

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 <i>et al.</i>	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo <i>et al.</i>	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Suou <i>et al.</i>	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 <i>et al.</i>	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin <i>et al.</i>	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong <i>et al.</i>	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 <i>et al.</i>	Case-control study	24 vs 33	69 vs 67	alpha	1 (4%)	36	14% vs 73% $P = 0.011$	100% vs 94% $P = 0.25$
Mazzafiero <i>et al.</i>	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu <i>et al.</i>	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo <i>et al.</i>	Case-control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% vs 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.<sup>144</sup> In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.<sup>145-147</sup> 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.<sup>114</sup> 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.<sup>138</sup>

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

**I**KEDA ET AL. elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.<sup>132</sup> At 5 and 10 years, hepatocarcinogenesis rates in the intermediate ( $100-140 \times 10^9/L$ ) and low platelet ( $<100 \times 10^9/L$ ) 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 ( $\geq 150 \times 10^9/L$ ) 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.<sup>148-150</sup>

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.<sup>101,151-155</sup> McHutchison et al. revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN- $\alpha$ -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.<sup>152</sup>

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.<sup>156</sup> As a result, the mean dose of PEG-IFN- $\alpha$ -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.<sup>157</sup> In the analysis of 472 patients responding to PEG-IFN- $\alpha$ -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- $\alpha$ -2a (135  $\mu$ g/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.<sup>158</sup> Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.<sup>159</sup>

**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- $\alpha$ -2a or over 1.2  $\mu$ g/kg per week of PEG-IFN- $\alpha$ -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- $\alpha$ -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.)

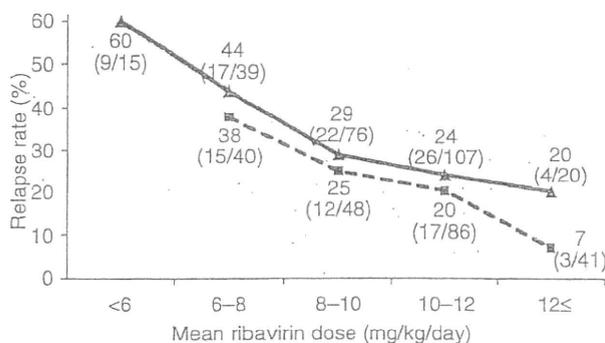


Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)- $\alpha$ -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses ( $\text{---}\blacktriangle\text{---}$ ). Group with the mean PEG-IFN dose  $< 1.4 \mu\text{g/kg/week}$  ( $\text{---}\blacksquare\text{---}$ ). Group with the mean PEG-IFN dose  $\ge 1.4 \mu\text{g/kg/week}$ . There was no significant difference between the two PEG-IFN- $\alpha$ -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 ( $\ge 80 \text{ IU/mL}$ ) than in those with a lower ALT value ( $< 80 \text{ IU/mL}$ ).<sup>70</sup> 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.<sup>160</sup>

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.<sup>161,162</sup> 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.<sup>163,164</sup>

Repeated phlebotomy has been shown to be effective for the improvement of serum ALT as well as progression of fibrosis,<sup>32</sup> however, it remains controversial whether the effects of IFN improve with extensive phlebotomy.<sup>165-169</sup>

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

**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.<sup>171,172</sup> 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- $\alpha$ -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

- 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.
- Shiffman RN, Shekelle P, Overhage J *et al.* Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann Intern Med* 2003; 139: 493-98.
- Pileri P, Uematsu Y, Campagnoli S *et al.* Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938-41.
- 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.
- Evans MJ, von Hahn T, Tschernie DM *et al.* Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; 446: 801-5.
- Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. *Cell Microbiol* 2008; 10: 821-7.
- 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.
- Flint M, von Hahn T, Zhang J *et al.* Diverse CD81 proteins support hepatitis C virus infection. *J Virol* 2006; 80: 11331-42.
- 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.
- 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.
- 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.
- 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.
- Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; 55: 255-81.
- 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.
- Gale M Jr, Foy EM. Evasion of intracellular host defense by hepatitis C virus. *Nature* 2005; 436: 939-45.
- 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.
- Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; 2: 410-6.
- 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.
- 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.
- Noguchi T, Satoh S, Noshi 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.
- 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.
- 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 Vitoria 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 or 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 DI, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 38: 1147-71.
- 39 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon- $\alpha$  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. *Intervirol* 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. IHT 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, METEVIR, 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, Kiriya S et al. Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. *Gut* 2007; 56: 738-9.
  - 72 Kumada T, Toyoda H, Kiriya 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. *Kantansui* 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, Eguchi 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 SUCCESS 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, Oliveira 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 F, 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 tripartite 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 Kamar 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 glycyrrhizic 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 carcinoma 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 depletion on 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 trial. *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.

## Original Article

# A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis

Masayuki Kurosaki,<sup>1</sup> Kotaro Matsunaga,<sup>2</sup> Itsuko Hirayama,<sup>1</sup> Tomohiro Tanaka,<sup>1</sup> Mitsuaki Sato,<sup>1</sup> Yutaka Yasui,<sup>1</sup> Nobuharu Tamaki,<sup>1</sup> Takanori Hosokawa,<sup>1</sup> Ken Ueda,<sup>1</sup> Kaoru Tsuchiya,<sup>1</sup> Hiroyuki Nakanishi,<sup>1</sup> Hiroki Ikeda,<sup>1</sup> Jun Itakura,<sup>1</sup> Yuka Takahashi,<sup>1</sup> Yasuhiro Asahina,<sup>1</sup> Megumu Higaki,<sup>4</sup> Nobuyuki Enomoto<sup>3</sup> and Namiki Izumi<sup>1</sup>

<sup>1</sup>Division of Gastroenterology and Hepatology and <sup>2</sup>Division of Pathology, Musashino Red Cross Hospital, Tokyo, <sup>3</sup>First Department of Internal Medicine, University of Yamanashi, Yamanashi, and <sup>4</sup>Department of Medical Science, Jikei Medical University, Tokyo, Japan

**Aim:** Early disappearance of serum hepatitis C virus (HCV) RNA is the prerequisite for achieving sustained virological response (SVR) in peg-interferon (PEG-IFN) plus ribavirin (RBV) therapy for chronic hepatitis C. This study aimed to develop a decision tree model for the pre-treatment prediction of response.

**Methods:** Genotype 1b chronic hepatitis C treated with PEG-IFN alpha-2b and RBV were studied. Predictive factors of rapid or complete early virological response (RVR/cEVR) were explored in 400 consecutive patients using a recursive partitioning analysis, referred to as classification and regression tree (CART) and validated.

**Results:** CART analysis identified hepatic steatosis (<30%) as the first predictor of response followed by low-density-lipoprotein cholesterol (LDL-C) ( $\geq 100$  mg/dL), age (<50 and <60 years), blood sugar (<120 mg/dL), and gamma-glutamyltransferase (GGT) (<40 IU/L) and built decision tree

model. The model consisted of seven groups with variable response rates from low (15%) to high (77%). The reproducibility of the model was confirmed by the independent validation group ( $r^2 = 0.987$ ). When reconstructed into three groups, the rate of RVR/cEVR was 16% for low probability group, 46% for intermediate probability group and 75% for high probability group.

**Conclusions:** A decision tree model that includes hepatic steatosis, LDL-C, age, blood sugar, and GGT may be useful for the prediction of response before PEG-IFN plus RBV therapy, and has the potential to support clinical decisions in selecting patients for therapy and may provide a rationale for treating metabolic factors to improve the efficacy of antiviral therapy.

**Key words:** data mining, decision tree, HCV, low-density-lipoprotein-cholesterol, steatosis

## INTRODUCTION

COMBINATION THERAPY WITH pegylated interferon (PEG-IFN) and ribavirin (RBV) is now recognized as a standard treatment for patients with chronic hepatitis C.<sup>1</sup> However, the rate of sustained virological response (SVR) to 48 weeks of PEG-IFN RBV combina-

tion therapy is only 50% in patients with hepatitis C virus (HCV) genotype 1b and high HCV RNA titer, so called difficult to treat chronic hepatitis C patients.<sup>2,3</sup> Within this difficult to treat group, the response to treatment sometimes can be highly heterogeneous for cases which are apparently equivalent in HCV RNA titer, making the prediction of response before treatment a difficult task. It has been suggested that early virological response (EVR), defined as either undetectable HCV RNA or a 2 log drop in HCV RNA at week 12, is a reliable means to predict SVR.<sup>2,4</sup> More recently, it has been suggested that patients with a rapid virological response (RVR: undetectable HCV RNA at week 4) and a complete EVR (cEVR: undetectable HCV RNA at week 12)

Correspondence: Dr Namiki Izumi, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. Email: nizumi@musashino.jrc.or.jp

Received 26 May 2009; revision 25 August 2009; accepted 26 August 2009.

achieve high SVR rates, while patients with a partial EVR (pEVR: 2 log drop in HCV RNA but still detectable at week 12) have lower rates of SVR.<sup>5</sup> Since PEG-IFN RBV combination therapy is costly and accompanied by potential adverse effects, the ability to predict the possibility of RVR or cEVR before therapy and identifying curable patients may significantly influence the selection of patients for therapy. Moreover, identification of baseline predictors of poor response is particularly important to establish a rationale for identifying therapeutic targets to improve the efficacy of antiviral therapy.

Data mining is a method of predictive analysis which explores tremendous volumes of data to discover hidden patterns and relationships in highly complex datasets and enables the development of predictive models. The classification and regression tree (CART) analysis is a core component of the decision tree tool for data mining and predictive modeling,<sup>6</sup> is deployed to decision makers in various fields of business, and currently is being used in the area of biomedicine.<sup>7-13</sup> The results of CART analysis are presented as a decision tree, which is intuitive and facilitates the allocation of patients into subgroups by following the flow-chart form.<sup>14</sup> CART has been shown to be competitive with other traditional statistical techniques such as logistic regression analysis.<sup>15</sup>

In the present study, we used the CART analysis to explore baseline predictors of response to PEG-IFN plus RBV therapy among clinical, biochemical, virological and histological pretreatment variables and to define a pre-treatment algorithm to discriminate chronic hepatitis C patients who are likely to respond to PEG-IFN plus RBV therapy.

## MATERIALS AND METHODS

### Patients

A TOTAL OF 419 chronic hepatitis C patients were treated with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital between December 2001 and December 2007. Among them, 400 patients who fulfilled the following inclusion criteria were enrolled in the present study. (i) infection by genotype 1b (ii) HCV RNA higher than 100 KIU/mL by quantitative PCR (Cobas Amplicor HCV Monitor, Roche Diagnostic systems, CA) which is usually used for the definition of high viral load in Japan (iii) lack of co-infection with hepatitis B virus or human immunodeficiency virus (iv) lack of other causes of liver disease such as autoimmune hepatitis, primary biliary cirrhosis, or alcohol intake of more than 20 g per day, and (v) having completed at

least 12 weeks of therapy with an early virological response that could be evaluated. Patients received PEG-IFN alpha-2b (1.5 microgram/kg) subcutaneously every week and were administered a weight adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg) which is the recommended dosage in Japan. Data from two third of patients (269 patients) were used for the model building set and the remaining one third of patients (131 patients) were used as a validation set. Consent in writing was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review committee.

### Laboratory tests

Blood samples were obtained before therapy, and at least once every month during therapy and analyzed for hematologic tests, blood chemistries, and HCV RNA. In the present study, RVR and cEVR was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/mL (Amplicor, Roche Diagnostic systems, CA) at week 4 and 12, respectively. SVR was defined as undetectable HCV RNA at week 24 after the completion of therapy.

### Histological examination

For all patients, liver biopsy specimens were obtained before therapy and were evaluated independently by three pathologists who were blinded to the clinical details. If there was a disagreement, the scores assigned by the majority of pathologists were used for the analysis. Fibrosis and activity were scored according to the METAVIR scoring system.<sup>16</sup> Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis) and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity) and A3 (severe activity). Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0–3: grade 0 (no steatosis), grade 1 (0–9%), grade 2 (10–29%), and grade 3 (over 30%) as we reported previously.<sup>17</sup>

### Database for analysis

A pretreatment database of 72 variables was created containing histological findings (grade of fibrosis, activity, and steatosis), laboratory tests including the quantity of HCV RNA by Cobas Amplicor, and clinical information (age, gender, body weight, and body mass index).

The baseline characteristics and test results are listed in Table 1. The overall rate of RVR/cEVR was 43% in the model building set and 48% in the validation set. There were no significant differences in the clinical backgrounds between these two groups. Hepatitis C viral mutations, such as mutations in interferon-sensitivity determining region or core amino acid residues 70 and 91, were not included in the present analysis. The dataset of laboratory tests was based on the digitized records in this hospital. Continuous data was split into categorized data by increment of 10; For example, age was categorized into <30, 30–39, 40–49, 50–59, 60–69, and ≥70.

### Statistical analysis

Based on this database, the recursive partitioning analysis algorithm referred to as CART was implemented to define meaningful subgroups of patients with respect to the possibility of achieving RVR/cEVR. The CART belongs to a family of nonparametric regression methods based on binary recursive partitioning of data. The software automatically explore the data to search for optimal split variables, builds a decision tree structure and finally classifies all subjects into particular subgroups that are homogeneous with respect to the outcome of interest.<sup>18</sup> During the CART analysis, first, the entire study population, and thereafter, all newly defined subgroups, were investigated at every step of the analysis to determine which variable at what cut-off point yielded the most significant division into two prognostic subgroups that were as homogeneous as possible with respect to estimates of RVR/cEVR possibilities. This algorithm uses the impurity function (Gini criterion function) for splitting.<sup>19</sup> A restriction was imposed on the tree construction such that terminal subgroups resulting from any given split must have at least 20 patients. The CART procedure stopped when either no additional significant variable was detected or when the sample size was below 20. The resulting final subgroups were most homogeneous with respect to the probability of achieving RVR/cEVR. For this analysis, data mining software Clementine version 12.0 (SPSS Inc, Chicago, IL) was utilized. SPSS 15.0 (SPSS Inc, Chicago, IL) was used for logistic regression analysis.

## RESULTS

### Factors associated with RVR/cEVR by standard statistical analysis

WE FIRST ANALYZED 72 variables by univariate and multivariate logistic regression analysis to find factors associated with RVR/cEVR (Table 2).

Patients with RVR/cEVR were significantly younger than those without. Among histological findings, grade of steatosis and stage of fibrosis was significantly lower in RVR/cEVR. Among hematologic tests, hemoglobin and hematocrit was significantly higher in RVR/cEVR. Among blood chemistry tests, creatinine and low-density lipoprotein cholesterol (LDL-C) was significantly higher and gamma-glutamyltransferase (GGT), low-density-lipoprotein cholesterol (LDL-C), and blood sugar were significantly lower in RVR/cEVR. The level of HCV RNA was significantly lower in RVR/cEVR. There were no significant differences in other tests.

Multivariate logistic regression analysis was performed on age, fibrosis stage, steatosis, HCV RNA, creatinine, hemoglobin, GGT, LDL-C, and blood sugar: hematocrit was not included since it is closely associated with hemoglobin. On multivariate analysis, age, grade of steatosis, level of HCV RNA, creatinine, hemoglobin, GGT, and LDL-cholesterol remained significant whereas stage of fibrosis, hemoglobin and blood sugar were not.

### The CART analysis

The CART analysis was carried out on the model building set of 269 patients using the same variables as logistic regression analysis. Figure 1 shows the resulting decision tree. The CART analysis automatically selected five predictive variables to produce a total of seven subgroups of patients. The grade of steatosis was selected as the variable of initial split with an optimal cut-off of 30%. The possibility of achieving RVR/cEVR was only 18% for patients with hepatic steatosis of 30% or more compared to 47% for patients with hepatic steatosis of less than 30%. Among patients with hepatic steatosis of less than 30%, the level of serum LDL-C, with an optimal cut-off of 100 mg/dL, was selected as the variable of second split. Patients with higher LDL-C level had the higher probability of RVR/cEVR (57% vs. 32%). Among patients with LDL-C of less than 100 mg/dL, age, with an optimal cut-off of 60, was selected as the third variable of split. Younger patients had the higher probability of RVR/cEVR (49% vs. 15%). Among patients younger than 60, the blood sugar, with an optimal cut-off of 120 mg/dL, was selected as the fourth variable of split. Patients with lower blood sugar level had the higher probability of RVR/cEVR (71% vs. 31%). Among patients with hepatic steatosis of less than 30% and LDL-C of 100 mg/dL or more, age, with an optimal cut-off of 50, was selected as the third variable of split, younger being the predictor of higher RVR/cEVR probability (77% vs. 50%). Among patients older than 50,

Table 1 Clinical characteristics of patients

	Model set n = 269	Validation set n = 131	P-value
Sex (M/F)	127/142	55/76	0.325
Age (years)	57.7 ± 10.1	57.6 ± 10.0	0.932
Body weight (kg)	59.6 ± 11.0	57.5 ± 9.5	0.094
Body mass index (kg/m <sup>2</sup> )	23.2 ± 3.1	23.3 ± 3.8	0.934
Total protein (g/dL)	7.6 ± 0.5	7.7 ± 0.6	0.558
Albumin (g/dL)	4.2 ± 0.3	4.2 ± 0.3	0.349
Globulin (g/dL)	3.4 ± 0.5	3.4 ± 0.6	0.989
Aspartate aminotransferase (IU/L)	58.1 ± 43.1	55.8 ± 37.5	0.601
Alanine aminotransferase (IU/L)	70.9 ± 49.2	66.4 ± 52.6	0.462
Gamma-glutamyltransferase (IU/L)	49.6 ± 44.0	45.2 ± 34.4	0.33
Lactate dehydrogenase (IU/L)	289.3 ± 112.3	301.5 ± 109.3	0.417
Total bilirubin (mg/dL)	0.71 ± 0.28	0.69 ± 0.23	0.317
Direct bilirubin (mg/dL)	0.23 ± 0.12	0.25 ± 0.10	0.147
Indirect bilirubin (mg/dL)	0.48 ± 0.21	0.44 ± 0.16	0.064
Alkaline phosphatase (IU/L)	290.9 ± 107.6	292.5 ± 107.6	0.917
Leucine aminopeptidase (IU/L)	64.3 ± 14.3	65.5 ± 12.3	0.543
Thymol turbidity test (KU)	7.1 ± 3.4	8.0 ± 3.7	0.062
Zinc sulfate turbidity test (KU)	15.4 ± 4.9	16.3 ± 5.4	0.188
Choline esterase (IU/L)	318.1 ± 81.7	321.1 ± 78.1	0.798
Ammonia (microg/dL)	39.7 ± 20.2	45.0 ± 15.6	0.668
Blood sugar (mg/dL)	125.9 ± 41.1	117.4 ± 47.9	0.081
Glycohemoglobin (%)	5.6 ± 1.6	5.4 ± 1.2	0.797
Total cholesterol (mg/dL)	170.8 ± 33.9	175.6 ± 36.8	0.170
Low-density-lipoprotein-cholesterol (mg/dL)	96.5 ± 25.2	100.9 ± 28.5	0.153
High-density-lipoprotein-cholesterol (mg/dL)	54.2 ± 15.9	55.2 ± 17.4	0.612
Triglyceride (mg/dL)	108.5 ± 47.8	102.8 ± 46.4	0.306
Creatinine (mg/dL)	0.72 ± 0.15	0.74 ± 0.17	0.236
Urea nitrogen (mg/dL)	14.1 ± 3.4	14.9 ± 3.9	0.123
Uric acid (mg/dL)	5.3 ± 1.2	5.2 ± 1.2	0.715
Sodium (mEq/L)	142.2 ± 2.0	142.4 ± 2.0	0.471
Potassium (mEq/L)	4.3 ± 0.3	4.3 ± 0.4	0.578
Chloride (mEq/L)	104.0 ± 2.2	104.0 ± 2.6	0.905
Calcium (mg/dL)	9.1 ± 0.4	9.2 ± 0.4	0.479
Phosphorus (mg/dL)	3.5 ± 0.5	3.5 ± 0.6	0.814
Magnesium (mg/dL)	2.2 ± 0.2	2.3 ± 0.3	0.390
Amylase (IU/L)	178.7 ± 125.8	175.1 ± 133.1	0.118
Creatine kinase (IU/L)	114.9 ± 147.6	119.3 ± 73.7	0.849
Iron (microg/dL)	104.7 ± 53.2	109 ± 37	0.726
Ferritin (ng/mL)	111.3 ± 103.3	59.7 ± 118.5	0.405
C-reactive peptide (mg/dL)	0.2 ± 1.1	0.1 ± 0.1	0.586
Immunoglobulin G (mg/dL)	1849 ± 426	1988 ± 525	0.129
Immunoglobulin M (mg/dL)	141 ± 69	205 ± 106	0.200
Immunoglobulin A (mg/dL)	323 ± 675	291 ± 81	0.784
Triiodothyronine (pg/mL)	2.3 ± 0.3	2.2 ± 0.3	0.358
Thyroxin (ng/dL)	0.9 ± 0.1	0.9 ± 0.1	0.872
Thyroid stimulating hormone (micro IU/mL)	1.8 ± 1.4	1.7 ± 0.7	0.939
White blood cell count (/microl)	5243 ± 1591	5286 ± 1101	0.843
Segmented neutrophils (%)	55.4 ± 10.8	57.0 ± 10.0	0.297
Band neutrophils (%)	1.5 ± 1.6	0.5 ± 0.6	0.250
Eosinophils (%)	2.9 ± 2.3	2.4 ± 1.4	0.127

Table 1 Continued

	Model set n = 269	Validation set n = 131	P-value
Basophiles (%)	0.6 ± 0.4	0.6 ± 0.3	0.727
Lymphocytes (%)	34.6 ± 9.6	34.0 ± 9.3	0.682
Monocytes (%)	6.6 ± 2.2	6.2 ± 2.6	0.149
Red blood cell count (10 <sup>4</sup> /microl)	458 ± 43	455 ± 47	0.643
Hemoglobin (g/dL)	14.4 ± 1.5	14.5 ± 1.5	0.618
Hematcrit (%)	42.7 ± 4.0	42.9 ± 4.4	0.717
Reticulocytes (%)	1.4 ± 0.4	1.4 ± 0.4	0.762
Mean corpuscular volume (fL)	93.3 ± 4.5	93.8 ± 5.41	0.466
Mean corpuscular hemoglobin concentration (pg)	31.5 ± 1.9	31.7 ± 2.3	0.583
Mean corpuscular hemoglobin concentration (g/dL)	33.8 ± 0.9	33.7 ± 1.3	0.910
Platelets (10 <sup>4</sup> /microl)	16.8 ± 5.4	16.3 ± 4.5	0.480
Prothrombin time (s)	11.7 ± 1.2	11.7 ± 0.9	0.762
Prothrombin time (activity %)	104.6 ± 14.4	102.6 ± 14.8	0.363
Prothrombin time (international normalized ratio)	1.0 ± 0.1	1.0 ± 0.1	0.387
Thrombin time (%)	97.2 ± 31.3	109 ± 31.5	0.231
Activated partial thromboplastin time (s)	29.7 ± 4.4	29.1 ± 2.7	0.260
Hepaplastin test (%)	97.8 ± 20.3	95.4 ± 19.4	0.523
Fibrinogen (%)	237 ± 44	225 ± 45	0.069
Hepatitis C virus RNA (<850/≥850 KIU/mL)	130/139	70/61	0.394
Histological grade of			
Activity (A1/A2/A3)	138/107/24	62/55/14	0.714
Fibrosis (F1/F2/F3/F4)	135/74/57/3	58/40/27/6	0.131
Steatosis (0%/1-9%/10-29%/30%≧)	89/109/37/34	49/45/21/16	0.643
Hepatitis C virus RNA negative at week 12 (yes/no)	116/153	63/68	0.349

the level of GGT, with an optimal cutoff of 40 U/L, were then selected as the fourth level of split, low levels being the predictor of higher RVR/cEVR probability (60% vs. 35%).

All five factors selected as significant variables in the CART analysis were also significantly associated with RVR/cEVR by univariate analysis (Table 2). In addition, steatosis, LDL-C, age and GGT were also independently

Table 2 Factors associated with rapid or complete early virological response by univariate and multivariate logistic regression analysis

Parameter	Category	Univariate			Multivariate		
		Odds	95% CI	P-value	Odds	95% CI	P-value
Age (years)	<50 vs. ≥50	2.65	1.51-4.65	<0.001	2.03	1.04-3.97	0.039
Fibrosis stage	F1-2 vs. F3-4	2.47	1.31-4.66	0.005	1.77	0.85-3.68	0.120
Steatosis (%)	<30 vs. ≥30	4.11	1.64-10.29	0.003	2.88	1.07-7.79	0.037
Hepatitis C virus RNA (KIU/mL)	<850 vs. ≥850	1.97	1.21-3.22	0.007	1.93	1.09-3.43	0.025
Creatinine (mg/dL)	≥0.8 vs. <0.8	3.30	1.96-5.56	<0.001	3.54	1.88-6.67	<0.001
Hemoglobin (g/dL)	≥14.5 vs. <14.5	1.76	1.08-2.87	0.023	1.38	0.74-2.57	0.320
Hematcrit (%)	≥43 vs. <43	1.75	1.07-2.84	0.003			
Gamma-glutamyltransferase (IU/L)	<40 vs. ≥40	2.06	1.26-3.37	0.004	2.45	1.32-4.56	0.005
Low-density-lipid cholesterol (mg/dL)	≥100 vs. <100	2.71	1.61-4.55	<0.001	2.21	1.21-4.06	0.010
Blood sugar (mg/dL)	<120 vs. ≥120	2.00	1.02-3.95	0.045	1.42	0.64-3.13	0.390

CI, confidence interval.

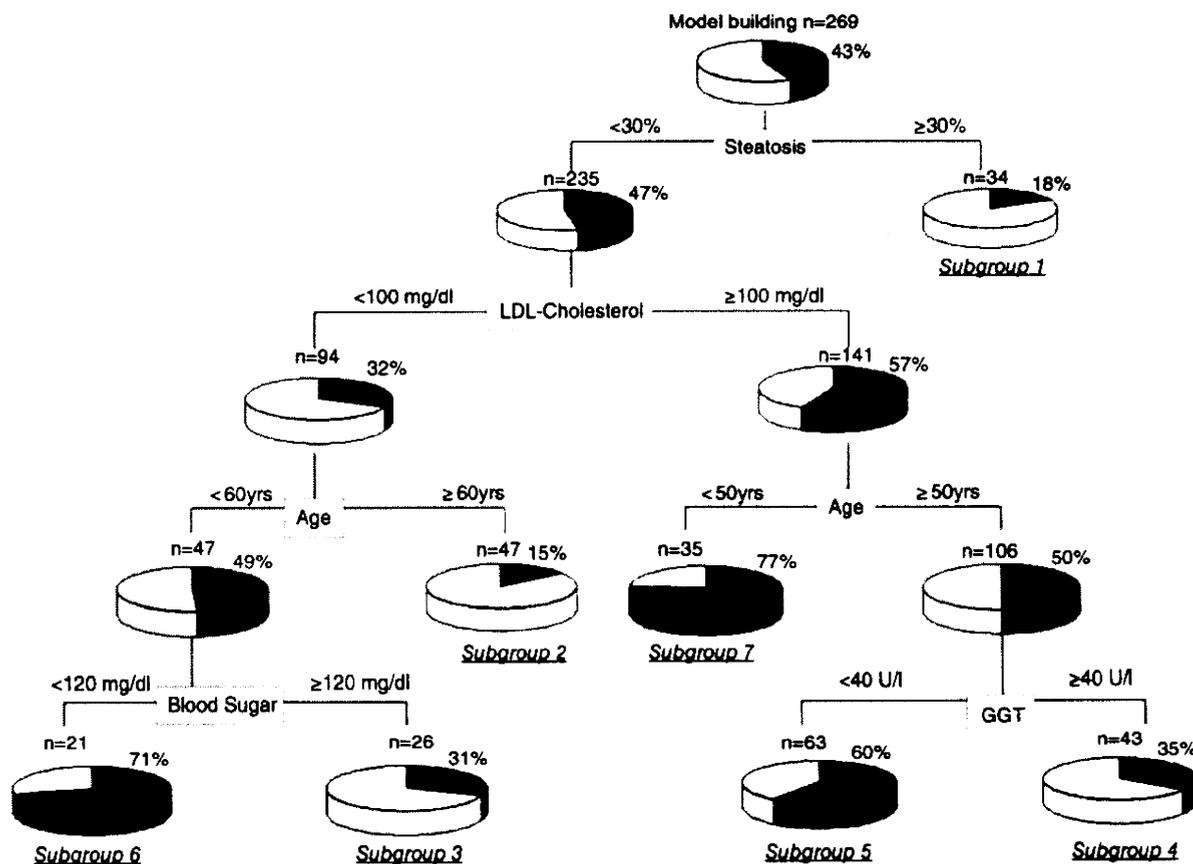


Figure 1 Classification and regression tree analysis. Boxes indicate the factors used for splitting and the cut-off value for the split. Pie charts indicate the rate of RVR/cEVR for each group of patients after splitting. Terminal subgroups of patients discriminated by the analysis are numbered from one to seven. GGT, gamma-glutamyltransferase; LDL, low-density-lipoprotein.

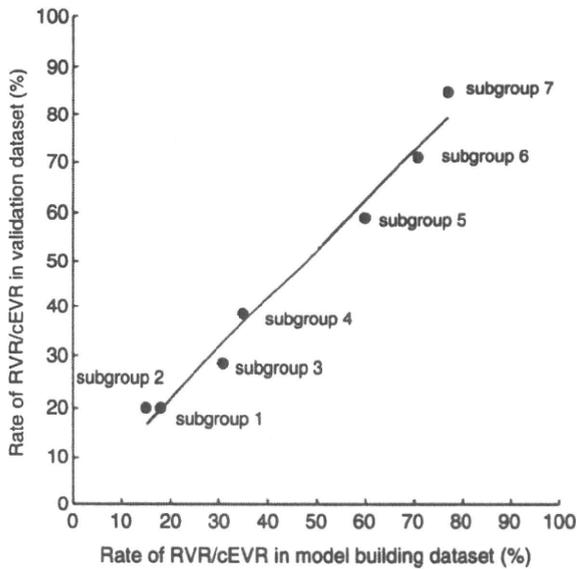
associated with RVR/cEVR by multivariate logistic regression analysis while blood sugar was not (Table 2). On the other hand, HCVRNA and creatinine which were significantly associated with RVR/cEVR by multivariate analysis were not selected as significant variables in CART analysis.

The probabilities of RVR/cEVR for the seven subgroups derived by this process were highly variable. The subgroup whose hepatic steatosis was less than 30%, serum LDL-C was 100 mg/dL or more and of an age less than 50 years (subgroup 7) showed the highest probability of RVR/cEVR (77%), while the subgroup whose hepatic steatosis more than 30% (subgroup 1) and the subgroup whose hepatic steatosis was less than 30% but serum LDL-C was less than 100 mg/dL and of an age

greater than 60 years (subgroup 2) showed the lowest probability of RVR/cEVR (18% and 15%, respectively).

#### Validation of the CART analysis

The results of the CART analysis were validated with a validation dataset of 131 cases which is independent of the model building dataset. Each patient in the validation set was allocated to subgroups 1–7 using the flow-chart form of the CART tree. The rates of RVR/cEVR were 20% for subgroups 1 and 2, 29% for subgroups 3, 38% for subgroup 4, 59% for subgroup 5, 71% for subgroup 6, and 85% for subgroups 7. The rates of RVR/cEVR for each subgroup of patients were closely correlated between the model building dataset and the validation dataset (Fig. 2).



**Figure 2** Validation of the classification and regression tree (CART) analysis: Subgroup stratified comparison of the rate of rapid or complete early virological response (RVR/cEVR) between the model building and validation datasets. Each patient in the validation set was allocated to subgroups 1–7 by following the flow-chart form of the CART tree and the rates of RVR/cEVR were calculated. The rate of RVR/cEVR in each subgroup was plotted. The x-axis represents the rate of RVR/cEVR in the model building datasets and the y-axis represents the rate of RVR/cEVR in the validation datasets. The rates of achieving RVR/cEVR in each subgroup of patients closely correlated between the model building dataset and the validation dataset ( $r^2 = 0.987$ ).

**Construction of 3 groups according to the probability of RVR/cEVR**

If the seven subgroups were reconstructed into three groups according to their rate of RVR/cEVR, the rate of RVR/cEVR was 16% for low probability group (subgroup 1 and 2), 46% for intermediate probability group (subgroup 3, 4, and 5) and 75% for high probability group (subgroup 6 and 7;  $P < 0.0001$ ).

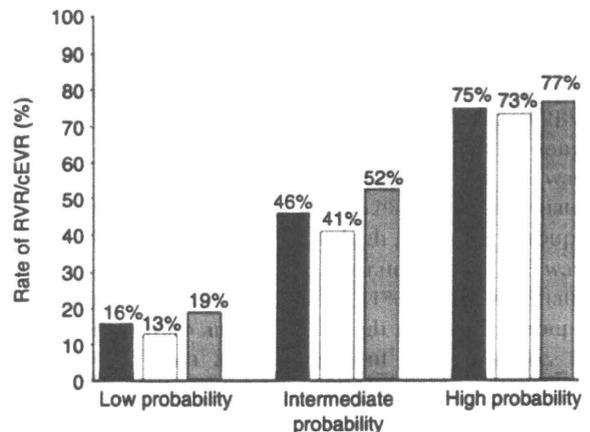
**Effect of adherence**

Adherence of PEG-IFN and RBV was not included as a variable of analysis since the present study aimed to develop a pre-treatment model for the prediction of response. To analyze the possible effect of adherence on the result of CART analysis, three groups of patients divided by CART (low, intermediate and high probability group) were further stratified according to adherence

of PEG-IFN and RBV. Poor adherence was defined as taking less than 80% planned dose of PEG-IFN or RBV at 12 weeks, and good adherence was defined as taking more than 80% planned dose of both PEG-IFN and RBV at 12 weeks. The result is shown in Figure 3. Among patients with good adherence, the rate of RVR/cEVR was 19% for low probability group, 52% for intermediate probability group and 77% for high probability group. Among poor adherence group, the rate of RVR/cEVR was 13% for low probability group, 41% for intermediate probability group and 73% for high probability group. Collectively, even after adjustment for adherence, 3 groups of patients divided by CART analysis still had low, intermediate and high probability of achieving RVR/cEVR, respectively.

**DISCUSSION**

**I**N THE PRESENT study, we performed the CART analysis and built a simple decision tree model for the pre-treatment prediction of response to PEG-IFN plus



**Figure 3** The rate of rapid or complete early virological response (RVR/cEVR) between the classification and regression tree (CART) groups stratified by adherence. The three groups of patients divided by CART (low, intermediate and high probability group) were further stratified according to adherence of peg-interferon (PEG-IFN) plus ribavirin (RBV). Black, white and gray boxes in the bar chart indicate total patients, patients with poor adherence (taking less than 80% planned dose of PEG-IFN or RBV at 12 weeks), and good adherence (taking more than 80% planned dose of both PEG-IFN and RBV at 12 weeks), respectively. Even after adjustment for adherence, 3 groups of patients divided by CART analysis still had low, intermediate and high probability of achieving RVR/cEVR, respectively.

RBV therapy. The analysis highlighted five host variables relevant to response: steatosis, LDL-C, age, blood sugar and GGT. Classification of patients based on these variables identified subgroups of patients with high probabilities of achieving RVR/cEVR among difficult to treat chronic hepatitis C patients. The reproducibility of the model was confirmed by the independent validation datasets. According to the result of the CART, patients were categorized into 3 groups: the rate of RVR/cEVR was 16% for low probability group, 46% for intermediate probability group and 75% for high probability group. The result of the CART analysis could be readily applicable to clinical practice because patients could be allocated to specific subgroups with a defined rate of response simply by following the flow-chart form. Although an early disappearance of serum HCV RNA is the prerequisite for achieving SVR, no reliable baseline predictors of response to PEG-IFN plus RBV therapy are established to date. Thus, this model may have the potential to support decisions in patient selection for PEG-IFN plus RBV therapy or to tailor treatment strategies for individual patients. Moreover, our result may provide a rationale for treating metabolic factors to improve the efficacy of antiviral therapy.

Among variables relevant to the prediction of RVR/cEVR, the grade of hepatic steatosis was selected as the variable of the first split. Previous studies suggested that steatosis induces resistance to IFN and RBV combination therapy<sup>20,21</sup> along with underlining metabolic factors such as insulin resistance or obesity.<sup>21-24</sup> In the present study, the grade of steatosis correlated positively with BMI and serum glucose level (data not shown) suggesting the etiologic role of metabolic factors. In addition, serum glucose level was selected as a predictor of RVR/cEVR at the fourth level of split. Serum GGT, which is associated with obesity,<sup>25</sup> insulin resistance<sup>26</sup> and response to IFN therapy,<sup>27-30</sup> was also selected as a predictor of RVR/cEVR at fourth level of splitting which may emphasize the importance of metabolic factors in therapeutic resistance. These findings raise the possibility that treatment of these metabolic factors may improve the virological response to the PEG-IFN plus RBV therapy. This hypothesis should be examined by a prospective study.

We and others have reported that steatosis, obesity and insulin resistance are associated with the progression of fibrosis,<sup>17,31-33</sup> which can interfere indirectly with the effect of IFN on hepatocytes. Other possible mechanisms of resistance by steatosis or metabolic factors include dysregulation of adipocytokines<sup>34</sup> or oxidative stress which may inhibit intracellular IFN signaling

pathway.<sup>35</sup> Despite these findings, the precise mechanism of resistance is not established and further investigation is needed.

Another factor relevant in the prediction of RVR/cEVR was LDL-C. LDL-C was selected as the second factor for splitting by CART, and was an independent predictor of RVR/cEVR by logistic regression analysis. LDL-C recently has attracted attention as a novel predictor of response to IFN or PEG-IFN plus RBV.<sup>30,36,37</sup> Since *in vitro* study showed that LDL-C receptor acts as a receptor for HCV and LDL-C competitively inhibit the binding of HCV,<sup>38</sup> high level of serum LDL-C may inhibit HCV entry to hepatocytes and attenuate replication. LDL-C and its receptor may be a future therapeutic target.

Not all factors selected as significant variables in the CART analysis were also significantly associated with response by standard statistical analysis: blood sugar was associated with response by univariate analysis but not by multivariate logistic regression analysis. On the other hand, HCV RNA and creatinine which were significantly associated with RVR/cEVR by multivariate analysis were not selected as significant variables in CART analysis. These differences may indicate both the unique feature and the limitations of the CART analysis. To note, blood sugar was significantly associated with RVR/cEVR within specialized subgroups of patients defined by the CART analysis: in subgroup of patients with steatosis <30%, LDL-C <100 mg/dL and younger than 60, which indicate the unique feature of the CART analysis that it could visualize significant predictors that specifically apply to selected patients. The limitation is that not all significant factors may be adopted in the decision tree since we applied the rule to stop CART procedure when the sample size was below 20. This rule was applied to avoid the generation of over-fit model which may lack universality. Therefore, it is possible that HCV RNA or creatinine may become a significant variable in the CART analysis if larger number of patients were included in the analysis. Stage of fibrosis was significantly associated with response to therapy by univariate analysis but not by multivariate analysis and not selected as a significant variable in the CART analysis. The possible reason is that advanced fibrosis is associated with older age as a confounding factor.

CART analyses are gaining acceptance in medical research in addition to biomedical field. Recent publications include the prediction of aggressive prostate cancer,<sup>8</sup> diabetic vascular complications,<sup>19</sup> prognosis of melanoma,<sup>7,39</sup> response to preoperative radiotherapy for rectal tumor,<sup>9</sup> prognostic groups in colorectal carcinoma,<sup>12</sup> and outcome after liver failure.<sup>11</sup> An advantage

of CART over traditional regression models is that it can identify prognostic subgroups that are useful in clinical practice. Because the results of CART analysis are presented as a decision tree, which is intuitive, they can be readily interpreted by medical professionals without any specific knowledge of statistics. The most important consideration is that five variables used in the decision tree were clinical parameters that are readily available by the usual work-up of patients before therapy. Especially, glucose, GGT and LDL-C are simple biochemical markers that are easily measured at a low cost. Using this model, we can rapidly develop an estimate of the response before treatment, which may facilitate clinical decision making.

In conclusion, we built a pre-treatment model for the prediction of virological response in PEG-IFN plus RBV therapy. Because this decision tree model was made up of simple host factors such as steatosis, LDL-C, age, blood sugar and GGT, it can be easily applied to clinical practice. This model may have the potential to support decisions in patient selection for PEG-IFN plus RBV therapy based on the possibility of response against a potential risk of adverse events or costs, and may provide a rationale for treating metabolic factors to improve the efficacy of antiviral therapy.

## ACKNOWLEDGEMENTS

THIS STUDY WAS supported by a grant-in-aid from Ministry of Health, Labor and Welfare, Japan. There exist no conflicts of interest.

## REFERENCES

- 1 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39: 1147-71.
- 2 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.
- 3 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.
- 4 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.
- 5 Lee SS, Ferenci P. Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. *Antivir Ther* 2008; 13 (Suppl 1): 9-16.
- 6 Breiman L, Friedman RA, Olshen CJ, Stone CM. *Classification and Regression Trees*. Calif: Wadsworth, 1980.
- 7 Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: Implications on the future of cancer staging. *Surg* 2002; 132: 589-602.
- 8 Garzotto M, Beer TM, Hudson RG *et al*. Improved detection of prostate cancer using classification and regression tree analysis. *J Clin Oncol* 2005; 23: 4322-9.
- 9 Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. *Clin Cancer Res* 2005; 11: 5440-3.
- 10 Jin H, Lu Y, Harris ST *et al*. Classification algorithms for hip fracture prediction based on recursive partitioning methods. *Med Decis Making* 2004; 24: 386-98.
- 11 Baquerizo A, Anselmo D, Shackleton C *et al*. Phosphorus ans an early predictive factor in patients with acute liver failure. *Transplantation* 2003; 75: 2007-14.
- 12 Valera VA, Walter BA, Yokoyama N *et al*. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. *Ann Surg Oncol* 2007; 14: 34-40.
- 13 Martin MA, Meyricke R, O'Neill T, Roberts S. Mastectomy or breast conserving surgery? Factors affecting type of surgical treatment for breast cancer - a classification tree approach. *BMC Cancer* 2006; 6: 98.
- 14 LeBlanc M, Crowley J. A review of tree-based prognostic models. *Cancer Treat Res* 1995; 75: 113-24.
- 15 Costanza MC, Paccaud F. Binary classification of dyslipidemia from the waist-to-hip ratio and body mass index: a comparison of linear, logistic, and CART models. *BMC Med Res Methodol* 2004; 4: 7.
- 16 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289-93.
- 17 Kurosaki M, Matsunaga K, Hirayama I *et al*. The presence of steatosis and elevation of alanine aminotransferase levels are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy. *J Hepatol* 2008; 48: 736-42.
- 18 Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. *Stat Med* 1989; 8: 539-50.
- 19 Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. *J Epidemiol* 2002; 12: 243-8.
- 20 Akuta N, Suzuki F, 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 tripartite interaction of viral, host and interferon treatment-related factors. *J Hepatol* 2002; 37: 831-6.

- 21 Poynard T, Ratziu V, McHutchison J *et al.* Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003; 38: 75–85.
- 22 Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for non-response to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; 38: 639–44.
- 23 Romero-Gomez M, Del Mar Vilorio 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.
- 24 Konishi I, Horiike N, Hiasa Y *et al.* Diabetes mellitus reduces the therapeutic effectiveness of interferon-alpha2b plus ribavirin therapy in patients with chronic hepatitis C. *Hepatol Res* 2007; 37: 331–6.
- 25 Marchesini G, Avagnina S, Barantani EG *et al.* Aminotransferase and gamma-glutamyltranspeptidase levels in obesity are associated with insulin resistance and the metabolic syndrome. *J Endocrinol Invest* 2005; 28: 333–9.
- 26 Fraser A, Ebrahim S, Smith GD, Lawlor DA. A comparison of associations of alanine aminotransferase and gamma-glutamyltransferase with fasting glucose, fasting insulin, and glycated hemoglobin in women with and without diabetes. *Hepatology* 2007; 46: 158–65.
- 27 Mazzella G, Salzetta A, Casanova S *et al.* Treatment of chronic sporadic-type non-A, non-B hepatitis with lymphoblastoid interferon: gamma GT levels predictive for response. *Dig Dis Sci* 1994; 39: 866–70.
- 28 Villela-Nogueira CA, Perez RM, de Segadas Soares JA, Coelho HS. Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-alpha and ribavirin. *J Clin Gastroenterol* 2005; 39: 728–30.
- 29 Berg T, Sarrazin C, Herrmann E *et al.* Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003; 37: 600–9.
- 30 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.
- 31 Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; 33: 1358–64.
- 32 Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. *Am J Gastroenterol* 2002; 97: 2408–14.
- 33 Muzzi A, Leandro G, Rubbia-Brandt L *et al.* Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. *J Hepatol* 2005; 42: 41–6.
- 34 Charlton MR, Pockros PJ, Harrison SA. Impact of obesity on treatment of chronic hepatitis C. *Hepatology* 2006; 43: 1177–86.
- 35 Di Bona D, Cippitelli M, Fionda C *et al.* Oxidative stress inhibits IFN-alpha-induced antiviral gene expression by blocking the JAK-STAT pathway. *J Hepatol* 2006; 45: 271–9.
- 36 Minuk GY, Weinstein S, Kaita KD. Serum cholesterol and low-density lipoprotein cholesterol levels as predictors of response to interferon therapy for chronic hepatitis C. *Ann Intern Med* 2000; 132: 761–2.
- 37 Gopal K, Johnson TC, Gopal S *et al.* Correlation between beta-lipoprotein levels and outcome of hepatitis C treatment. *Hepatology* 2006; 44: 335–40.
- 38 Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci USA* 1999; 96: 12766–71.
- 39 Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the german dermatological society. *J Clin Oncol* 2004; 22: 3660–7.