviral drugs, such as protease inhibitors and polymerase inhibitors, should be taken into account as a candidate of treatment for the patients who can wait for the oncoming drugs.

Recommendation 28: Novel antiviral drugs, such as a protease inhibitor or a polymerase inhibitor, in combination with PEG-IFN plus RBV, can improve the SVR rates in genotype 1 CH-C patients. (Level 2a, Grade A.)

REFERENCES

1 Ghany MG, Strader DB, Thomas DL *et al.* AASLD practice guidelines. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335–74.
2 Shiffman RN, Shekelle P, Overhage J *et al.* Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standadization. *Ann Intern Med* 2003; 139: 493–98.
3 Pileri P, Hematsu Y, Campagnoli S *et al.* Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938–41.
4 Scarselli E, Ansuini H, Cerino R *et al.* The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002; 21: 5017–25.
5 Evans MJ, von Hahn T, Tscherne DM *et al.* Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; 446: 801–5.
6 Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. *Cell Microbiol* 2008; 10: 821–7.
7 Ploss A, Evans MJ, Gaysinskaya VA *et al.* Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009; 457: 882–6.
8 Flint M, von Hahn T, Zhang J *et al.* Diverse CD81 proteins support hepatitis C virus infection. *J Virol* 2006; 80: 11331–42.
9 Sumpter R Jr, Loo YM, Foy E *et al.* Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, *RIG-I*. *J Virol* 2005; 79: 2689–99.
10 Yoneyama M, Kikuchi M, Natsukawa T *et al.* The RNA helicase *RIG-I* has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; 5: 730–7.
11 Li K, Chen Z, Kato N, Gale M Jr, Lemon SM. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. *J Biol Chem* 2005; 280: 16739–47.
12 Kawai T, Takahashi K, Sato S *et al.* IPS-1, an adaptor triggering *RIG-I*- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005; 6: 981–8.
13 Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; 55: 255–81.
14 Loo YM, Owen DM, Li K *et al.* Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. *Proc Natl Acad Sci USA* 2006; 103: 6001–6.
15 Gale M Jr, Foy EM. Evasion of intracellular host defense by hepatitis C virus. *Nature* 2005; 436: 939–45.
16 Bode JG, Ludwig S, Ehrhardt C *et al.* IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. *FASEB J* 2003; 17: 488–90.
17 Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; 2: 410–6.
18 Polyak SJ, Khabar KS, Paschal DM *et al.* Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *J Virol* 2001; 75: 6095–106.
19 Taylor DR, Shi ST, Romano PR *et al.* Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999; 285: 107–10.
20 Noguchi T, Satoh S, Koshi T *et al.* Effects of mutation in hepatitis C virus nonstructural protein 5A on interferon resistance mediated by inhibition of PKR kinase activity in mammalian cells. *Microbiol Immunol* 2001; 45: 829–40.
21 Moriya K, Nakagawa K, Santa T *et al.* Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* 2001; 61: 4365–70.
22 Okuda M, Li K, Beard MR *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; 122: 366–75.

23 Korenaga M, Wang T, Li Y *et al.* Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* 2005; 280: 37481–8.
24 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; 126: 840–8.
25 Kawaguchi T, Yoshida T, Harada M *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; 165: 1499–508.
26 Perlemuter G, Sabile A, Letteron P *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; 16: 185–94.
27 Moriishi K, Mochizuki R, Moriya K *et al.* Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2007; 104: 1661–6.
28 Nishina S, Hino K, Korenaga M *et al.* Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* 2008; 134: 226–38.
29 Miura K, Taura K, Kodama Y, Schnabl B, Brenner DA. Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* 2008; 48: 1420–9.
30 Veldt BJ, Chen W, Heathcote EJ *et al.* Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; 47: 1856–62.
31 Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; 97: 3036–43.
32 Kato J, Kobune M, Nakamura T *et al.* Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* 2001; 61: 8697–702.
33 Furutani T, Hino K, Okuda M *et al.* Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. *Gastroenterology* 2006; 130: 2087–98.
34 Romero-Gomez M, Del Mar Viloria M, Andrade RJ *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; 128: 636–41.
35 Harrison SA, Brunt EM, Qazi RA *et al.* Effect of significant histologic steatosis on steatohepatitis on response to antiviral therapy in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2005; 3: 604–9.
36 Martinot-Peignoux M, Boyer N, Cazals-Hatem D *et al.* Prospective study on anti-hepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. *Hepatology* 2001; 34: 1000–5.
37 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343–50.
38 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39: 1147–71.
39 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon- therapy in hepatitis C virus infection. *Hepatology* 1994; 19: 1088–94.
40 Lau JY, Davis GL, Kniffen J *et al.* Significance of serum hepatitis C virus RNA in chronic hepatitis C. *Lancet* 1993; 341: 1501–4.
41 Yamada G, Takatani M, Kishi F *et al.* Efficacy of interferon alfa therapy in chronic hepatitis C patients depends primarily on hepatitis C virus RNA level. *Hepatology* 1995; 22: 1351–4.
42 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. *J Clin Invest* 1995; 96: 224–30.
43 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.
44 Pascu M, Martus P, Hohne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; 53: 1345–54.
45 Shirakawa H, Matsumoto A, Joshita S *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; 48: 1753–60.
46 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; 48: 38–47.
47 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48: 372–80.
48 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: 403–10.
49 Donlin MJ, Cannon NA, Yao E *et al.* Pretreatment sequence diversity differences in the full-length hepatitis

C virus open reading frame correlate with early response to therapy. *J Virol* 2007; **81**: 8211–24.
50 Akuta N, Suzuki F, Kawamura Y *et al.* Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; **46**: 1357–64.
51 Fishman SL, Factor SH, Balestrieri C *et al.* Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 2009; **15**: 3205–13.
52 Ogata S, Florese RH, Nagano-Fujii M *et al.* Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. *J Clin Microbiol* 2003; **41**: 2835–41.
53 Nishise Y, Saito T, Sugahara K *et al.* Risk of hepatocellular carcinoma and secondary structure of hepatitis C virus (HCV) NS3 protein amino-terminus, in patients infected with HCV subtype 1b. *J Infect Dis* 2007; **196**: 1006–9.
54 Mattsson L, Sonnerborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-years follow-up study of hepatitis C virus markers. *Liver* 1993; **13**: 274–8.
55 Barrera JM, Bruguera M, Ercilla MG *et al.* Persistent hepatitis C viremia after self-limiting posttransfusion hepatitis C. *Hepatology* 1995; **21**: 639–44.
56 Amoroso P, Rapicetta M, Tosti ME *et al.* Correlation between virus genotype and chronicity rate in acute hepatitis C. *J Hepatol* 1998; **28**: 939–44.
57 Tanaka E, Kiyosawa K. Natural history of acute hepatitis C. *J Gastroenterol Hepatol* 2000; **15**: E97–104.
58 Seeff LB, Buskell-Bales Z, Wright EC *et al.* Long-term mortality after transfusion-associated non-A, Non B hepatitis. *N Engl J Med* 1992; **327**: 1906–11.
59 Seeff LB, Miller RN, Rabkin CS *et al.* 45-year follow-up of hepatitis C virus infection in healthy young adults. *Ann Intern Med* 2000; **132**: 105–11.
60 Kenny-Walsh E, the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Eng J Med* 1999; **340**: 1228–33.
61 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671–5.
62 Tong MJ, El-Farra NS, Reikes AR *et al.* Clinical outcomes after transfusion-associated hepatitis C. *N Eng J Med* 1995; **332**: 1463–6.
63 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47–53.
64 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999; **131**: 174–81.
65 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. *J Hepatol* 1999; **30**: 653–9.
66 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. *Lancet* 1997; **349**: 825–32.
67 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517–24.
68 Persico M, Persico E, Suozzo R *et al.* Natural history of hepatitis C virus carriers with persistently normal aminotransferase. *Gastroenterology* 2000; **118**: 760–4.
69 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; **43**: 599–605.
70 Tarao K, Rino Y, Ohkawa S *et al.* Association between high serum ALT and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* 1999; **86**: 589–94.
71 Kumada T, Toyoda H, Kiriyama S *et al.* Relation between incidence of hepatic cardcinhogenesis and integration value of alanine aminmotransferase in patients with hepatitis C virus infection. *Gut* 2007; **56**: 738–9.
72 Kumada T, Toyoda H, Kiriyama S *et al.* Long-term follow-up of patients with hepatitis C with a normal alanine aminotransferase. *J Med Virol* 2009; **81**: 446–51.
73 Suruki R, Hayashi K, Kusumoto K *et al.* Alanine aminotransferase level as a predictor of hepatitis C virus-associated hepatocellular carcinoma incidence in a community-based population in Japan. *Int J Cancer* 2006; **119**: 192–5.
74 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; **101**: 1616–22.
75 Bruno S, Stroffolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579–87.
76 George S, Bacon BR, Brunt EM *et al.* Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729–38.

77 Iino S, Okita K, Omata M *et al.* Clinical efficacy of PEG-Interferon alfa-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load - retrospective comparison with Interferon alfa-2b and ribavirin combination therapy for 24 weeks. *Kanzo* 2004; 49: 1099–121

78 Yamada G, Iino S, Okuno T *et al.* Virological response in patients with hepatitis C virus genotype 1b and a high viral load: impact of peginterferon-alpha-2a plus ribavirin dose reductions and host-related factors. *Clin Drug Investig* 2008; 28: 9–16.

79 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; 81: 452–8.

80 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; 352: 2609–17.

81 Sezaki H, Suzuki F, Kawamura Y *et al.* Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. *Dig Dis Sci* 2009; 54: 1317–24.

82 Kogure T, Ueno Y, Fukushima K *et al.* Pegylated interferon plus ribavirin for genotype 1b chronic hepatitis C in Japan. *World J Gastroenterol* 2008; 14: 7225–4230.

83 Akuta N, Suzuki F, Kawamura Y *et al.* Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2007; 79: 1686–95.

84 Mizuta T, Kawaguchi Y, Iguchi Y *et al.* Whole-body insulin sensitivity index is a highly specific predictive marker for virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients with genotype 1b and high viral load. *Dig Dis Sci* 2010; 55: 183–9.

85 Tsukada H, Ochi H, Maekawa T *et al.* A Polymorphism in MAPKAPK3 Affects Response to Interferon Therapy for Chronic Hepatitis C. *Gastroenterology* 2009; 136: 1796–805.

86 Asahina Y, Izumi N, Hirayama I *et al.* Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. *Gastroenterology* 2008; 134: 1396–405.

87 Asahina Y, Izumi N, Umeda N *et al.* Pharmacokinetics and enhanced PKR response in patients with chronic hepatitis C treated with pegylated interferon alpha-2b and ribavirin. *J Viral Hepat* 2007; 14: 396–403.

88 Sezaki H, Suzuki F, Kawamura Y *et al.* Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res* 2007; 37: 787–92.

89 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon Alfa-2a plus ribavirin. *Gastroenterology* 2006; 130: 1086–109

90 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; 131: 451–60.

91 Ferenci P, Laferl H, Scherzer TM *et al.* Customizing treatment with peginterferon alfa-2a (40kD) (PEGASYS®) plus ribavirin (COPEGUS®) in patient with HCV genotype 1 or 4 infection: interim results of a prospective randomized trial. *Hepatology* 2006; 44: 336a.

92 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; 46: 1688–94.

93 Buti M, Lurie Y, Zakharova NG *et al.* Extended treatment duration in chronic hepatitis C genotype 1-infected slow responders: final results of the SLICCESS study (abstract #141). *J Hepatol* 2009; 50 (Suppl 1): S58.

94 Ide T, Hino T, Ogata K *et al.* A randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative status in patients with genotype 1b chronic hepatitis C. *Am J Gastroenterol* 2009; 104: 70–5.

95 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; 129: 522–7

96 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; 357: 124–34.

97 Dalgard O, Bjøro K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; 47: 35–42

98 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; 47: 1837–45.

99 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; 56: 553–9.

100 Mangia A, Minerva N, Bacca D *et al.* Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. *Hepatology* 2009; 49: 358–63.

101 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–65.

102 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–82.
103 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: 346–55.
104 Helbling B, Jochum W, Stamenic I *et al.* HCV-related advanced fibrosis/cirrhosis: randomized controlled trial of pegylated interferon alpha-2a and ribavirin. *J Viral Hepat* 2006; 13: 762–9.
105 Bergmann JF, Vrolijk JM, van der Schaar P *et al.* Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007; 27: 1217–25.
106 Diago M, Crespo J, Olveira A *et al.* Clinical trial: pharmacodynamics and pharmacokinetics of re-treatment with fixed-dose induction of peginterferon alpha-2a in hepatitis C virus genotype 1 true non-responder patients. *Aliment Pharmacol Ther* 2007; 26: 1131–8.
107 Carr C, Hollinger FB, Yoffe B *et al.* Efficacy of interferon alpha-2b induction therapy before retreatment for chronic hepatitis C. *Liver Int* 2007; 27: 1111–8.
108 Mathew A, Peiffer LP, Rhoades K *et al.* Sustained viral response to pegylated interferon alpha-2b and ribavirin in chronic hepatitis C refractory to prior treatment. *Dig Dis Sci* 2006; 51: 1956–61.
109 Jacobson IM, Gonzalez SA, Ahmed F *et al.* A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. *Am J Gastroenterol* 2005; 100: 2453–62.
110 Herrine SK, Brown RS Jr, Bernstein DE *et al.* Peginterferon alpha-2a combination therapies in chronic hepatitis C patients who relapsed after or had a viral breakthrough on therapy with standard interferon alpha-2b plus ribavirin: a pilot study of efficacy and safety. *Dig Dis Sci* 2005; 50: 719–26.
111 Shiffman ML, Di Bisceglie AM, Lindsay KL *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; 126: 1015–23.
112 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009; 150: 528–40.
113 Poynard T, Colombo M, Bruix J *et al.* Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009; 136: 1618–28.
114 Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; 359: 2429–41.
115 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1485–92.
116 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; 352: 1426–32.
117 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1493–9.
118 Reichard O, Norkrans G, Frydén A *et al.* Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. *Lancet* 1998; 351: 83–7.
119 Zeuzem S, Feinman SV, Rasenack J *et al.* Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; 343: 1666–72.
120 Lindsay KL, Trepo C, Heintges T *et al.* A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; 34: 395–403.
121 Pockros PJ, Carithers R, Desmond P *et al.* Efficacy and safety of two-dose regimens of peginterferon alpha-2a compared with interferon alpha-2a in chronic hepatitis C: a multicenter, randomized controlled trial. *Am J Gastroenterol* 2004; 99: 1298–305.
122 Akuta N, Suzuki I, Tsubota A *et al.* Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of triparite interaction of viral, host and interferon treatment-related factors. *J Hepatol* 2002; 37: 831–6.
123 Iwasaki Y, Shiratori Y, Hige S, Nishiguchi S *et al.* A randomized trial of 24 versus 48 weeks of peginterferon a-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatol Int* 2009; 3: 468–79.
124 Masuko K, Okuda K, Meguro T *et al.* Hepatitis C virus antibodies, viral RNA and genotypes in sera from patients on maintenance haemodialysis. *J Viral Hepat* 1994; 1: 65–71.
125 Fabrizi F, Martin P, Dixit V, Bunnapradist S, Dulai G. Meta-analysis: effect of hepatitis C virus infection on mortality in dialysis. *Aliment Pharmacol Ther* 2004; 20: 1271–7.
126 Rostaing L, Chatelut E, Payen JL *et al.* Pharmacokinetics of alphaIFN-2b in chronic hepatitis C virus patients undergoing chronic hemodialysis or with normal renal function: clinical implications. *J Am Soc Nephrol* 1998; 9: 2344–8.

127 Russo MW, Goldsweig CD, Jacobson IM, Brown RS Jr. Interferon monotherapy for dialysis patients with chronic hepatitis C: an analysis of the literature on efficacy and safety. *Am J Gastroenterol* 2003; 98: 1610–5.

128 Fabrizi F, Dixit V, Messa P, Martin P. Interferon monotherapy of chronic hepatitis C in dialysis patients: meta-analysis of clinical trials. *J Viral Hepat* 2008; 15: 79–88.

129 Kumar N, Ribes D, Izopet J, Rostaing L. Treatment of hepatitis C virus infection (HCV) after renal transplantation: implications for HCV-positive dialysis patients awaiting a kidney transplant. *Transplantation* 2006; 82: 853–6.

130 KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int Suppl* 2008; 109: S1–99.

131 Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; 13: 34–41.

132 Ikeda K, Arase Y, Kawamura Y *et al.* Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C. *Am J Med* 2009; 122: 479–86.

133 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; 32: 228–32.

134 Kubo S, Nishiguchi S, Hirohashi K *et al.* Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001; 134: 963–7.

135 Shiratori Y, Shiina S, Teratani T *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; 138: 299–306.

136 Mazzaferro V, Romito R, Schiavo M *et al.* Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; 44: 1543–54.

137 Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002; 89: 418–22.

138 Shiratori Y, Ito Y, Yokosuka O *et al.* Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; 142: 105–14.

139 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; 346: 1051–5.

140 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–30.

141 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; 129: 94–9.

142 Arase Y, Ikeda K, Suzuki F *et al.* Prolonged interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. *J Med Virol* 2007; 79: 1095–102.

143 Nomura H, Kashiwagi Y, Hirano R *et al.* Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: A pilot study. *Hepatol Res* 2007; 37: 490–7.

144 Shiffman ML, Hofmann CM, Contos MJ *et al.* A randomized, controlled trial of maintenance interferon therapy for patients with chronic hepatitis C virus and persistent viremia. *Gastroenterology* 1999; 117: 1164–72.

145 Saito Y, Saito H, Tada S *et al.* Effect of long-term interferon therapy for refractory chronic hepatitis c: preventive effect on hepatocarcinogenesis. *Hepatogastroenterology* 2005; 52: 1491–6.

146 Arase Y, Ikeda K, Suzuki F *et al.* Interferon induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. *J Med Virol* 2007; 79: 1485–90.

147 Akuta N, Suzuki F, Kawamura Y *et al.* Efficacy of low dose intermittent interferon-alpha monotherapy in patients infected with hepatitis C virus genotype 1b who were predicted or failed to respond to pegylated interferon plus ribavirin combination therapy. *J Med Virol* 2008; 80: 1363–9.

148 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; 39: 1069–77.

149 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; 43: 54–63.

150 Hiramatsu N, Oze T, Tsuda N *et al.* Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? *Hepatol Res* 2006; 35: 185–9.

151 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645–52.

152 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123: 1061–9.

153 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; 132: 103–12.
154 Reddy KR, Shiffman ML, Morgan TR *et al.* Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007; 5: 124–9.
155 Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007; 46: 371–9.
156 Oze T *et al.* Pegylated interferon alpha-2b affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN alpha-2b plus ribavirin. *J Viral Hepat* 2009; 16: 578–85.
157 Hiramatsu N *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; 16: 586–94.
158 Weiland O, Hollamder A, Mattsson L *et al.* Lower-than standard dose peg-IFN alfa-2a for chronic hepatitis C caused by genotype 2 and 3 is sufficient when given in combination with weight-based ribavirin. *J Viral Hepat* 2008; 15: 641–5.
159 Inoue Y, Hiramatsu N, Oze T *et al.* Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses. *J Viral Hepat* (in press).
160 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; 56: 1747–53.
161 Suzuki H, Ohta Y, Takino T *et al.* Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Double blind trial. *Asian Med J* 1983; 26: 423–38.
162 Wildhirt E. Experience in Germany with glycyrrhizinic acid for the treatment of chronic viral hepatitis. In: Nishioka K, Suzuki H, Mishiro S, Oda T, eds. *Viral Hepatitis and Liver Disease*. Tokyo, Springer-Verlag, 1994; 658–61.
163 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494–500.
164 Ikeda K, Arase Y, Kobayashi M *et al.* A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1249 patients. *Dig Dis Sci* 2006; 51: 603–9.
165 Piperno A, Sampietro M, D'Alba R *et al.* Iron stores, response to alpha-interferon therapy and effects of iron depletion in chronic hepatitis C. *Liver* 1996; 16: 248–54.
166 Fong TL, Han SH, Tsai NC *et al.* A pilot randomized, controlled trial of the effect of iron depletionon long-term response to alpha-interferon in patients with chronic hepatitis C. *J Hepatol* 1998; 28: 369–74.
167 Herrera JL. Iron depletion is not effective in inducing a virologic response in patients with chronic hepatitis C who failed to respond to interferon therapy. *Am J Gastroenterol* 1999; 94: 3571–5.
168 Fontana RJ, Israel J, LeClair P *et al.* Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. *Hepatology* 2000; 31: 730–6.
169 Di Bisceglie AM, Bonkovsky HL, Chopra S *et al.* Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who previously not responded to interferon: a multicenter, prospective randomized, controlled trail. *Hepatology* 2000; 32: 135–8.
170 Yano M, Hayashi H, Yoshioka K *et al.* A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. *J Gastroenterol* 2004; 39: 570–4.
171 Marchesini G, Bianchi G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; 124: 1792–801.
172 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: 1827–38.

J Gastroenterol
DOI 10.1007/s00535-010-0301-x

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Real-time tissue elastography as a tool for the noninvasive assessment of liver stiffness in patients with chronic hepatitis C

Hiroyasu Morikawa · Katsuhiko Fukuda · Sawako Kobayashi · Hideki Fujii · Shuji Iwai · Masaru Enomoto · Akihiro Tamori · Hiroki Sakaguchi · Norifumi Kawada

Received: 6 May 2010 / Accepted: 15 July 2010

Abstract
Background Although histopathological examination by "invasive" liver biopsy remains the gold standard for evaluating disease progression in chronic liver disease, noninvasive tools have appeared and have led to great progress in diagnosing the stage of hepatic fibrosis. The aim of this study was to assess the value of real-time tissue elastography, using an instrument made in Japan, for the visible measurement of liver elasticity; in particular, comparing the results with those of transient elastography (Fibroscan).
Methods Real-time tissue elastography (RTE), transient elastography (Fibroscan), liver biopsy, and routine laboratory analyses were performed in 101 patients with chronic hepatitis C. The values for tissue elasticity obtained using novel software (Elasto_ver 1.5.1) connected to RTE were transferred to four image features, Mean, Standard Deviation (SD), Area, and Complexity. Their association with the stage of fibrosis at biopsy and with liver stiffness (kPa) obtained by Fibroscan was analyzed.
Results Colored images obtained by RTE were classified into diffuse soft, intermediate, and patchy hard patterns and the calculated elasticity differed significantly between patients according to and correlated with the stages of fibrosis ($p < 0.0001$). Mean, SD, Area, and Complexity showed significant differences between the stages of fibrosis (Tukey–Kramer test, $p < 0.05$). In discriminating patients with cirrhosis, the areas under the receiver operating characteristic curves (AUC) were 0.91 for Mean, 0.84 for SD, 0.91 for Area, 0.93 for Complexity, and 0.95 for Fibroscan.
Conclusions RTE is a noninvasive instrument for the colored visualization of liver elasticity in patients with chronic liver disease.

Keywords Liver fibrosis · Transient elastography · Ultrasound · Liver biopsy

H. Morikawa · S. Kobayashi · H. Fujii · S. Iwai · M. Enomoto · A. Tamori · H. Sakaguchi · N. Kawada (✉)
Department of Hepatology, Graduate School of Medicine, Osaka City University, 1-4-3 Asahimachi, Abeno, Osaka 545-8585, Japan
e-mail: kawadanori@med.osaka-cu.ac.jp

K. Fukuda
Department of Gastroenterology, PL Hospital, Tondabayashi, Japan

Published online: 10 August 2010

Introduction

Hepatitis C virus (HCV) infects approximately 170 million individuals worldwide, according to a report from the World Health Organization [1]. Chronic liver damage attributable to HCV infection results in hepatic fibrosis, which is characterized by an unusual accumulation of extracellular matrix materials produced by fibroblast-like cells including stellate cells in the hepatic parenchyma. Hepatic fibrosis progresses towards cirrhosis, an end-stage liver injury, leading to hepatic failure, hepatocellular carcinoma, and finally death. Thus, precise evaluation of the stage of chronic hepatitis C with respect to fibrosis has become an important issue to prevent the occurrence of cirrhosis and to initiate appropriate therapeutic intervention such as viral eradication using pegylated interferon (PEG-IFN) plus ribavirin [2].

Although liver biopsy is acknowledged as the gold standard for staging disease progression, there are some limitations, including its invasiveness, risk of complications, sampling error, variability in histopathological interpretation, and the reluctance of patients to submit to repeated examinations [3]. Because of these disadvantages, there is a

growing shift in clinical practice to utilize or develop 'non-invasive' methodologies to reflect the stage of liver fibrosis.

Several noninvasive approaches have appeared, such as serum fibrosis markers, transient elastography (Fibroscan®; Echosens SA, Paris, France), and real-time tissue elastography (RTE). Serum fibrosis markers include direct tests, such as hyaluronic acid and type IV collagen, and indirect approaches, which detect alterations in hepatic function but do not directly reflect hepatic extracellular matrix metabolism; these include platelet counts, coagulation studies, and hepatic transaminases, or their combinations in indices/scores, such as the aspartate aminotransferase-to-platelet ratio index (APRI) [4, 5].

Transient elastography, which has been developed for the measurement of liver stiffness, is considered to reflect more directly than other means the fibrotic evolution of chronic liver trauma [6–10]. In 2005, Castera et al. and Ziol et al. reported that liver stiffness measurements could be useful in assessing the presence of significant fibrosis (F2–4) and in suggesting the presence of cirrhosis in cohorts of patients with chronic hepatitis C; the areas under the receiver operating characteristic curves (AUCs) ranged from 0.79 to 0.83 for the prediction of F2–4 and were over 0.95 for the identification of cirrhosis [11, 12]. Friedrich-Rust et al. [13] assessed the overall performance of transient elastography for the diagnosis of liver fibrosis by a meta-analysis which included fifty articles; the mean AUCs for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis were 0.84, 0.89, and 0.94, respectively. The limitations of this method have also been discussed; intraobserver agreement is influenced by variables such as body mass index (BMI, particularly when ≥28), hepatic steatosis, and flares of transaminases [11–14].

RTE is a method developed in Japan for the visual assessment of tissue elasticity integrated in a sonography machine, based on a Combined Autocorrelation Method that calculates the relative hardness of tissue rapidly from the degree of tissue distortion and which displays this information as a color image. The distortion of tissue is color-coded according to its magnitude and superimposed translucently on a conventional B-mode image. This simultaneous display enables us to evaluate the anatomical correspondence between tissue elasticity and B-mode images. The RTE image is constructed by the amount of displacement of the reflected ultrasound echoes under compression. Ultrasound elastography does not demonstrate physical elasticity directly, but shows the relative degree of tissue strain when subtle compression is applied. In hard tissue, the amount of displacement of the reflected ultrasound echoes is low, whereas in soft tissue, the amount of displacement is higher because soft tissue can be compressed more than hard tissue [15, 16]. This technology has already been proved to be diagnostically valuable in detecting space-occupying lesions in the breast, prostate, and pancreas [17–20]. Friedrich-Rust et al. [21] attempted to determine the elasticity of liver tissue in 79 patients with chronic viral hepatitis. They developed an elasticity score from the color-coded bit-map image produced by the computer program Mathlab version 6 (Math Works, Natick, MA, US). However, the diagnostic accuracy of this semi-quantitative assessment for the prediction of significant fibrosis (METAVIR scoring system ≥F2), severe fibrosis (≥F3), and cirrhosis (F4) was not satisfactory; the AUCs were 0.75, 0.73, and 0.69, respectively [21].

We report here an updated RTE system as a tool for the noninvasive assessment of liver stiffness in patients with chronic hepatitis C. In this new system, all pixel data in the colored image were transformed into a histogram and a binary image for more accurate quantification, using an exclusive software program.

Methods

Patients

Ten healthy adult volunteers with no evident liver disease were recruited after giving their oral informed consent. One hundred and one patients with chronic hepatitis C, whose disease was defined by the presence of serum anti-hepatitis C virus (HCV) antibodies and serum HCV RNA, with serum levels of alanine aminotransferase above the upper limit of normal, were included in this study. Percutaneous liver biopsy or laparoscopy was performed on the patients within 1 week following Fibroscan and RTE analysis at the Department of Hepatology, Osaka City University Hospital, between September 2007 and September 2009. The study protocol accorded with the Helsinki Declaration and was approved by the ethics committee of our institution. Patients were enrolled and liver biopsy was performed after informed consent was obtained.

Liver histology and quantification of liver fibrosis

Liver biopsy was carried out using a 15-gauge Tru-Cut needle biopsy apparatus (Hakko, Tokyo, Japan). The median length of liver samples obtained at biopsy was 18 mm (range 10–25 mm). Tissue specimens obtained by liver biopsy were fixed immediately in 10% formalin solution and embedded in paraffin. After cutting, sections were stained with hematoxylin and eosin or Azan Mallory and the stage of fibrosis and grade of inflammatory activity in the liver were determined according to the METAVIR scoring system [22, 23]. All biopsy specimens were examined independently by two experienced pathologists who were blinded to the clinical data and the measurements

of liver stiffness. Fibrosis was staged on a 0–4 scale as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; F4, cirrhosis. The chronic hepatitis activity was graded as follows: A0, none; A1, mild; A2, moderate; and A3, severe.

Real-time elastography

We used ultrasonography (Hitachi EUB-8500; Hitachi Medical, Chiba, Japan) and an EUP-L52 Linear probe (3–7 MHz; Hitachi Medical) for real-time tissue elastography (RTE). This system is commercially available currently for the diagnosis of mammary neoplasms [17].

Patients were examined in a supine position with the right arm elevated above the head, and were instructed to hold their breath. The examination was performed on the right lobe of the liver through the intercostals, because liver biopsy and Fibroscan were also performed at the same site. The RTE equipment displays two images simultaneously; the RTE image showing the region of interest (ROI) as a colored area and the conventional B-mode image (Fig. 1a). An area was chosen where the tissue was free of large vessels and near the biopsy point. The measurement was fixed to a rectangle with 30 mm length × 20 mm breadth placed 5 mm below the surface of the liver (Fig. 1a). The color in the ROI was graded from blue to red (Fig. 1b). We stored the RTE images as moving digital images for 20–40 s. Then ten static images, captured by the observer at random from the moving images, using AVI2JPG v6.10 converter software (Novo, Tokyo, Japan), were analyzed using the novel software Elasto_ver 1.5.1 (which was developed and donated by Hitachi Medical) on a personal computer. Numerical values of pixels were from 0 to 255 (256 stepwise grading) according to color mapping from blue (0) to red (255), and a histogram of the distribution was generated (Fig. 1c). The scale ranged from red for components with the greatest strain (i.e., softest components) to blue for those with no strain (i.e., hardest components). Green indicated average strain in the ROI, and therefore intact liver tissue displayed a diffuse homogeneous green pattern. An appearance of unevenness in the color pattern was considered to reflect a change in the liver stiffness. For quantification, all pixel data in the colored image were transformed into a histogram and binary image (Fig. 1c, d).

Colored RTE images are usually classified into several patterns in the diagnosis of breast disease [17]. In this study, we evaluated liver fibrosis as three patterns: a diffuse soft pattern, an intermediate pattern, and a patchy hard pattern. The diffuse soft pattern was a relatively homogeneously spread, light-green colored image (Fig. 2a; the corresponding histology is shown in Fig. 2d). The intermediate pattern was typified by a partially mottled, dotted image with blue spots on a light green background (Fig. 2b; the corresponding histology is shown in Fig. 2e). The patchy hard pattern comprised mixed images with a patchwork effect of light green, red, and blue (Fig. 2c; the corresponding histology is shown in Fig. 2f).

Transient elastography

Liver stiffness was also measured by transient elastography (Fibroscan®; Echosens SA, Paris, France). Briefly, patients were placed on the bed in the horizontally supine position. A probe was placed on the skin above the right intercostal space. The velocity of shear waves, which were generated temporarily and passed though the liver, was combined with Young's modulus for the automated calculation of elasticity [9]. Ten successful acquisitions were performed on each patient. The results that obtained ten valid measurements with a success rate of at least 60% and an interquartile range under 30% were considered successful. A median of 10 valid measurements was regarded as the liver stiffness for a given subject, and expressed in kilopascals (kPa).

APRI

The APRI was calculated as follows: aspartate aminotransferase (/upper limit of normal) × 10/platelet count (10^4/mm^3).

Statistical analysis

Box plots were used to study the distribution of the RTE values according to the stage of fibrosis. The trends were evaluated using the Jonckheere–Terpstra test. The Tukey–Kramer test was used to compare the data between the groups. The diagnostic performance of RTE parameters and transient elastography was assessed with receiver operating characteristic (ROC) curves. The ROC curve is a plot of the sensitivity versus 1-specificity for all possible cutoff values. The most commonly used index of accuracy is the area under the receiving operating characteristic curve (AUC), with values close to 1.0 indicating high diagnostic accuracy. Analyses were performed using JMP-8 software (SAS Institute, Cary, USA).

Results

Patients

The characteristics of our 101 patients with chronic HCV infection at the time of RTE and transient elastography are summarized in Table 1. In 91 of them, liver biopsy was performed successfully. Ten patients, who had no clinically

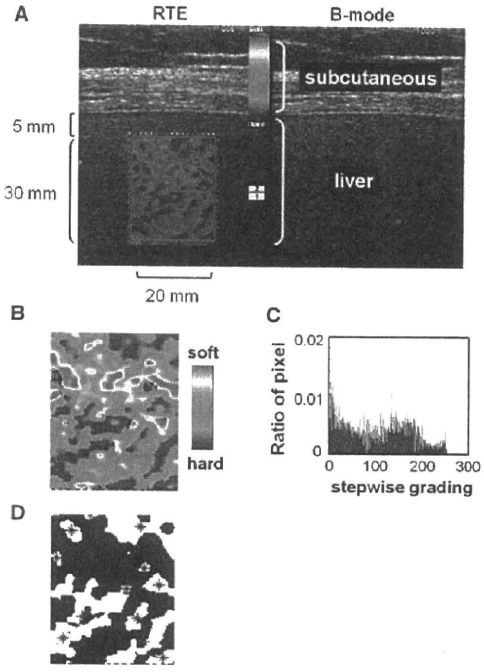

Fig. 1 Procedure of image analyses for real-time tissue elastography (*RTE*). **a** The region of interest (ROI) was fixed to a rectangle with about 30 mm length × about 20 mm breadth with a 400–600 mm^2 area placed 5 mm below the surface of the liver. **b** The color-coded images from the ROI of RTE were analyzed by the software Elasto_ver 1.5.1. The colors ranged from *blue to red*, indicating the relative gradients from hardness to softness. **c** Mean and Standard Deviation were calculated by a histogram, which was generated by 256 stepwise grading derived from the color image obtained in **b**. **d** Area and Complexity were calculated from the binary image. Area was derived from the percentage of *white regions* (*asterisks*, i.e., hard area). Complexity was calculated by the following equation, $periphery^2/Area$. Median value of the data was kept as representative of RTE parameters

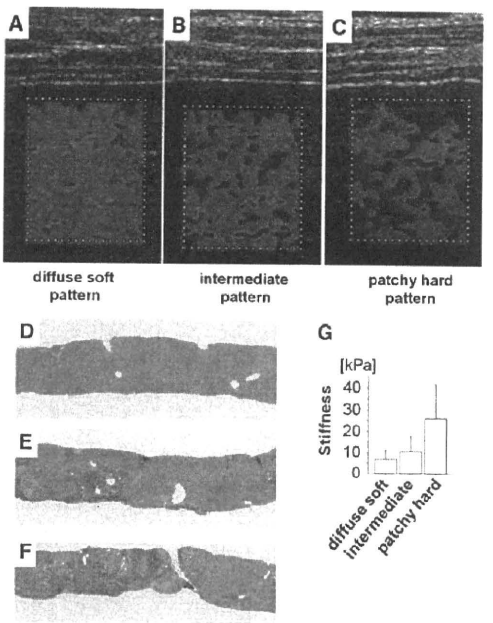

Fig. 2 Representative colored images of real-time tissue elastography. **a** A case of F1 whose histology is shown in **d**. Relatively homogeneous image colored *light green* indicates a diffuse soft pattern in RTE. Liver stiffness measured by transient elastography (Fibroscan) was 4.1 kPa. **b** A case of F3 whose histology is shown in **e**. Partially mottled and dotted image with blue and red spots in the light green background indicates an intermediate pattern in RTE. Liver stiffness measured by Fibroscan was 9.6 kPa. **c** A case of F4 whose histology is shown in **f**. Mixed image with *light green*, *blue*, and *red colors* indicates patchy hard pattern. Liver stiffness measured by Fibroscan was 36.3 kPa. **g** Correlation of the averages of liver stiffness measured by Fibroscan with the three patterns of RTE images. The transition of these three patterns correlated positively with the liver stiffness ($p < 0.01$). **d–f** H&E staining. *Yellow bars* 2 mm

overt sign of decompensated cirrhosis, were diagnosed with cirrhosis (F4) by the appearance of the liver surface at laparoscopy without liver biopsy. RTE was performed successfully in all patients, but five patients (F1, 2; F3, 1; F4, 2) failed to receive transient elastography measurements due to obesity and liver atrophy.

Representative images of real-time tissue elastography

The results were described as the Mean, which indicates the mean of the histogram and ranges from 73.0 to 139.8 (median 111.9), and Standard Deviation (SD), which indicates the standard deviation of the histogram and ranges from 36.5 to 76.6 (median 60.8). In another analysis, the data were transformed into a binary image and the results were described as Area, which indicates the percentage of hard tissue and represents the hard tissue domain divided by the ROI and ranges from 5.0 to 54.7% (median 24.8%), and Complexity, which indicates the complex ratio of the shape of an extracted hard tissue domain in the ROI and is calculated as $periphery^2/area$ of the hard tissue domain and ranges from 15.9 to 40.21 (median 22.9) (Fig. 1d). The four image features were calculated automatically by Elasto_ver 1.5.1 (Hitachi Medical). Mean, SD, and Complexity were described in arbitrary units [a.u.].

The colored RTE images were classified into three patterns according to their visual appearance. The values for liver stiffness measurement by transient elastography (Fibroscan) were 6.9 ± 4.5, 10.9 ± 6.8, and 26.0 ± 15.8 kPa in the diffuse soft pattern group, intermediate pattern group, and patchy

Table 1 Characteristics of the patients at the time of real-time tissue elastography examination

Characteristics	Patients (n = 101)
Sex: male/female	43/58
Age (years)	54 ± 13 (range 24–80)
BMI (kg/m^2)	22.1 ± 3.1 (range 15.1–32.3)
Platelet count ($\times 10^4$/mm^3)	16.4 ± 6.6
Total bilirubin (mg/dL)	0.9 ± 0.4
Prothrombin time (INR)	1.02 ± 0.1
ALT (IU/L)	58.2 ± 41.2
Fibrosis stage	
F0	6
F1	48
F2	15
F3	16
F4	16
Histological activity of 91 patients with liver biopsy	
A0	2
A1	24
A2	45
A3	20

Values are means ± SEM

BMI body mass index, *ALT* alanine aminotransferase

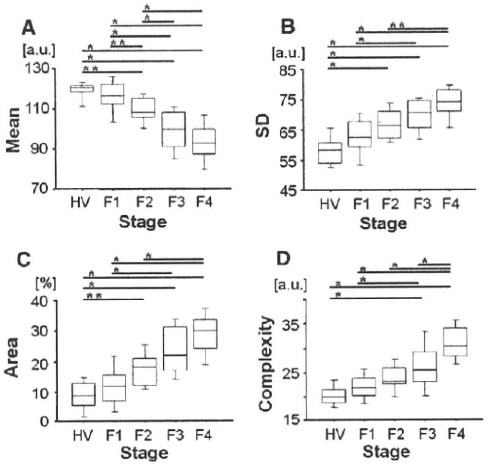

Fig. 3 Parameter analyses measured by real-time tissue elastography (RTE) for each fibrosis stage. Box plots of each RTE value corresponding to fibrosis stages F1–4 and the healthy volunteer group (*HV*). The *tops and bottoms of the boxes* indicate the 1st and 3rd quartiles. The *length of the box* represents the interquartile range within which 50% of values are located. The *lines through the middles of the boxes* represent the medians. **a** Mean, **b** SD, **c** Area, and **d** Complexity. HV, n = 10. F1–4, n = 95. *$p < 0.01$, and **$p < 0.05$

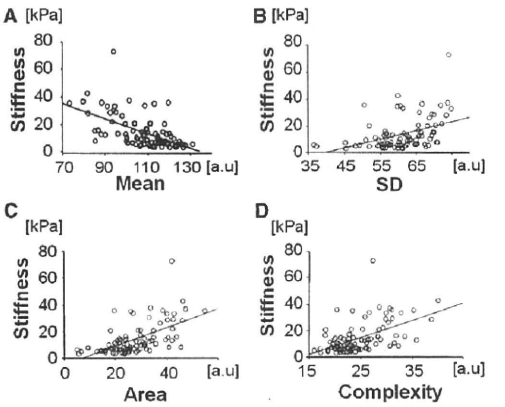

Fig. 4 Correlation between liver stiffness measured by transient elastography (Fibroscan) and the parameters of real-time tissue elastography. **a** Mean was negatively correlated with liver stiffness (kPa) ($p < 0.01$). Correlation coefficient was −0.585. **b** SD was significantly correlated with liver stiffness (kPa) ($p < 0.01$). Correlation coefficient was 0.425. **c** Area was significantly correlated with liver stiffness (kPa) ($p < 0.01$). Correlation coefficient was 0.590. **d** Complexity was significantly correlated with liver stiffness (kPa) ($p < 0.01$). Correlation coefficient was 0.532 (n = 96). *a.u.* arbitrary units

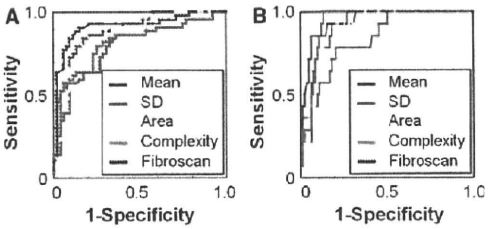

Fig. 5 Receiver operating characteristic curves of each parameter obtained by RTE. **a** No significant fibrosis (F0–1). **b** Cirrhosis (F4). **a** The areas under the receiver operating characteristic curves (AUC) for no significant fibrosis (F0–1) were 0.89, 0.81, 0.87, 0.81, and 0.92 for Mean (*red*), SD (*blue*), Area (*yellow*), Complexity (*pink*), and transient elastography (Fibroscan, *black*), respectively. **b** The AUCs for cirrhosis were 0.91, 0.84, 0.91, 0.93, and 0.95 for Mean, SD, Area, and Complexity, and transient elastography, respectively (n = 96)

hard pattern group, respectively. Thus, these three patterns correlated significantly with the kPa values obtained by transient elastography (Jonckheere–Terpstra test, $p < 0.0001$) (Fig. 2g).

Relationship between real-time tissue elastography and histological parameters

Figure 3a–d shows box plots of the RTE values corresponding to fibrosis stage and includes the healthy volunteer (HV) group. The Mean decreased with increasing fibrosis

score (Jonckheere–Terpstra test, $p < 0.0001$). SD, Area, and Complexity increased with increasing fibrosis score (Jonckheere–Terpstra test, $p < 0.0001$). The significant differences between each fibrosis stage were as follows: HV versus F3 and F4, F1 versus F3 and F4, and F2 versus F4 at every parameter; HV versus F2 at Mean, SD, and Area; F1 versus F2 at Mean; F2 versus F3 at Mean; F3 versus F4 at Complexity (Tukey–Kramer test, $p < 0.05$) No significant difference was found between the chronic hepatitis activity grades with same fibrosis stage at all parameters (Tukey–Kramer test).

Relationship between real-time tissue elastography and liver stiffness

Figure 4a–d, shows linear regression analysis of the values obtained by RTE compared to the liver stiffness values (kPa) obtained by transient elastography (Fibroscan). Simple regression analyses indicated that Mean, SD, Area, and Complexity were all significantly correlated with liver stiffness measured by Fibroscan (Mean: $r = -0.585$, $p < 0.001$; SD: $r = 0.425$, $p < 0.001$; Area: $r = 0.590$, $p < 0.001$; Complexity: $r = 0.532$, $p < 0.001$).

Relationship between real-time tissue elastography and platelet count, APRI, and other laboratory parameters

Simple regression analyses indicated that all Mean, SD, Area, and Complexity values were significantly correlated with the platelet count (Mean: $r = 0.432$, $p < 0.001$; SD: $r = -0.332$, $p = 0.001$; Area: $r = -0.402$, $p < 0.001$; Complexity: $r = -0.393$, $p < 0.001$). In addition, simple regression analyses indicated that Mean, SD, Area, and Complexity were all significantly correlated with APRI (Mean: $r = -0.442$, $p < 0.001$; SD: $r = 0.373$, $p < 0.001$; Area: $r = 0.425$, $p < 0.001$; Complexity: $r = 0.418$, $p < 0.001$). Furthermore, the correlation coefficient was significant for prothrombin time (Mean: $r = -0.404$, $p < 0.001$; SD: $r = 0.343$, $p < 0.005$; Area: $r = 0.435$, $p < 0.001$; Complexity: $r = 0.440$, $p < 0.001$), while no significant correlation was found for the four image features and total bilirubin, age, BMI, or alanine aminotransferase.

Diagnostic value of real-time tissue elastography and transient elastography

Figure 5 shows the ROC curves of RTE parameters for no significant fibrosis (F0–1) and cirrhosis (F4) in ninety-six patients who were also examined successfully by transient elastography. The AUCs for the stage of no significant fibrosis (F0–1) were 0.89, 0.81, 0.87, and 0.81 for Mean, SD, Area, and Complexity, respectively. The AUCs for severe fibrosis (≥F3) were 0.93, 0.84, 0.91, and 0.86 for Mean, SD, Area, and Complexity, respectively. The AUCs for cirrhosis (F4) were 0.91, 0.84, 0.91, and 0.93 for Mean, SD, Area, and Complexity, respectively. In transient elastography (Fibroscan), the AUCs were 0.92, 0.95, and 0.95 for stages F0–1, ≥F3s and F4, respectively. The corresponding sensitivities, specificities, and positive and negative predictive values are detailed in Table 2.

Discussion

Recently, various techniques based on ultrasound or magnetic resonance imaging have been developed to quantify

Table 2 Cutoff values of real-time tissue elastography (image features) and transient elastography for the diagnosis of fibrosis stages (*F*)

	$F = 0$–1	$F \geq 3$	$F = 4$
Mean (AUC)	0.89	0.93	0.91
Cutoff (a.u.)	110.1	106.9	101.5
Sensitivity (%)	84.1	82.8	85.7
Specificity (%)	82.7	85.1	82.9
Positive predictive value (%)	80.4	70.6	46.2
Negative predictive value (%)	86.0	91.9	97.1
SD (AUC)	0.81	0.84	0.84
Cutoff (a.u.)	61.2	63.0	65.7
Sensitivity (%)	70.5	75.9	78.6
Specificity (%)	73.1	77.6	79.3
Positive predictive value (%)	68.9	59.5	39.3
Negative predictive value (%)	74.5	88.1	95.6
Area (AUC)	0.87	0.91	0.91
Cutoff (%)	25.8	29.5	33.7
Sensitivity (%)	81.8	79.3	85.7
Specificity (%)	80.8	80.6	86.6
Positive predictive value (%)	78.3	63.9	52.2
Negative predictive value (%)	79.6	90.0	97.3
Complexity (AUC)	0.81	0.86	0.93
Cutoff (a.u.)	23.2	24.9	27.8
Sensitivity (%)	77.3	79.3	85.7
Specificity (%)	75.0	80.6	87.8
Positive predictive value (%)	72.3	63.9	54.5
Negative predictive value (%)	79.6	90.0	97.3
Transient elastography (AUC)	0.92	0.95	0.95
Cutoff (kPa)	10.1	13.3	16.3
Sensitivity (%)	88.6	89.7	85.7
Specificity (%)	86.5	86.6	85.4
Positive predictive value (%)	84.8	74.3	50.0
Negative predictive value (%)	90.0	95.1	97.2

AUC the area under the receiver operating characteristic curves, *a.u.* arbitrary units

liver stiffness, and thereby liver fibrosis, noninvasively. Among them, transient elastography (Fibroscan) has been used most frequently worldwide and has become established in clinical practice to detect advanced fibrosis without liver biopsy, although several limitations and disadvantages of the modality have been discussed [8, 24]. Another novel imaging modality is magnetic resonance elastography (MRE). The technique typically is added to a conventional MR examination of the upper abdomen [25]. A pneumatic or electromechanical driver is placed in contact with the abdominal wall and is used to generate propagating mechanical waves in the liver at frequencies between 40 and 120 Hz. Although MRE was shown to be superior to APRI and transient elastography for determining the stage of fibrosis in patients with various underlying liver diseases [26], MRE cannot be performed on an iron-overloaded liver because of noise. In addition, MRE takes a longer time and costs more than the ultrasound-based elastographies [2].

We paid attention in our analysis to the pattern change of RTE color images according to the progression of fibrosis. Normal or minimally fibrotic liver exhibited a homogeneous RTE image that was colored light green (Fig. 2a). According to the progression of liver fibrosis, the homogeneous pattern transited to a patchy pattern consisting of a blue-colored area (Fig. 2c), which may suggest a collapse of homogeneity. In the present study, for semi-quantification of the RTE image, we used a histogram and binary image produced using an exclusive software program that was developed by Hitachi Medical. This is the first report demonstrating the utility of Mean, SD, Area, and Complexity as RTE parameters. We speculate that Mean and Area may directly represent liver elasticity, while SD and Complexity may imply the collapse of the uniform architecture of the liver parenchyma concomitant with progressing hepatic fibrosis (Figs. 3, 5).

After the report by Friedrich-Rust et al. [21] other investigators criticized the intraobserver variability and the lack of interobserver agreement in hepatic RTE [27–29]. In general practice, an operator presses lightly on the surface of the liver through the skin with a transducer when the elastogram is generated. Thus, the pressure generated by the operator's compression is assumed to influence both the image of elasticity and the resulting elasticity score. To avoid this source of error, we used here the latest and most sensitive probe that was produced by Hitachi Medical and did not require extra external stress. Accordingly, we were able to improve the acquisition of the color image representing the distortion of liver tissue under the heartbeat or abdominal aorta. We also adopted ten individual frames for semi-quantitative analysis. On the other hand, Saftoiu et al. and Gulizia et al. have proposed that the ROI should include the surrounding tissues, such as adipose tissue, diaphragm, and intercostal muscles, in order to clearly compare and distinguish the strain between the liver and these organs [27, 30]. However, we placed the ROI inside the liver at 5 mm below the surface of the liver, because the new probe used in this study is sensitive and the deep attenuation of the ultrasound image could be disregarded. We avoided including the liver surface inside the ROI because the liver surface is hard and therefore is assessed as a harder area, influencing the histogram analysis. Recently, Tatsumi et al. [31] also reported the results of RTE using the ROI inside the liver in a similar fashion to our study.

We note that all four image features of RTE, comprising Mean, SD, Area, and Complexity, were significantly correlated with the kPa value obtained by Fibroscan [8, 11–13]. In particular, Mean and Area had a high correlation. In addition, the AUC values were similar between RTE and Fibroscan. Mean and Area were highly accurate for the diagnosis of significant fibrosis (i.e., >F0–1) and for the diagnosis of cirrhosis (F4). Although the performance of Fibroscan has been demonstrated in many studies to have high accuracy [4, 8], the machine is used solely for elasticity measurements. In contrast, with the new equipment used in the present study, RTE can be used simultaneously with conventional B-mode ultrasonography. Moreover, as reported by Obara et al. [32], liver stiffness measurement by Fibroscan was unsuccessful in 5.3% of Japanese cases of chronic liver disease, similar to our experience in the present study (5.0%). Thus, RTE is considered to be superior to Fibroscan at points where measurements by Fibroscan are difficult to perform in obese patients and are impossible to perform in patients with ascites [32, 33], and where RTE images are unaffected by steatosis, as suggested previously by Friedrich-Rust et al. [29]. Furthermore, because Fibroscan measures liver stiffness between 25 and 65 mm below the surface of the skin [10], knowledge of the relative thickness of the liver is necessary for the measurements.

The diagnostic performance of RTE is similar to that of other noninvasive laboratory tests, such as the FibroTest (BioPredictive, Paris, France), APRI, and the Forns score reported in the literature [4, 8]. The FibroTest is based on five serological parameters; bilirubin, gamma-glutamyl transpeptidase (GGT), apolipoprotein A1, α2-macroglobulin, and haptoglobin. While the diagnostic accuracy was high (AUC 0.87) for significant fibrosis (METAVIR, ≥F2), the FibroTest costs more than APRI and the Forns score, and needs two uncommon parameters [34]. In APRI, using the cutoffs proposed by Wai et al. [5], approximately 50% of patients could be correctly classified as having cirrhosis without a liver biopsy5. With the Forns' index, the AUC for the prediction of significant fibrosis (Scheuer classification, ≥F2) was 0.86 in the test set and 0.81 in the validation set [35]. It is, however, known that the determination of the

severity of liver fibrosis by serum markers is confounded by acute inflammation, hemolysis, cholestasis, and renal failure [4, 5, 34, 35]. Castera et al. [11] compared the performance of transient elastography with that of the FibroTest, APRI, and liver biopsy for the assessment of liver fibrosis in a large number of patients with hepatitis C. Interestingly, they reported that the best performance was achieved by a combination of transient elastography with the FibroTest [11]. Friedrich-Rust et al. reported that the best diagnostic accuracy was obtained by combining the variables used for the calculation of the RTE elasticity score with the platelet count and GGT [18]. Thus, RTE, in combination with serological parameters, can further improve the accuracy of differentiating fibrosis stages.

One of the major limitations of the present study was that the number of patients with F1 was higher than the number of those with the other stages, because most of our patients received liver biopsy prior to interferon treatment. However, our study compared the performance of RTE with that of transient elastography in the same patients. Although the AUC for RTE in this study was higher than that in the studies by Friedrich-Rust et al. [21, 29], the AUC for transient elastography was approximately equivalent to that in one of these studies [29].

In the METAVIR and Desmet's histological scoring systems, cirrhosis is classified as a single category (i.e., F4) [22, 23]. However, the degree of fibrosis; for example, the content of collagen and extracellular matrix materials that may be closely associated with the function of hepatocytes and portal hypertension, may vary among patients with cirrhosis. Foucher et al. [36] reported that the kPa measured by transient elastography in cirrhotic patients correlated well with clinical parameters indicating the severity of cirrhosis; 27.5 kPa was the cutoff value for the presence of esophageal varices stage 2 or 3, 37.5 kPa for liver function Child B or C, 49.1 kPa for a past history of ascites, and 62.7 kPa for esophageal variceal bleeding. Thus, because cirrhosis can be staged in greater detail with clinical relevance based on liver stiffness with RTE, RTE may be useful for this staging of cirrhosis and for detecting and assessing the risk of cirrhotic complications [36].

In summary, we have shown a convenient and noninvasive procedure, RTE, for the visual assessment of liver stiffness. The performance of RTE compares favorably with that of transient elastography (Fibroscan) for detecting the presence of significant liver fibrosis in patients with chronic hepatitis C. We suggest that RTE could be used as a routine imaging method to evaluate the degree of liver fibrosis in patients with liver disease. Future studies of larger patient cohorts will be necessary for the validation of the four RTE parameters, and the combination of these parameters will enable improvement of accuracy in assessing hepatic fibrosis.

Acknowledgments We thank Ms. Akiko Tonomura and Mr. Junji Warabino, Hitachi Medical Co., for the technical support for RTE. Hiroyasu Morikawa was supported by a research grant from Osaka City University (2009). Norifumi Kawada was supported by Research on Hepatitis, Health and Labour Science Research Grants from the Ministry of Health, Labour and Welfare of Japan (2008–2009) and by a Thrust Area Research Grant from Osaka City University (2008–2009).

References

1. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat. 1999;6:35–47.
2. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358:958–65.
3. Sporea I, Popescu A, Sirli R. Why, who and how should perform liver biopsy in chronic liver diseases. World J Gastroenterol. 2008;14:3396–402.
4. Manning DS, Afdhal NH. Diagnosis and quantitation of fibrosis. Gastroenterology. 2008;134:1670–81.
5. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518–26.
6. Sandrin L, Tanter M, Gennisson JL, Catheline S, Fink M. Shear elasticity probe for soft tissues with 1D transient elastography. IEEE Trans Ultrason Ferroelectr Freq Control. 2002;49:436–46.
7. Ganne-Carrié N, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, et al. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. Hepatology. 2006;44:1511–7.
8. Pinzani M, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. Nat Clin Pract Gastroenterol Hepatol. 2008;5:95–106.
9. Yeh WC, Li PC, Jeng YM, Hsu HC, Kuo PL, Li ML, et al. Elastic modulus measurements of human liver and correlation with pathology. Ultrasound Med Biol. 2002;28:467–74.
10. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29:1705–13.
11. Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology. 2005;128:343–50.
12. Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology. 2005;41:48–54.
13. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology. 2008;134:960–74.
14. Arena U, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology. 2008;47:380–4.
15. Nitta N, Yamakawa M, Shiina T. Tissue elasticity imaging based on combined autocorrelation method and 3D tissue model. In: Proceedings of the IEEE Ultrasonics Symposium. Savoy, Ill: