treatment among HBV genotypes.[53] Adefovir showed no resistance after 1 year, but the rate of resistance increased to 1–3%, 11%,18% and 28% at years 2, 3, 4 and 5, respectively.[24,54,55]

Prolonged therapy with adefovir in HBeAg positive patients resulted in viral load below the LLD, 10*3 copies/mL, in 28% at year 1, 45% and 56% of patients in years 2 and 3, respectively. ALT levels became normal in 48%, 71% and 81% after 1, 2 and 3 years of treatment, respectively. Rates of HBeAg loss increased to 42% and 52% and HBe seroconversion rates increased 29% to 42% at years 2 and 3.[56]

In HBeAg negative patients, prolonged adefovir therapy for 2 years showed little additional decline in viral load but consolidated the response to adefovir, as 71–75% of the patients had a viral load below the LLD, 10*3 copies/mL, and ALT normalization in 73–79%. In the long-term, up to 5 years of continuous treatment resulted in a viral load below the LLD, 10*3 copies/mL, in 78–79% at year 3, 65–68% at year 4 and 67% after 5 years. ALT levels were normal in 69–78% at year 3, 70–75% at year 4 and 69% after 5 years.[30]

Longer term results indicate that continued therapy with adefovir resulted in the suppression of HBV-DNA with lower rates of resistance than lamivudine, although adefovir monotherapy may not suffice for patients with high levels of viral replication and requiring rapid suppression of HBV-DNA.

Adefovir for lamivudine resistant patients

Adefovir has proven to be effective for lamivudine resistant mutants.[15,57–60] The current interest surrounds adefovir monotherapy vs. combination therapy with lamivudine, for lamivudine resistant patients. Adefovir monotherapy is able to suppress viral load by 2.4–4.0 $\log_{10}$ copies/mL, while adefovir and lamivudine combination therapy suppressed viral load by 2.4–6.5 $\log_{10}$ copies/mL in 1 year.[26,61] A randomized study found no difference in viral decline between adefovir monotherapy and combination therapy, although other studies have found that after long-term treatment, combination therapy showed a stronger viral decline (4.3 $\log_{10}$ copies/mL) than adefovir monotherapy (3.4 $\log_{10}$ copies/mL) and higher rates of HBV-DNA negativity by PCR, 81% in combination therapy versus 40% in adefovir monotherapy, in patients with baseline viral load ≥5 $\log_{10}$ copies/mL.[62]

Most of the clinical reports showed no difference in adefovir treatment outcome between lamivudine resistant patients and naïve patients,[26,27,63] although adefovir was reported to have some degree of cross-resistance with lamivudine *in vitro*.[64–66] However, in lamivudine resistant patients, compared to combination therapy with lamivudine, adefovir monotherapy showed a higher rate of resistance.[24,26,29,67]

As we can see from the above, recent reports of the comparative study of adefovir monotherapy versus adefovir and lamivudine combination therapy showed that adding adefovir to ongoing lamivudine therapy is preferable to switching to adefovir monotherapy in terms of the potent antiviral effect and a lower rate of resistance.[24,26,29,35,40]

ENTECAVIR

ENTECAVIR IS A cyclopentyl guanosine analog and is known to inhibit all three activities of the HBV polymerase/reverse transcriptase: base priming, reverse transcription of the negative strand from the pregenomic messenger RNA and synthesis of the positive strand of HBV-DNA. Entecavir has been shown to reduce the viral antigens and cccDNA levels in liver samples of HBV-infected woodchucks.[30]

Entecavir for naïve patients

With 1 year of entecavir (0.5 mg) therapy for HBeAg positive patients, HBeAg loss was reported in 22%, HBe seroconversion in 21% and HBsAg loss in 2%, respectively, of patients.[31] The decline of viral load was 6.9 $\log_{10}$ copies/mL for HBeAg positive patients and 5.0 $\log_{10}$ copies/mL for HBeAg negative patients.[32] ALT normalization was achieved in 68% of HBeAg positive patients and 78% of HBeAg negative patients. HBV-DNA negativity, less than the LLD of 300 copies/mL by PCR, was achieved in 67% of HBeAg positive patients and 90% of HBeAg negative patients. Entecavir showed no resistance up to 2 years,[33] but a cumulative resistance rate of approximately 1.2% after 4 years has been reported at the 42nd Meeting of the European Association for the Study of Liver Diseases.

Entecavir for lamivudine resistant patients

Entecavir shows efficacy for lamivudine resistant patients, although higher doses of entecavir (1.0 mg) are required and virologic rebound occurs due to resistance to entecavir. In a phase III trial, lamivudine resistant patients were treated with entecavir for 48 weeks. HBV-DNA was suppressed to less than the LLD, 300 copies/mL by PCR, in 19% of entecavir treated patients and 1% of lamivudine treated patients.[43] Another study shows entecavir suppressed HBV–DNA less than the LLD, 300 copies/mL, in 21% and 34% of patients by weeks 48 and 96, respectively.[72]

Entecavir is known to have cross-resistance with lamivudine and approximately 9% of lamivudine resistant patients treated with entecavir develop a resistance to entecavir after 2 years of therapy.[44] Virologic rebound due to entecavir resistance required pre-existing lamivudine resistance, HBV reverse transcriptase substitutions M204V and L180M, and additional changes at T184, S202, or M250.[73] A recent study showed T184, S202, or M250 substitution, which was considered to occur during entecavir therapy, emerged during lamivudine therapy before entecavir.[45]

Entecavir is a potent inhibitor of HBV replication and for naïve patients entecavir showed a very low rate of resistance. Thus far entecavir can be considered the first line drug for naïve patients, although we may not put entecavir as the first line therapy for lamivudine resistant patients because of a relatively higher risk of cross-resistance and rebound. For lamivudine pretreated patients, screening of mutations may be necessary before switching from lamivudine to entecavir. Recent case reports suggest that entecavir resistant chronic hepatitis B can be successfully treated with adefovir and lamivudine combination therapy.[75]

LIMITATIONS OF THE CURRENT THERAPIES AND NEWER NUCLEOSIDE/NUCLEOTIDE ANALOGS

Telbivudine

ANOTHER WELL-REPORTED nucleoside/nucleotide analog is telbivudine, a cytosine nucleoside analog. A dose-finding study revealed that this drug is a potent antiviral drug and dose-dependent antiviral activity is evident up to a dose of 400mg or more. In the 800 mg/d cohort, the mean reduction of HBV–DNA was 3.75 log_{10} copies/ml at week 4.[76] A one-year trial of telbivudine 400mg/d or 600 mg/day, lamivudine 100mg/day, and the combination of these in HBeAg positive patients revealed the reduction of 6.43 or 6.09 log_{10} copies/ml by telbivudine, respectively, compared to 4.66 of lamivudine, and 6.40 and 6.05 of combination at 52 weeks.[77] The randomized control trial of telbivudine or adefovir showed telbivudine demonstrated a greater reduction of HBV–DNA than adefovir at 24 weeks. After 52 weeks, the suppression was greater in patients who had received continuous telbivudine than in those who received continuous adefovir. This thus showed telbivudine as a potent antiviral agent for HBV,[78] although it is associated with a rather high rate of resistance, 21.6% of HBeAg positive and 8.6% of HBeAg negative patients who received telbivudine for 2 years, and telbivudine-resistant mutations are cross-resistant with lamivudine.[79]

Durability of response

Durability of response after discontinuation of therapy is one of the limitations of nucleoside/nucleotide analog therapy. In HBeAg positive patients who seroconverted during therapy, the response, which is assessed by HBeAg loss or HBe seroconversion, is durable in over half of the patients,[42,80–83] but in HBeAg negative patients the durability of the response, which is the suppression of HBV–DNA, is quite poor. Patients treated with lamivudine for 2 years and who had undetectable HBV–DNA, less than 200 copies/mL, showed a 50% relapse rate 12 months after discontinuation.[84] With adefovir 96-week long-term therapy, 71% of HBeAg negative patients showed HBV–DNA levels that were less than 1000 copies/mL, but after discontinuation the majority of patients lost the benefit of treatment and only 8% of patients had levels below 1000 copies/mL at week 96 of follow up. Data on the durability of response to entecavir is lacking.

Clevudine is a pyrimidine analog and is reported to have relatively high sustained antiviral activity against HBV, or slow viral rebound after therapy. At the American Association for the Study of Liver Diseases (AASLD) in 2006, it was reported that 31% of HBeAg positive patients and 92% of HBeAg negative patients had undetectable HBV 12 weeks after the end of treatment. In HBeAg positive patients, median serum HBV–DNA reductions from baseline at week 24 were 5.10 log_{10} and viral suppression in the clevudine group was sustained after therapy, with 3.73 log_{10} reduction at week 34 and 2.02 log_{10} reduction at week 48. At week 24, 59.0% of patients in the clevudine group had HBV–DNA less than the LLD, 300 copies/mL.[85,86] In HBeAg negative patients, median changes of HBV–DNA were −4.25 log_{10} copies/ml and 92% of the patients had undetectable HBV–DNA by PCR assay at 24 weeks of 30mg/day of clevudine treatment and viral suppression was 3.11 log_{10} reduction 24 weeks after the end of treatment.[87] Although further studies are needed to confirm this, analysis of the mechanism of slow viral rebound in clevudine therapy could lead to the development of new drugs that provide better a sustainability of response.

Viral clearance: HBs seroconversion

With the currently available nucleoside/nucleotide analogs, HBsAg loss is rare – less than 2% – which is almost the same rate observed in the natural history of the disease, as compared to IFN therapy, which shows a 3–10% HBsAg loss within 1 year of therapy and 11–32% in sustained responders.[5,88–93]

However, Tenofovir, another new nucleoside/nucleotide analog, was reported to have achieved a 14% HBsAg loss, although only in a small cohort of 35 patients.[94] Tenofovir, which has related molecules and a similar mechanism of action to adefovir, inhibits reverse transcription by competing with the substrate deoxyadenosine 5'-triphosphate. A phase III controlled trial comparing the efficacy of adefovir and tenofovir in HBeAg positive and negative patients is ongoing and data from the previous studies with lamivudine resistant patients showed that tenofovir had a faster and greater suppression of HBV-DNA than adefovir.[95]

Tenofovir is expected to be an important next applicable drug in HBV infection and also HIV/HBV coinfection.[96,97] More studies have to be carried out to investigate the effects of the drug on HBsAg loss and that mechanism is to be explored.

CONCLUSIONS

OUR KNOWLEDGE OF HBV and the therapeutic options has been broadened over the past decades, but much of the natural history of HBV remains unknown and none of the current registered therapies for chronic HBV can be said to be ideal in terms of tolerability and efficacy.

Nucleoside/nucleotide analog therapy has a high relapse rate after discontinuation and results in long-term therapy, which may put unborn children or the patients themselves at risk. Identifying the factors that contribute to the sustained response will help us to select patients who would be able to stop the therapy without relapse.

The occurrence of resistance to nucleoside/nucleotide analogs is currently unavoidable. Several more nucleoside/nucleotide analogs will be available in the future and switching from one drug to another will rescue patients who develop a resistance, but to pick the best drug for the patients, we should accumulate a large number of data concerning mutations, drug resistance and cross-resistance, and establish the data base to make it possible to select drugs for the patients based on mutational patterns or genotypes.[24]

In HBV therapy, viral clearance is the ultimate aim of treatment, although current therapies do not suffice for that purpose. It will be important to establish treatment strategies based on both viral characteristics and host immune systems, which are necessary for HBV eradication.

REFERENCES

1 Kane M. Global programme for control of hepatitis B infection. *Vaccine* 1995; **13**(Suppl. 1): S47–9.

2 Lok AS. Chronic hepatitis B. *N Engl J Med* 2002; **346**: 1682–3.

3 Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678–86.

4 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65–73.

5 Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29–60.

6 Bertoletti A, Gehring A. Immune response and tolerance during chronic hepatitis B virus infection. *Hepatol Res* 2007; **37**(Suppl 3): S331–8.

7 Ayada M, Ishikawa T, Okumura A *et al.* Alteration of serum cytokine balances among different phases of chronic hepatitis B virus infection. *Hepatol Res* 2006; **34**(4): 214–21.

8 Guo H, Jiang D, Zhou T, Cuconati A, Block TM, Guo JT. Characterization of the intracellular deproteinized relaxed circular DNA of hepatitis B virus: an intermediate of covalently closed circular DNA formation. *J Virol* 2007; **81**(22): 12472–84.

9 Yokosuka O, Omata M, Imazeki F, Okuda K. Active and inactive replication of hepatitis B virus deoxyribonucleic acid in chronic liver disease. *Gastroenterology* 1985; **89**(3): 610–6.

10 Yokosuka O, Omata M, Imazeki F, Ito Y, Okuda K. Hepatitis B virus RNA transcripts and DNA in chronic liver disease. *N Engl J Med* 1986; **315**(19): 1187–92.

11 Summers J, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell* 1982; **29**(2): 403–15.

12 Wright TL. Antiviral therapy and primary and secondary prevention of hepatocellular carcinoma. *Hepatol Res* 2007; **37**(Suppl 2): S294–8.

13 Gish RG. Improving outcomes for patients with chronic hepatitis B. *Hepatol Res* 2007; **37**(s1): S67–78.

14 Koga H, Ide T, Oho K *et al.* Lamivudine treatment-related morphological changes of esophageal varices in patients with liver cirrhosis. *Hepatol Res* 2007; **37**(7): 503–9.

15 Kubo S, Tanaka H, Takemura S *et al.* Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus. *Hepatol Res* 2007; **37**(2): 94–100.

16 Akbar F, Yoshida O, Abe M, Hiasa Y, Onji M. Engineering immune therapy against hepatitis B virus. *Hepatol Res* 2007; **37**(Suppl 3): S351–6.

17 Niederau C, Heintges T, Lange S *et al.* Long-term follow-up of HBeAg-positive patients treated with interferon alpha for chronic hepatitis B. *N Engle J Med* 1996; **334**: 1422–7.

18 Kao JH. Appropriate use of interferon for treatment of chronic hepatitis B. *Hepatol Res* 2007; **37**(s1): S47–54.

19 Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; **33**: 998–1002.

20 Wai CT, Chu CJ, Hussain M, Lok AS *et al.* HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002; **36**: 1425–30.

21 Erhardt A, Reineke U, Blondin D *et al.* Mutations of the core promoter and response to interferon treatment in chronic replicative hepatitis B. *Hepatology* 2000; **31**: 716–25.

22 Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 2006; **101**: 297–303.

23 Orito E, Mizokami M. Differences of HBV genotypes and hepatocellular carcinoma in Asian countries. *Hepatol Res* 2007; **37**(s1): S33–5.

24 Shin-I T, Tanaka Y, Tateno Y, Mizokami M. Development and public release of a comprehensive hepatitis virus database. *Hepatol Res* 2008; **38**: 234–43.

25 Orito E, Mizokami M, Sakugawa H *et al.* A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. *Hepatology* 2001; **33**: 218–33.

26 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37**: 19–26.

27 Orito E, Sugauchi F, Tanaka Y *et al.* Differences of hepatocellular carcinoma patients with hepatitis B virus genotypes of Ba, Bj or C in Japan. *Intervirology* 2005; **4**: 239–45.

28 Matsuura K, Michitaka K, Yamauchi K *et al.* Characteristics of geographic distributions and route of infection for hepatitis B virus genotype D in Ehime area in western Japan. *Hepatol Res* 2007; **37**(4): 255–62.

29 Sugauchi F, Orito E, Ohno T *et al.* Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. *Hepatol Res* 2006; **36**(2): 107–14.

30 Yokosuka O, Omata M, Imazeki F, Okuda K, Summers J. Changes of hepatitis B virus DNA in liver and serum caused by recombinant leukocyte interferon treatment: analysis of intrahepatic replicative hepatitis B virus DNA. *Hepatology* 1985; **5**(5): 728–34.

31 Hatakeyama T, Noguchi C, Hiraga N *et al.* Serum HBV RNA is a predictor of early emergence of the YMDD mutant in patients treated with lamivudine. *Hepatology* 2007; **45**(5): 1179–86.

32 Orito E, Fujiwara K, Tanaka Y *et al.* A case-control study of response to lamivudine therapy for 2 years in Japanese and Chinese patients chronically infected with hepatitis B virus of genotypes Bj, Ba and C. *Hepatol Res* 2006; **35**: 127–34.

33 Enomoto M, Tamori A, Nishiguchi S. Hepatitis B virus genotypes and response to antiviral therapy. *Clin Lab* 2006; **52**(1–2): 43–7.

34 Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. *Hepatology* 2004; **39**: 857–61.

35 Minami M, Okanoue T. Management of HBV infection in Japan. *Hepatol Res* 2007; **37**(s1): S79–82.

36 Tassopoulos NC, Volpes R, Pastore G *et al.* Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. *Hepatology* 1999; **29**: 889–96.

37 Chen CH, Lee CM, Lu SN *et al.* Comparison of clinical outcome between patients continuing and discontinuing lamivudine therapy after biochemical breakthrough of YMDD mutants. *J Hepatol* 2004; **41**: 454–61.

38 Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen – negative chronic hepatitis B. *Hepatology* 2000; **32**: 847–51.

39 Papatheodoridis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthroughs under long-term lamivudine in HBeAg-negative precore mutant HBV liver disease. *Hepatology* 2002; **36**: 219–26.

40 Di Marco V, Marzano A, Lampertico P *et al.* Clinical outcome of HBeAg negative chronic hepatitis B in relation to virological response to lamivudine. *Hepatology* 2004; **40**: 883–91.

41 Orito E, Fujiwara K, Tanaka Y *et al.* A case-control study of response to lamivudine therapy for 2 years in Japanese and Chinese patients chronically infected with hepatitis B virus of genotypes Bj, Ba and C. *Hepatol Res* 2006; **35**: 127–34.

42 Kobayashi M, Suzuki F, Akuta N. Response to long-term lamivudine treatment in patients infected with hepatitis B virus genotypes A, B, and C. *J Med Virol* 2006; **78**: 1276–83.

43 Marcellin P, Lada O, Asselah T. Treatment of chronic hepatitis B with the combination of pegylated interferon with lamivudine. *Hepatol Res* 2007; **37**(s1): S55–61.

44 Ishikawa T, Kakumu S. Combination therapy with lamivudine and HB vaccine on chronic hepatitis B. *Hepatol Res* 2007; **37**(s1): S62–6.

45 Matsumoto A, Tanaka E, Minami M *et al.* Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol Res* 2007; **37**(8): 661–6.

46 Shinkai N, Tanaka Y, Orito E *et al.* Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 2006 Dec; **36(4)**: 272–6. Sep 12 [Epub 2006].

47 Kramata P, Votruba I, Otova B, Holy A. Different inhibitory potencies of acyclic phosphonomethoxyalkyl nucleotide analogs toward DNA polymerases alpha, delta and epsilon. *Mol Pharmacol* 1996; **46**: 1005–11.

48 Heijtink RA, De Wilde GA, Kruining J *et al.* Inhibitory effect of 9-(2-phosphonylmethoxyethyl) -adenine (PMEA) on human and duck hepatitis B virus infection. *Antiviral Res* 1993; **21**: 141–53.

49 Marcellin P, Chang TT, Lim SG *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; **348**: 808–16.

50 Bzowej N, Chan H, Lai CL *et al.* A randomized trial of telbivudine vs. adefovir for HBeAg-positive chronic hepatitis B: week 52 results. *Hepatology* 2006; **44**: 563A.

51 Lee YS, Suh DJ, Lim YS *et al.* Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006; **43**: 1385–91.

52 Zeng M, Mao Y, Yao G *et al.* A double-blind randomized trial of adefovir dipivoxil in Chinese subjects with HBeAg-positive chronic hepatitis B. *Hepatology* 2006; **44**: 108–16.

53 Westland C, Delaney W, Yang H *et al.* Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. *Gastroenterology* 2002; **125**: 107–16.

54 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; **348**: 800–7.

55 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005; **352**: 2673–81.

56 Macellin P, Chang TT, Lim S *et al.* Long term efficacy and safety of adefovir dipivoxil 10 mg in HBeAg + chronic hepatitis B patients: increasing serologic, virologic and biochemical response over time. *J Hepatol* 2004; **40**(Suppl. 4): 655A.

57 Takamura M, Ichida T, Ohkoshi S *et al.* Decompensated lamivudine-resistant hepatitis B virus-related cirrhosis treated successfully with adefovir dipivoxil allowing surgery for hepatocellular carcinoma. *Intern Med* 2007; **46**(7): 367–71.

58 Suzuki F, Kumada H, Nakamura H. Changes in viral loads of lamivudine-resistant mutants and evolution of HBV sequences during adefovir dipivoxil therapy. *J Med Virol* 2006; **78**(8): 1025–34.

59 Perrillo R, Schiff E, Yoshida E *et al.* Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000; **32**: 129–34.

60 Walsh KM, Woodall T, Lamy P, Wight DG, Bloor S, Alexander GJ. Successful treatment with adefovir dipivoxil in a patient with fibrosing cholestatic hepatitis and lamivudine resistant hepatitis B virus. *Gut* 2001; **49**: 436–40.

61 Peters MG, Hann Hw H, Martin P *et al.* Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2004; **126**: 91–101.

62 Marzano A, Gaia S, Barbon V *et al.* Therapy with adefovir alone or combined with lamivudine in patients with lamivudine-resistant chronic hepatitis B: clinical and virological aspects. *Hepatology* 2006; **44**: 231A. (Abstract 113).

63 Lee YS, Chung Y, Ryu S *et al.* Hepatitis B virus with rtL80V/I mutation associates with poor response to adefovir dipivoxil therapy. *Hepatology* 2005; **42**(Suppl. 1): 575A.

64 Lada O, Benhamou Y, Cahour A *et al.* In vitro susceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir. *Antivir Ther* 2004; **9**: 353–63.

65 Ono SK, Kato N, Shiratori Y *et al.* The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. *J Clin Invest* 2001; **102**: 449–55.

66 Yang H, Qi X, Sabogal A, Miller M, Xiong S, Delaney WE 4th. Cross-resistance testing of next-generation nucleoside and nucleotide analogues against lamivudine-resistant HBV. *Antivir Ther* 2005; **10**: 625–33.

67 Fung SK, Chae HB, Fontana RJ *et al.* Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; **44**: 283–90.

68 Colonno RJ, Genovesi EV, Medina I *et al.* Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. *J Infect Dis* 2001; **184**: 1236–45.

69 Chang TT, Gish RG, de Man R *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001–10.

70 Lai CL, Shouval D, Lok AS *et al.* Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *New Engl J Med* 2006; **354**: 1011–20.

71 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naive patients with hepatitis B. *Hepatology* 2006; **44**: 1656–65.

72 Tenney DJ, Rose RE, Baldick CJ. Two-year assessment of entecavir resistance in lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother* 2007; **51**: 902–11.

73 Tenney DJ, Levine SM, Rose RE *et al.* Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. *Antimicrob Agents Chemother* 2004; **48**: 3498–507.

74 Nagasaki F, Niitsuma H, Ueno Y, Inoue J, Kogure T, Fukushima K, Shimosegawa T. The high incidence of the emergence of entecavir-resistant mutants among

patients infected with lamivudine-resistant hepatitis B virus. *Tohoku J Exp Med* 2007; **213**(2): 181–6.
75 Yatsuji H, Hiraga N, Mori N *et al*. Successful treatment of an entecavir-resistant hepatitis B virus variant. *J Med Virol* 2007; **79**(12): 1811–7.
76 Lai CL, Lim SG, Brown NA *et al*. A dose-finding study of once-daily oral telbivudine in HBeAg-positive patients with chronic hepatitis B virus infection. *Hepatology* 2004; **40**(3): 719–26.
77 Lai CL, Leung N, Teo EK *et al*. A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. *Gastroenterology* 2005; **129**(2): 528–36.
78 Chan HL, Heathcote EJ, Marcellin P *et al*. and the 018 Study Group*. Treatment of Hepatitis B e Antigen-Positive Chronic Hepatitis with Telbivudine or Adefovir: A Randomized Trial. *Ann Intern Med* 2007; **147**: 745–54.
79 Lai CL, Leung NW, Teo EK *et al*. Phase IIb extended-treatment trial of telbivudine (LdT) vs lamivudine vs. combination treatment in hepatitis B patients: two year results. *Gastroenterology* 2005; **128**: A692 (Abstract).
80 Colonno RJ, Rose FE, Baldick CJ *et al*. High barrier to resistance results in no emergence of entecavir resistance in nucleoside-naïve subjects during the first two years of therapy. *J Hepatol* 2006; **44**(Suppl. 2): S182.
81 Song BC, Suh DJ, Lee HC *et al*. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. *Hepatology* 2000; **30**: 803–6.
82 Ryu SH, Chung YH, Choi MH *et al*. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. *J Hepatol* 2003; **39**: 614–19.
83 Chang TT, Schiffman ML, Tong M *et al*. Durability of HBeAg seroconversion following adefovir dipivoxil treatment for chronic hepatitis B. *J Hepatol* 2006; **44**(Suppl. 2): S187.
84 Fung SK, Wong F, Hussain M, Lok AS *et al*. Sustained response after a 2-year course of lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. *J Viral Hepat* 2004; **11**: 432–8.
85 Yoo BC, Kim JH, Chung YH *et al*. Twenty-four-week clevudine therapy showed potent and sustained antiviral activity in HBeAg-positive chronic hepatitis B. *Hepatology* 2007; **45**: 1172–8.
86 Lee HS, Chung YH, Lee K *et al*. A 12-week clevudine therapy showed potent and durable antiviral activity in HBeAg-positive chronic hepatitis B. *Hepatology* 2006; **43**(5): 982–8.
87 Yoo BC, Kim JH, Kim TH *et al*. Clevudine is highly efficacious in hepatitis B e antigen-negative chronic hepatitis B with durable off-therapy viral suppression. *Hepatology* 2007; **46**(4): 1041–8.
88 Janssen HL, van Zonneveld M, Senturk H *et al*. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomized trial. *Lancet* 2005; **365**: 123–9.
89 Lau GK, Piratvisuth T, Luo KX *et al*. Peg interferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682–95.
90 Marcellin P, Lau GK, Bonino F *et al*. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004; **351**: 1206–17.
91 Bortolotti F, Jara P, Barbera C *et al*. Long term effect of alpha interferon in children with chronic hepatitis B. *Gut* 2000; **46**: 715–18.
92 Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2001; **34**: 306–13.
93 Brunetto MR, Oliveri F, Coco B *et al*. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long-term cohort study. *J Hepatol* 2002; **36**: 263–70.
94 van Bommel F, Wunsche T, Mauss S *et al*. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. *Hepatology* 2004; **40**: 1421–5.
95 van Bömmel F, Zöllner B, Sarrazin C *et al*. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV-DNA level during adefovir therapy. *Hepatology* 2006; **44**(2): 318–25.
96 Peters MG, Andersen J, Lynch P *et al*. Randomized controlled study of tenofovir and adefovir in chronic hepatitis B virus and HIV infection: ACTG A5127 *Hepatology* 2006; **44**(5): 1110–6.
97 Benhamou Y, Fleury H, Trimoulet P *et al*. TECOVIR Study Group. Anti-hepatitis B virus efficacy of tenofovir disoproxil fumarate in HIV-infected patients. *Hepatology* 2006; **43**(3): 548–55.

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TOPIC HIGHLIGHT

Harry HX Xia, PhD, MD, *Series Editor*

Current role of ultrasound for the management of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) has a decisive influence on the prognosis of cirrhotic patients. Although α-fetoprotein (AFP) is a known and specific tumor maker for HCC, it is not suitable for the screening and surveillance of HCC because of its poor predictive value and low sensitivity. The use of imaging modalities is essential for the screening, diagnosis and treatment of HCC. Ultrasound (US) plays a major role among them, because it provides real-time and non-invasive observation by a simple and easy technique. In addition, US-guided needle puncture methods are frequently required for the diagnosis and/or treatment process of HCC. The development of digital technology has led to the detection of blood flow by color Doppler US, and the sensitivity for detecting tumor vascularity has shown remarkable improvement with the introduction of microbubble contrast agents. Moreover, near real-time 3-dimensional US images are now available. As for the treatment of HCC, high intensity focused ultrasound (HIFU) was developed as a novel technology that provides a transcutaneous ablation effect without needle puncture. These advancements in the US field have led to rapid progress in HCC management, and continuing advances are expected. This article reviews the current application of US for HCC in clinical practice.

Key words: Ultrasound; Contrast agent; Hepatocellular carcinoma; Liver; Surveillance; Treatments

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INTRODUCTION

Hepatocellular carcinoma (HCC) is increasing worldwide and is one of the most common carcinomas in the eastern part of Asia[1]. As the prognosis of cirrhotic patients depends on the occurrence and progression of HCC, management of this neoplasm is a major issue in clinical practice. The recent popularization of periodic surveillance and the development of diagnostic capabilities have resulted in the discovery of increasing numbers of patients with small HCC nodules[2,3]. Although tumor markers may be helpful for the diagnosis of HCC, imaging modalities are essential for finding and characterizing this neoplasm[4,5].

On the basis of the continuing development of digital technologies, ultrasound (US) has also shown significant improvements within the last decade[6]. As for grey-scale imaging, tissue harmonic imaging (THI) has improved both lateral resolution and contrast resolution by narrowing the width of the US beam, with the reduction of reverberation and side-lobe artifacts. Since the margin and structure of tumor nodules have become clear, with distinct delineation[7-10], THI has become popular as part of the routine work of grey-scale US examination.

Color Doppler imaging provides real-time evaluation of the hemodynamics in liver tumors, and power Doppler mode has contributed to a better detectability of blood flow[11-15]. However, limitations in the detection of slow flow and vessels located deeply from the skin surface have prevented the wider application of Doppler mode in the evaluation of tumor hemodynamics[16-18]. Furthermore, artifacts caused by respiratory or cardiac motion sometimes affect the precise evaluation of hemodynamic information.

With these backgrounds, US contrast agents have been expected to improve the detectability of blood flow in liver tumors, since the first report about a US contrast agent by Gramiak *et al*[19]. From the late 1980s to the 1990s, grey-scale contrast-enhanced US with carbon dioxide gained broad attention as an echo-enhancing technique, with high sensitivity for detecting tumor vascularity and high performance for the characterization of liver tumors[20,21]. However, this method requires an arteriography procedure because carbon dioxide is easily soluble in blood. The development of microbubble contrast agents together with peripheral venous injection was expected for practical use. In the late 1990's, a galactose-based US contrast agent (SHU 508, Levovist) was made available by Schering, Germany[22,23]. It was a long-awaited material that could provide a stable enhancement effect in abdominal organs with a peripheral injection. Subsequently, many microbubble contrast agents have been produced or are currently under development. At present, the application of Doppler mode alone for detecting tumor blood flow is rare, as contrast-enhanced US with microbubble contrast agents provides details of the hemodynamics that are useful for the detection and characterization of liver tumors. Additionally, three-dimensional US images are now easily available due to the development of advanced digital technologies[24,25], and high intensity focused ultrasound (HIFU) was developed as a novel treatment method for tumors[26]. This article reviews the current development and application of US for the diagnosis and treatment of HCC.

SURVEILLANCE FOR HCC

Viral-related and/or alcoholic chronic liver disease is a high-risk factor for developing HCC that limits the prognosis. There is no question about the importance of periodic surveillance for HCC in these high-risk patients[27-29]. Some serum markers are known for HCC, and α-fetoprotein (AFP) is widely used for its diagnosis[30-32]. Ishii *et al* reported that sensitivity and specificity of AFP was 13.8% and 97.4% at a cut-off value of 200 ng/mL, respectively, and 62.1% and 78.3%, at a cut-off value of 20 ng/mL, respectively[31]. They added that when AFP and another tumor maker, protein induced by vitamin K absence or antagonist Ⅱ (PIVKA-Ⅱ), were combined with cut-off values of 40 ng/mL for AFP and 80 mAU/mL for PIVKA-Ⅱ, sensitivity was 65.5% and specificity was 85.5%. The study by Tong *et al* showed that the positive predictive value for AFP to detect HCC was only 12% or less for all AFP cut-off values, and the maximum joint sensitivity and specificity as determined by receiver operator characteristic (ROC) analysis were approximately 65% and 90%, respectively. Meanwhile, the positive predictive value for US to detect HCC was 78%, while sensitivity and specificity were 100% and 98%, respectively[33]. They concluded that AFP should not be used as the only test for screening and surveillance for HCC because of its poor predictive value and low sensitivity. Larcos *et al* also mentioned that US screening was superior to AFP assay for detection of HCC[34]. Novel serum markers with improved sensitivity are awaited for screening tests for HCC.

US is the most common method for the screening of HCC because of its advantages - simple, non-invasive and real-time observation[4,5]. However, there has been a variety of results in the application of US for HCC surveillance (Table 1). Sherman *et al* reported that US showed a sensitivity of 71.4%, a specificity of 93.8%, with only 14% of positive predictive value, as a screening test in chronic HBsAg carriers[35]. Chalasani *et al* compared the sensitivity in a screening program between US and computed tomography (CT), and the sensitivity of US (59%) was much lower than that of CT (91%)[36]. Two other studies in the diagnosis of HCC before liver transplantation resulted in similar sensitivity between US and CT, 79.4% for US and 81.6% for CT[37], 58% for US and 53% for CT[38], respectively, with the latter claiming that US is preferable to CT for routine screening of HCC before liver transplantation because of its lower cost. Meanwhile, Teefey *et al* mentioned that the sensitivity of US (89%) was much higher than CT (67%) and magnetic resonance imaging (MRI, 56%)[39]. Evaluation of the actual sensitivity of US and other imaging techniques from the published studies on screening and surveillance is quite difficult because of the lack of a defined gold standard, as was also noted in the review article by Bolondi[28]. In addition, Chalasani *et al* described in their study that the lesser steatosis to change liver echogenecity in Asian patients with predominantly viral cirrhosis, leaner body habitus in Japanese patients resulting in better visualization of the liver by US, and differences in US technique between physicians (Japan) and technologists (USA) were the causes for the high detection rates by US in Japanese reports[4,36,40]. Although it is natural that US results depend on the physical size of the patients and the operator's skill, medical staffs and engineers who engage in US should not accept the current situation. Further technical and technological improvements are required to overcome these problems.

Tumor detectability between US without enhancement and contrast-enhanced spiral CT has been compared in some previous studies. The comparison may not be on an equal footing, as US has now acquired collaboration with microbubble contrast agents. The application of contrast-enhanced CT for screening of HCC would be expensive and invasive, and MRI has the limitation of a low availability rate of the equipment. Although contrast-enhanced US may not be cheap, it is much less invasive and more convenient than contrast-enhanced CT. The

Table 1 Sensitivity and specificity of US and other imaging modalities for the screening of HCC

Authors	US		Other modalities	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Sherman *et al*[35]	71.4	93.8	-	-
Chalasani *et al*[36]	59	93	91 (CT)	96 (CT)
[1]Yao *et al*[37]	79.4	-	81.6 (CT)	-
			88.9 (MRI)	-
Gambarin-Gelwan *et al*[38]	58	94	53 (CT)	94 (CT)
[2]Teefey *et al*[39]	89	75	67 (CT)	75 (CT)
			56 (MRI)	81 (MRI)
			0 (PET)	88 (PET)

[1]Sensitivity of radiologic procedures in the diagnosis and staging of known HCC before liver transplantation. [2]The higher value was presented from two data obtained between two observers.

establishment of surveillance based on both non-contrast US and contrast-enhanced US may be necessary for the screening procedure of HCC.

According to clinical studies concerning the doubling time of tumor, median days were reported as 117 d (29-398 d) by Sheu *et al*[27] or 171.6 d (27.2-605.6 d) by Barbara *et al*[41], and the former study called for a suitable screening interval for the early detection of HCC of 4-5 mo. Solmi *et al* reported that the percentage of detected unifocal tumors with a diameter less than or equal to 3 cm was significantly higher in the group followed-up every six months by both US and AFP than the group without this follow-up protocol[42]. Depending on the risk factors, a score based on certain clinical findings may be predictive for the doubling time of HCC[41,43]. The latter report recommended a regular US follow-up of a 3- or 6-mo interval according to the risk of HCC development, sex (male), alkaline phosphatase, AFP, γ-glutamyltransferase and albumin[43]. The study by Izzo *et al* also supported the 6-month surveillance by AFP and US for patients with severe chronic active hepatitis or liver cirrhosis[44]. However, Fasani *et al* reported that screening with US every six months may be inadequate for early detection of liver cancer in patients with multiple risk factors because multinodular HCC was under detected by US[45]. A tailor-made surveillance interval may be required according to the risk of HCC development.

Bolondi *et al* examined their surveillance program based on US and AFP at six-month intervals in 313 cirrhotic patients, reporting that the cumulative survival of the 61 patients with liver tumors detected by the surveillance program was significantly longer than that of controls not participating in any specific surveillance program, with incidentally detected HCC, and multivariate analysis showed an association between surveillance and survival[46]. Other studies showed that surveillance based on US and AFP every 6-12 mo improved the survival of patients[47,48].

As described above, the method and appropriate interval of surveillance have been discussed from the aspect of growth speed of HCC, detected number and size of HCC, and the risk of developing HCC. Furthermore, the significance of surveillance is well-supported by the improved survival rate. US should play a main role in the screening procedure of HCC.

DIAGNOSIS OF HCC

Imaging diagnosis of HCC is based on the presentation of characteristic hypervascular appearances in nodules. The European Association for the Study of the Liver (EASL) has documented the diagnostic criteria for HCC in a report for the clinical management of HCC[49]. Nodules larger than 2 cm with an arterial hypervascular pattern by two imaging techniques or by one imaging technique associated with an AFP level higher than 400 ng/mL was considered to be HCC in cirrhotic patients without needing confirmation by a positive biopsy. Four imaging modalities, US, spiral CT, MRI, and angiography, were recommended for evaluation of the vascularity of hepatic nodules in that article.

The advantages of US imaging consist of the simple and non-invasive demonstration of blood flow by real-time observation. US is a unique method that can evaluate blood flow direction under physiological condition. In contrast to focal nodular hyperplasia (FNH) with a centrifugal blood flow appearance (Figure 1), HCC has a characteristic hypervascular appearance with centripetal blood flow, and a basket pattern is one of the typical findings of HCC by color Doppler imaging[50-52]. The clinical application of microbubble contrast agents has resulted in remarkable improvement in blood flow detection by US examination. It was reported that the same enhancement pattern was found between contrast-enhanced harmonic grey-scale imaging with Levovist and contrast-enhanced helical CT in 53 of 61 (87%) HCC nodules[53]. Other studies have also shown over 80% concordance of tumor vascularity[54,55] between contrast-enhanced US with SonoVue (Bracco Diagnostics, Princeton, NJ, USA) and contrast-enhanced helical CT. Thus, the application of Doppler mode alone for detecting tumor blood flow is rare, as the more recent availability of microbubble contrast agents has assisted in overcoming the limitations of Doppler methods.

Figure 1 Contrast-enhanced harmonic imaging with Sonazoid in focal nodular hyperplasia (FNH). The centrifugal blood flow appearance like "spoke-wheel sign" was clearly demonstrated in the center of the nodules (arrows).

The diagnostic performance of contrast-enhanced US is not limited to the demonstration of tumor vascularity. Some microbubble contrast agents have a characteristic property of organ-specific accumulation[56-59]. Although the precise mechanism remains unclear, the reticuloendothelial system (i.e., phagocytosis by Kupffer cells) may be involved in this phenomenon. Both Levovist and Sonazoid (Nycomed-Amersham, Oslo, Norway) accumulate in the liver, and sonograms in this phase (late liver-specific parenchymal phase) are frequently used for the detection or characterization of liver tumors. In contrast, Definity (Bristol-Myers Squibb, N. Billerica, MA, USA) and SonoVue do not accumulate in the liver. The characterization of liver tumors by contrast-enhanced US has been carried out using accumulation images as well as vascular enhancement images (Figure 2A and B).

Concerning the discrimination of malignant versus benign liver lesions by contrast-enhanced US, recent literature has reported sensitivity of 98% to 100% and specificity of 63% to 93% with Levovist[60-63], and sensitivity of 98% and accuracy of 92.7% with SonoVue[64]. Furthermore, in a clinical study with two independent image reviewers, Kim *et al*[65] described that contrast-enhanced US (agent detecting imaging mode with Levovist) provided a specific diagnosis in 75%-79% of 75 patients with focal hepatic lesions, and that the technique

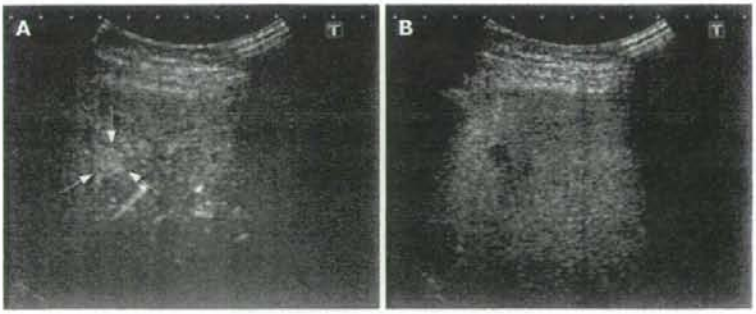

Figure 2 Contrast-enhanced harmonic imaging with Sonazoid in small HCC (9.8 mm, arrows). A: Early-phase image (22 s after the injection); B: Late-phase image (10 min after the injection). The early-phase image showed positive enhancement and the late-phase image showed negative enhancement in the nodule. These findings could easily diagnose this lesion as HCC.

was successful as a confirmatory imaging technique in 63%-72% of the patients.

Hypervascular hepatic lesions do not always reflect the fact that the final diagnosis of the nodule is HCC in heavy drinkers[66], since benign hypervascular nodules sometimes occur in their liver. A recent report has shown that the ring-shaped appearance on liver-specific contrast-enhanced sonograms with Levovist may be a useful sign for the differential diagnosis of benign nodule from HCC in heavy drinkers[67]. Since contrast-enhanced CT hardly differentiates these benign nodules from HCC, this characteristic finding may prevent unnecessary treatments under misdiagnosis. Moreover, it could be expected to lead to a reduction in the application of percutaneous needle biopsy, an invasive procedure, for the precise diagnosis.

Non-hypervascular and/or small (< 2 cm) nodules

Well-differentiated HCC, dysplastic nodule (DN) and regenerative nodule (RN) do not always reveal the specific hypervascular pattern on contrast-enhanced CT such as typical HCC[68-71]. The characterization of such non-hypervascular nodules is very important in clinical practice[72,73] because high-grade DN are considered potentially pre-malignant lesions. However, as these non-hypervascular nodules have Kupffer cell distribution[74,75], observation of the superparamagnetic iron oxide-enhanced (SPIO) MR images or liver-specific images on contrast-enhanced US could not easily characterize them.

According to the EASL report, percutaneous needle biopsy has until now been a standard method for the diagnosis of non-hypervascular hepatic nodules or small hepatic nodules of 1 cm to 2 cm[49], because characterization of these nodules by imaging modalities alone is difficult[76-79]. As for nodules smaller than 1 cm, EASL recommended repeated US observation every 3 mo until the lesion grows to 1 cm, at which point additional diagnostic techniques can be applied[49].

Thanks to the establishment of US-guided needle puncture technique[80], percutaneous needle biopsy has a quite high diagnostic accuracy. Caturelli *et al* found that the typing accuracy of fine-needle aspiration biopsy was 88.6% for nodules with diameters < 10 mm, 86.2% for nodules with diameters of 11-15 mm, and 91.3% for nodules with diameters of 16-20 mm[81]. Durand *et al* reported that US-guided FNB diagnosed HCC nodules with a sensitivity of 91%[82]. However, liver biopsy for small nodules always has the possibility of sampling error, and a negative biopsy of a nodule visible with imaging techniques in a cirrhotic liver can never be taken as a criterion to rule out malignancy[83]. Additionally, as rapid progression is rare in these kinds of nodules, repeated observations in their clinical course would determine their management. Therefore contrast-enhanced US can be expected to be an effective diagnostic tool for these non-hypervascular lesions because of its high resolution and non-invasive procedure.

TREATMENT SUPPORT AND EVALUATION OF THERAPEUTIC EFFECT

US-guided treatment

Since the majority of HCC patients have poor liver function and recurrence is not rare, surgical treatment is not always an appropriate choice[2,3,49]. With such backgrounds, percutaneous ethanol injection (PEI)[84-86] and radio-frequency ablation (RFA)[87,88] were developed and came to be widely used in clinical practice as minimally invasive methods[89]. They are now a first-line, favored approach with an efficient therapeutic effect on HCC[90-93].

Treatment for recurrent lesions

Although percutaneous US-guided treatments provide sufficient therapeutic effect, recurrence often plagues many HCC patients. According to long-term study results, cumulative recurrence rates of the treated site of post-PEI lesions were 3.4% at 1 year, 7.1% at 2 years, and 10% at 3 years, and those of the untreated sites in liver were 18.7% at 1 year, 62.1% at 3 years, and 81.7% at 5 years, respectively[94]. Thus, many HCC patients have to receive repeated treatments during their clinical course. In order to minimize adverse effects to the liver, less invasive treatment such as PEI or RFA is preferable for these patients. However, localization of lesions on sonograms is sometimes problematic in patients with cirrhotic liver and/ or repeated treatment history[95,96]. Although percutaneous treatment under CT guidance is a well-established technique and a useful method for lesions undetected by US, the method lacks convenience and exposes both patients and physicians to radiation[97-100]. Microbubble contrast agents are also useful in such a case. A recent study showed that contrast-enhanced US with Levovist could localize 24/32 (75%) of HCC lesions that were invisible by non-contrast US[101]. Application of the next-generation US contrast agents, SonoVue and Sonazoid, is expected to improve the localization result.

Evaluation of therapeutic effect

US examination is eligible for the evaluation of the therapeutic effect after percutaneous treatments such as PEI and RFA, because they are usually performed under US guidance. In fact, contrast-enhanced US has come to be frequently applied for evaluation of the therapeutic response in HCC nodules with improved sensitivity and specificity for detecting tumor blood flow (Table 2). According to the results by Bartolozzi *et al*, color Doppler US with Levovist showed sensitivity of 92%, specificity of 100%, and accuracy of 98% compared to the results of spiral CT and biopsy, in the detection of residual tumor tissue in 47 HCC lesions after PEI[102]. Wen *et al* examined the efficacy of coded harmonic angio mode with Levovist for detecting residual tumor in 91 HCC nodules about one week after RFA in comparison with contrast-enhanced CT, and they found that sensitivity, specificity, and diagnostic accuracy of US were 95.3%, 100%, and 98.1%, respectively[103]. Meloni *et al* reported that sensitivity and specificity of pulse inversion harmonic imaging with Levovist were 83.3% and 100%, respectively, for detecting residual non-ablated tumor at 4 mo after treatment in 35 patients with 43 HCC nodules, compared with helical CT findings[104]. Immediate evaluation of the therapeutic effect is often desirable after RFA for the management of HCC, and Choi *et al* mentioned that diagnostic agreement between power Doppler with Levovist about half or one day after ablation therapy and CT just after ablation was achieved in 100% of the 45 HCC nodules in 40 patients[105]. Another study showed that diagnostic concordance between agent detection imaging with Levovist performed within 24 h after RFA and 1-mo follow-up CT was 99% in 90 patients with 97 HCC nodules[106]. Thus, estimation of the therapeutic response in HCC after percutaneous treatments would become more efficient on the basis of this non-invasive imaging method. Although artificial signals caused by the RFA procedure affect an early detailed observation[105-107], monitoring by contrast-enhanced US during RFA would likely be applied to the assessment of the therapeutic effect as well as the detection of viable tumor.

It is well known that contrast-enhanced CT can hardly evaluate intratumoral contrast enhancement when partial retention of iodized oil is present in the tumor after transcatheter arterial chemoembolization (TACE). Therefore, the therapeutic effect of TACE is usually assessed by the distribution of iodized oil in the tumor on non-contrast CT images, though these findings are an indirect presentation. As MRI findings are not affected by the presence of iodized oil, contrast-enhanced MRI is favorable for the assessment of the therapeutic effect after TACE. However, the equipment has not yet come into wide-spread use, the procedure is not convenient, and evaluation of the findings in small lesions is sometimes difficult due to the low resolution and influence of motion artifacts. Contrast-enhanced US has the advantage of not being limited by iodized oil deposition that affects the evaluation of contrast-enhanced CT findings. Some clinical studies have shown the magnitude of contrast-enhanced US for evaluation of the therapeutic effect after TACE[108,109]. According to the report by Pompili *et al*, contrast-enhanced US with SonoVue resulted in diagnostic agreement in 53/56 cases (94.6%), with 87.0% sensitivity and 98.4% specificity compared with contrast-enhanced CT findings, after non-surgical treatments for HCC[110]. Another study showed that contrast-enhanced US resulted in considerably higher sensitivity in detecting residual tumor blood flow after TACE than dynamic CT or dynamic MRI[111]. Meanwhile, Lim *et al* described that a reliable assessment of intratumoral blood flow by contrast-enhanced US may not be possible in many instances, particularly in small lesions or in lesions located deep within the liver parenchyma[112]. They concluded that CT is the standard imaging technique for monitoring the effectiveness of TACE and RFA, and contrast-enhanced US and MRI can complement CT in evaluating the therapeutic response. Although the performance of the US examination may depend on the operator's skill, location of the tumor and system capability, quite a few radiologists and hepatologists may believe that contrast-enhanced US plays a major role in evaluation of the therapeutic effect after TACE. The recent developments in this technology would allow contrast-enhanced US to be positioned as the standard method for evaluation of the therapeutic effect in many HCC patients (Figure 3A and B).

Table 2 Assessment of therapeutic response after percutaneous treatment for HCC using contrast-enhanced US

Author	Treatment	No. of patients/ No. of lesions	Results[1] (contrast agent)
Bartolozzi *et al*[102]	PEI	40/47	Sensitivity 92% Specificity 100% Accuracy 98% (Levovist)
Wen *et al*[103]	RFA	67/91	Sensitivity 95.30% Specificity 100% Accuracy 98.10% (Levovist)
Meloni *et al*[104]	RFA	25/43	Sensitivity 83.30% Specificity 100% (Levovist)
Choi *et al*[105]	RFA	40/45	Diagnostic agreement 100% (Levovist)
Kim *et al*[106]	RFA	90/94	Diagnostic concordace[2] 99% (Levovist)
Solbiati *et al*[107]	RFA	20/20[3]	Sensitivity 50% Specificity 100% Diagnostic agreement 85% (Levovist)
Pompili *et al*[110]	PEI, RFA, TACE Combined treatments	47/56	Sensitivity 87% Specificity 98.40% Diagnostic agreement 94.60% (SonoVue)

[1]Comparison with contrast-enhanced helical CT; [2]1-mo follow-up CT; [3]Solitary colorectal liver metastases.

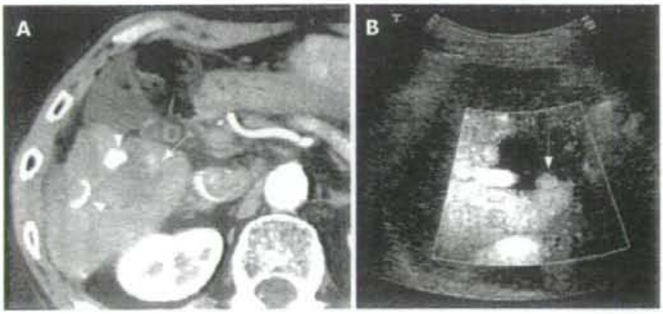

Figure 3 Assessment of therapeutic response after PEI for HCC. **A**: Contrast-enhanced CT with dynamic study; **B**: Contrast-enhanced US (Advanced Dynamic Flow with Levovist). Contrast-enhanced CT showed enhancement appearance which needed additional treatment within the treated area (arrow), and contrast-enhanced US could demonstrate a similar finding. Arrow heads: Lipiodol.

ADVANCED TECHNOLOGY

Recent US systems have provided three-dimensional visualization of the combined tissue structures and color blood-flow appearance under easy handling[24,25]. Additional anatomical information of the tumor with tumor-associated vessels is available at any plane from multiple directions[113-116]. With the remarkable progress in microelectronic technology, the US transducer has achieved full digital specification (Matrix transducer, iu22, Philips) with 3000 elements[117,118]. Including built-in micro-beamforming composed of a 150-computer board, it can visualize "Live 3D", which presents real-time three-dimensional anatomical views visible from any angle with volume rendering for pyramidal volume (90*70 angles). Contrast-enhanced 3D or 4D ultrasonographies using microbubble contrast agents might become a standard method for the characterization and/or evaluation of the therapeutic effect on liver tumors (Figure 4)[119].

Figure 4 Real-time three-dimensional imaging of HCC (contrast-enhanced LIVE 3D with Sonazoid, iu22, Philips). Abundant tumor vessels were dramatically demonstrated in the HCC nodule. (Arrows: HCC nodule).

HIFU is a novel technology that enables transcutaneous ablation effect without needle puncture[120,121]. While controlling the energy and focusing of US, successful HIFU results in necrosis of the tumor in the focal area with less damage of surrounding tissues. A number of clinical studies have been carried out using HIFU for the treatment of liver tumors as well as breast cancer and myoma uteri. In regard to liver tumors, it was reported that the anti-tumor effect and survival time by HIFU combined with TACE were superior to those by TACE alone in 50 patients with advanced HCC[122]. Although some of the subjects seemed to have a complete ablation effect, the precise effect for complete tumor necrosis by HIFU was not clear in this study. Furthermore, as the background of the HCCs showing sufficient ablation effect was not fully analyzed, it remains to be solved whether HIFU is valuable as a reliable method for curative treatment of small HCC. Nonetheless, this non-invasive method is really expected to be used for HCC treatment, as an alternative to PEI or RFA, because needle puncture is an invasive procedure for cirrhotic patients.

Normal ventilation is one of the serious problems in the completion of HIFU treatment for liver tumor, as movement of the liver may cause ablation failure that results on non-tumor tissue damage and/or incomplete therapeutic effect for the tumor. Wu *et al* reported that three-dimensional US images were used as a monitor to localize the tumor during HIFU treatment, and changes in echogenicity of the tumor just after the treatment were evaluated by US[123]. Advances in imaging technology for real-time 3D sonography would help the improvement of the therapeutic ability of HIFU.

In conclusion, US has made amazing strides in the last decades because of digital technology progress, and it will continue to grow. The advancement of imaging methods is expected to support the clinical management of patients with HCC.

REFERENCES

1 **Bosch FX**, Ribes J, Borras J. Epidemiology of primary liver cancer. *Semin Liver Dis* 1999; **19**: 271-285
2 **Okuda K**. Hepatocellular carcinoma: recent progress. *Hepatology* 1992; **15**: 948-963
3 **Okuda K**. Hepatocellular carcinoma. *J Hepatol* 2000; **32**: 225-237
4 **Oka H**, Kurioka N, Kim K, Kanno T, Kuroki T, Mizoguchi Y, Kobayashi K. Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *Hepatology* 1990; **12**: 680-687
5 **Takayasu K**, Moriyama N, Muramatsu Y, Makuuchi M, Hasegawa H, Okazaki N, Hirohashi S. The diagnosis of small hepatocellular carcinomas: efficacy of various imaging procedures in 100 patients. *AJR Am J Roentgenol* 1990; **155**: 49-54
6 **Kremkau FW**. Diagnostic ultrasound: Principles and Instruments. 4th edition. Philadelphia: WB Saunders, 1993
7 **Harvey CJ**, Albrecht T. Ultrasound of focal liver lesions. *Eur Radiol* 2001; **11**: 1578-1593
8 **Shapiro RS**, Wagreich J, Parsons RB, Stancato-Pasik A, Yeh HC, Lao R. Tissue harmonic imaging sonography: evaluation of image quality compared with conventional sonography. *AJR Am J Roentgenol* 1998; **171**: 1203-1206
9 **Hann LE**, Bach AM, Cramer LD, Siegel D, Yoo HH, Garcia

R. Hepatic sonography: comparison of tissue harmonic and standard sonography techniques. *AJR Am J Roentgenol* 1999; **173**: 201-206
10 **Whittingham TA**. Tissue harmonic imaging. *Eur Radiol* 1999; 9 Suppl 3: S323-S326
11 **Taylor KJ**, Ramos I, Morse SS, Fortune KL, Hammers L, Taylor CR. Focal liver masses: differential diagnosis with pulsed Doppler US. *Radiology* 1987; **164**: 643-647
12 **Nino-Murcia M**, Ralls PW, Jeffrey RB Jr, Johnson M. Color flow Doppler characterization of focal hepatic lesions. *AJR Am J Roentgenol* 1992; **159**: 1195-1197
13 **Choi BI**, Kim TK, Han JK, Chung JW, Park JH, Han MC. Power versus conventional color Doppler sonography: comparison in the depiction of vasculature in liver tumors. *Radiology* 1996; **200**: 55-58
14 **Lencioni R**, Pinto F, Armillotta N, Bartolozzi C. Assessment of tumor vascularity in hepatocellular carcinoma: comparison of power Doppler US and color Doppler US. *Radiology* 1996; **201**: 353-358
15 **Gaiani S**, Volpe L, Piscaglia F, Bolondi L. Vascularity of liver tumours and recent advances in doppler ultrasound. *J Hepatol* 2001; **34**: 474-482
16 **Mitchell DG**. Color Doppler imaging: principles, limitations, and artifacts. *Radiology* 1990; **177**: 1-10
17 **Foley WD**, Erickson SJ. Color Doppler flow imaging. *AJR Am J Roentgenol* 1991; **156**: 3-13
18 **Rubin JM**, Bude RO, Carson PL, Bree RL, Adler RS. Power Doppler US: a potentially useful alternative to mean frequency-based color Doppler US. *Radiology* 1994; **190**: 853-856
19 **Gramiak R**, Shah PM. Echocardiography of the normal and diseased aortic valve. *Radiology* 1970; **96**: 1-8
20 **Matsuda Y**, Yabuuchi I. Hepatic tumors: US contrast enhancement with CO2 microbubbles. *Radiology* 1986; **161**: 701-705
21 **Kudo M**, Tomita S, Tochio H, Mimura J, Okabe Y, Kashida H, Hirasa M, Ibuki Y, Todo A. Small hepatocellular carcinoma: diagnosis with US angiography with intraarterial CO2 microbubbles. *Radiology* 1992; **182**: 155-160
22 **Schlief R**, Staks T, Mahler M, Rufer M, Fritzsch T, Seifert W. Successful opacification of the left heart chambers on echocardiographic examination after intravenous injection of a new saccharide based contrast agent. *Echocardiography* 1990; **7**: 61-64
23 **Goldberg BB**. Ultrasound contrast agents. London: Martin Dunitz Ltd, 1997: 169
24 **Nelson TR**, Pretorius DH. Three-dimensional ultrasound imaging. *Ultrasound Med Biol* 1998; **24**: 1243-1270
25 **Downey DB**, Fenster A, Williams JC. Clinical utility of three-dimensional US. *Radiographics* 2000; **20**: 559-571
26 **Kennedy JE**, Ter Haar GR, Cranston D. High intensity focused ultrasound: surgery of the future? *Br J Radiol* 2003; **76**: 590-599
27 **Sheu JC**, Sung JL, Chen DS, Yang PM, Lai MY, Lee CS, Hsu HC, Chuang CN, Yang PC, Wang TH. Growth rate of asymptomatic hepatocellular carcinoma and its clinical implications. *Gastroenterology* 1985; **89**: 259-266
28 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084
29 **Collier J**, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998; **27**: 273-278
30 **Sato Y**, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; **328**: 1802-1806
31 **Izzo F**, Cremona F, Delrio P, Leonardi E, Castello G, Pignata S, Daniele B, Curley SA. Soluble interleukin-2 receptor levels in hepatocellular cancer: a more sensitive marker than alfa fetoprotein. *Ann Surg Oncol* 1999; **6**: 178-185
32 **Ishii M**, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, Toyota T, Takahashi T, Kasukawa R. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. *Am J Gastroenterol* 2000; **95**: 1036-1040
33 **Tong MJ**, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. *J Gastroenterol Hepatol* 2001; **16**: 553-559
34 **Larcos G**, Sorokopud H, Berry G, Farrell GC. Sonographic screening for hepatocellular carcinoma in patients with chronic hepatitis or cirrhosis: an evaluation. *AJR Am J Roentgenol* 1998; **171**: 433-435
35 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438
36 **Chalasani N**, Horlander JC Sr, Said A, Hoen H, Kopecky KK, Stockberger SM Jr, Manam R, Kwo PY, Lumeng L. Screening for hepatocellular carcinoma in patients with advanced cirrhosis. *Am J Gastroenterol* 1999; **94**: 2988-2993
37 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403
38 **Gambarin-Gelwan M**, Wolf DC, Shapiro R, Schwartz ME, Min AD. Sensitivity of commonly available screening tests in detecting hepatocellular carcinoma in cirrhotic patients undergoing liver transplantation. *Am J Gastroenterol* 2000; **95**: 1535-1538
39 **Teefey SA**, Hildeboldt CC, Dehdashti F, Siegel BA, Peters MG, Heiken JP, Brown JJ, McFarland EG, Middleton WD, Balfe DM, Ritter JH. Detection of primary hepatic malignancy in liver transplant candidates: prospective comparison of CT, MR imaging, US, and PET. *Radiology* 2003; **226**: 533-542
40 **Tanaka S**, Kitamura T, Nakanishi K, Okuda S, Yamazaki H, Hiyama T, Fujimoto I. Effectiveness of periodic checkup by ultrasonography for the early diagnosis of hepatocellular carcinoma. *Cancer* 1990; **66**: 2210-2214
41 **Barbara L**, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, Rigamonti A, Barbara C, Grigioni W, Mazziotti A. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. *Hepatology* 1992; **16**: 132-137
42 **Solmi L**, Primerano AM, Gandolfi L. Ultrasound follow-up of patients at risk for hepatocellular carcinoma: results of a prospective study on 360 cases. *Am J Gastroenterol* 1996; **91**: 1189-1194
43 **Zoli M**, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. *Cancer* 1996; **78**: 977-985
44 **Izzo F**, Cremona F, Ruffolo F, Palaia R, Parisi V, Curley SA. Outcome of 67 patients with hepatocellular cancer detected during screening of 1125 patients with chronic hepatitis. *Ann Surg* 1998; **227**: 513-518
45 **Fasani P**, Sangiovanni A, De Fazio C, Borzio M, Bruno S, Ronchi G, Del Ninno E, Colombo M. High prevalence of multinodular hepatocellular carcinoma in patients with cirrhosis attributable to multiple risk factors. *Hepatology* 1999; **29**: 1704-1707
46 **Bolondi L**, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, Piscaglia F, Gramantieri L, Zanetti M, Sherman M. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut* 2001; **48**: 251-259
47 **Sangiovanni A**, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004; **126**: 1005-1014
48 **Trevisani F**, Cantarini MC, Labate AM, De Notariis S, Rapaccini G, Farinati F, Del Poggio P, Di Nolfo MA, Benvegnu L, Zoli M, Borzio F, Bernardi M. Surveillance for hepatocellular carcinoma in elderly Italian patients with cirrhosis: effects on cancer staging and patient survival. *Am J Gastroenterol* 2004; **99**: 1470-1476
49 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J. Clinical management of hepatocellular carcinoma. Conclusions

of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430
50 **Tanaka S**, Kitamura T, Fujita M, Nakanishi K, Okuda S. Color Doppler flow imaging of liver tumors. *AJR Am J Roentgenol* 1990; **154**: 509-514
51 **Kudo M**, Tomita S, Tochio H, Kashida H, Hirasa M, Todo A. Hepatic focal nodular hyperplasia: specific findings at dynamic contrast-enhanced US with carbon dioxide microbubbles. *Radiology* 1991; **179**: 377-382
52 **Golli M**, Mathieu D, Anglade MC, Cherqui D, Vasile N, Rahmouni A. Focal nodular hyperplasia of the liver: value of color Doppler US in association with MR imaging. *Radiology* 1993; **187**: 113-117
53 **Numata K**, Tanaka K, Kiba T, Saito S, Ikeda M, Hara K, Tanaka N, Morimoto M, Iwase S, Sekihara H. Contrast-enhanced, wide-band harmonic gray scale imaging of hepatocellular carcinoma: correlation with helical computed tomographic findings. *J Ultrasound Med* 2001; **20**: 89-98
54 **Giorgio A**, Ferraioli G, Tarantino L, de Stefano G, Scala V, Scarano F, Coppola C, Del Viscovo L. Contrast-enhanced sonographic appearance of hepatocellular carcinoma in patients with cirrhosis: comparison with contrast-enhanced helical CT appearance. *AJR Am J Roentgenol* 2004; **183**: 1319-1326
55 **Bolondi L**, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34
56 **Blomley M**, Albrecht T, Cosgrove D, Jayaram V, Butler-Barnes J, Eckersley R. Stimulated acoustic emission in liver parenchyma with Levovist. *Lancet* 1998; **351**: 568
57 **Marelli C**. Preliminary experience with NC100100, a new ultrasound contrast agent for intravenous injection. *Eur Radiol* 1999; 9 Suppl 3: S343-S346
58 **Morel DR**, Schwieger I, Hohn L, Terrettaz J, Llull JB, Cornioley YA, Schneider M. Human pharmacokinetics and safety evaluation of SonoVue, a new contrast agent for ultrasound imaging. *Invest Radiol* 2000; **35**: 80-85
59 **Maruyama H**, Matsutani S, Saisho H, Mine Y, Yuki H, Miyata K. Different behaviors of microbubbles in the liver: time-related quantitative analysis of two ultrasound contrast agents, Levovist and Definity. *Ultrasound Med Biol* 2004; **30**: 1035-1040
60 **von Herbay A**, Vogt C, Haussinger D. Late-phase pulse-inversion sonography using the contrast agent levovist: differentiation between benign and malignant focal lesions of the liver. *AJR Am J Roentgenol* 2002; **179**: 1273-1279
61 **Bryant TH**, Blomley MJ, Albrecht T, Sidhu PS, Leen EL, Basilico R, Pilcher JM, Bushby LH, Hoffmann CW, Harvey CJ, Lynch M, MacQuarrie J, Paul D, Cosgrove DO. Improved characterization of liver lesions with liver-phase uptake of liver-specific microbubbles: prospective multicenter study. *Radiology* 2004; **232**: 799-809
62 **Dietrich CF**, Ignee A, Trojan J, Fellbaum C, Schuessler G. Improved characterisation of histologically proven liver tumours by contrast enhanced ultrasonography during the portal venous and specific late phase of SHU 508A. *Gut* 2004; **53**: 401-405
63 **von Herbay A**, Vogt C, Willers R, Haussinger D. Real-time imaging with the sonographic contrast agent SonoVue: differentiation between benign and malignant hepatic lesions. *J Ultrasound Med* 2004; **23**: 1557-1568
64 **Nicolau C**, Vilana R, Catalá V, Bianchi L, Gilabert R, García A, Brú C. Importance of evaluating all vascular phases on contrast-enhanced sonography in the differentiation of benign from malignant focal liver lesions. *AJR Am J Roentgenol* 2006; **186**: 158-167
65 **Kim SH**, Lee JM, Lee JY, Han JK, An SK, Han CJ, Lee KH, Hwang SS, Choi BI. Value of contrast-enhanced sonography for the characterization of focal hepatic lesions in patients with diffuse liver disease: receiver operating characteristic analysis. *AJR Am J Roentgenol* 2005; **184**: 1077-1084
66 **Kim SR**, Maekawa Y, Ninomiya T, Imoto S, Matsuoka T, Ando K, Mita K, Ku K, Koterazawa T, Nakajima T, Fukuda K, Yano Y, Nakaji M, Kudo M, Kim KI, Hirai M, Hayashi Y. Multiple hypervascular liver nodules in a heavy drinker of alcohol. *J Gastroenterol Hepatol* 2005; **20**: 795-799
67 **Maruyama H**, Matsutani S, Kondo F, Yoshizumi H, Kobayashi S, Okugawa H, Ebara M, Saisho H. Ring-shaped appearance in liver-specific image with Levovist: a characteristic enhancement pattern for hypervascular benign nodule in the liver of heavy drinkers. *Liver Int* 2006; **26**: 688-694
68 **Amano S**, Ebara M, Yajima T, Fukuda H, Yoshikawa M, Sugiura N, Kato K, Kondo F, Matsumoto T, Saisho H. Assessment of cancer cell differentiation in small hepatocellular carcinoma by computed tomography and magnetic resonance imaging. *J Gastroenterol Hepatol* 2003; **18**: 273-279
69 **Sakabe K**, Yamamoto T, Kubo S, Hirohashi K, Hamuro M, Nakamura K, Inoue Y, Kaneda K, Suehiro S. Correlation between dynamic computed tomographic and histopathological findings in the diagnosis of small hepatocellular carcinoma. *Dig Surg* 2004; **21**: 413-420
70 **Takayasu K**, Muramatsu Y, Mizuguchi Y, Moriyama N, Ojima H. Imaging of early hepatocellular carcinoma and adenomatous hyperplasia (dysplastic nodules) with dynamic ct and a combination of CT and angiography: experience with resected liver specimens. *Intervirology* 2004; **47**: 199-208
71 **Libbrecht L**, Desmet V, Roskams T. Preneoplastic lesions in human hepatocarcinogenesis. *Liver Int* 2005; **25**: 16-27
72 **Terminology for hepatic allograft rejection. International Working Party**. *Hepatology* 1995; **22**: 648-654
73 **Borzio M**, Fargion S, Borzio F, Fracanzani AL, Croce AM, Stroffolini T, Oldani S, Cotichini R, Roncalli M. Impact of large regenerative, low grade and high grade dysplastic nodules in hepatocellular carcinoma development. *J Hepatol* 2003; **39**: 208-214
74 **Tanaka M**, Nakashima O, Wada Y, Kage M, Kojiro M. Pathomorphological study of Kupffer cells in hepatocellular carcinoma and hyperplastic nodular lesions in the liver. *Hepatology* 1996; **24**: 807-812
75 **Imai Y**, Murakami T, Yoshida S, Nishikawa M, Ohsawa M, Tokunaga K, Murata M, Shibata K, Zushi S, Kurokawa M, Yonezawa T, Kawata S, Takamura M, Nagano H, Sakon M, Monden M, Wakasa K, Nakamura H. Superparamagnetic iron oxide-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading. *Hepatology* 2000; **32**: 205-212
76 **Quaglia A**, Bhattacharjya S, Dhillon AP. Limitations of the histopathological diagnosis and prognostic assessment of hepatocellular carcinoma. *Histopathology* 2001; **38**: 167-174
77 **Roncalli M**. Hepatocellular nodules in cirrhosis: focus on diagnostic criteria on liver biopsy. A Western experience. *Liver Transpl* 2004; **10**: S9-S15
78 **Bolondi L**, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34
79 **Takayasu K**, Muramatsu Y, Mizuguchi Y, Okusaka T, Shimada K, Takayama T, Sakamoto M. CT Evaluation of the progression of hypoattenuating nodular lesions in virus-related chronic liver disease. *AJR Am J Roentgenol* 2006; **187**: 454-463
80 **Ohto M**, Karasawa E, Tsuchiya Y, Kimura K, Saisho H, Ono T, Okuda K. Ultrasonically guided percutaneous contrast medium injection and aspiration biopsy using a renal-time puncture transducer. *Radiology* 1980; **136**: 171-176
81 **Caturelli E**, Solmi L, Anti M, Fusilli S, Roselli P, Andriulli A, Fornari F, Del Vecchio Blanco C, de Sio I. Ultrasound guided fine needle biopsy of early hepatocellular carcinoma complicating liver cirrhosis: a multicentre study. *Gut* 2004; **53**: 1356-1362
82 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of

the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258

83 **Nakashima T**, Kojiro M. Hepatocellular carcinoma. Tokyo: Springer-Verlag, 1987: 105-115

84 **Ebara M**, Ohto M, Sugiura N, Kita K, Yoshikawa M, Okuda K, Kondo F, Kondo Y. Percutaneous ethanol injection for the treatment of small hepatocellular carcinoma. Study of 95 patients. *J Gastroenterol Hepatol* 1990; **5**: 616-626

85 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929

86 **Redvanly RD**, Chezmar JL, Strauss RM, Galloway JR, Boyer TD, Bernardino ME. Malignant hepatic tumors: safety of high-dose percutaneous ethanol ablation therapy. *Radiology* 1993; **188**: 283-285

87 **Goldberg SN**, Gazelle GS, Solbiati L, Rittman WJ, Mueller PR. Radiofrequency tissue ablation: increased lesion diameter with a perfusion electrode. *Acad Radiol* 1996; **3**: 636-644

88 **Solbiati L**, Goldberg SN, Ierace T, Livraghi T, Meloni F, Dellanoce M, Sironi S, Gazelle GS. Hepatic metastases: percutaneous radio-frequency ablation with cooled-tip electrodes. *Radiology* 1997; **205**: 367-373

89 **Ryu M**, Shimamura Y, Kinoshita T, Konishi M, Kawano N, Iwasaki M, Furuse J, Yoshino M, Moriyama N, Sugita M. Therapeutic results of resection, transcatheter arterial embolization and percutaneous transhepatic ethanol injection in 3225 patients with hepatocellular carcinoma: a retrospective multicenter study. *Jpn J Clin Oncol* 1997; **27**: 251-257

90 **Lencioni R**, Bartolozzi C, Caramella D, Paolicchi A, Carrai M, Maltinti G, Capria A, Tafi A, Conte PF, Bevilacqua G. Treatment of small hepatocellular carcinoma with percutaneous ethanol injection. Analysis of prognostic factors in 105 Western patients. *Cancer* 1995; **76**: 1737-1746

91 **Livraghi T**, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. *Radiology* 1999; **210**: 655-661

92 **Lencioni RA**, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228**: 235-240

93 **Giorgio A**, Tarantino L, de Stefano G, Scala V, Liorre G, Scarano F, Perrotta A, Farella N, Aloisio V, Mariniello N, Coppola C, Francica G, Ferraioli G. Percutaneous sonographically guided saline-enhanced radiofrequency ablation of hepatocellular carcinoma. *AJR Am J Roentgenol* 2003; **181**: 479-484

94 **Ebara M**, Okabe S, Kita K, Sugiura N, Fukuda H, Yoshikawa M, Kondo F, Saisho H. Percutaneous ethanol injection for small hepatocellular carcinoma: therapeutic efficacy based on 20-year observation. *J Hepatol* 2005; **43**: 458-464

95 **Takayasu K**, Muramatsu Y, Asai S, Muramatsu Y, Kobayashi T. CT fluoroscopy-assisted needle puncture and ethanol injection for hepatocellular carcinoma: a preliminary study. *AJR Am J Roentgenol* 1999; **173**: 1219-1224

96 **Sato M**, Watanabe Y, Tokui K, Kawachi K, Sugata S, Ikezoe J. CT-guided treatment of ultrasonically invisible hepatocellular carcinoma. *Am J Gastroenterol* 2000; **95**: 2102-2106

97 **Schweiger GD**, Brown BP, Pelsang RE, Dhadha RS, Barloon TJ, Wang G. CT fluoroscopy: technique and utility in guiding biopsies of transiently enhancing hepatic masses. *Abdom Imaging* 2000; **25**: 81-85

98 **Shibata T**, Iimuro Y, Yamamoto Y, Ikai I, Itoh K, Maetani Y, Ametani F, Kubo T, Konishi J. CT-guided transthoracic percutaneous ethanol injection for hepatocellular carcinoma not detectable with US. *Radiology* 2002; **223**: 115-120

99 **Kickuth R**, Laufer U, Hartung G, Gruening C, Stueckle C, Kirchner J. 3D CT versus axial helical CT versus conventional tomography in the classification of acetabular fractures: a ROC analysis. *Clin Radiol* 2002; **57**: 140-145

100 **Solomon SB**, Bohlman ME, Choti MA. Percutaneous gadolinium injection under MR guidance to mark target for CT-guided radiofrequency ablation. *J Vasc Interv Radiol* 2002; **13**: 419-421

101 **Maruyama H**, Kobayashi S, Yoshizumi H, Okugawa H, Akiike T, Yukisawa S, Fukuda H, Matsutani S, Ebara M, Saisho H. Application of percutaneous ultrasound-guided treatment for ultrasonically invisible hypervascular hepatocellular carcinoma using microbubble contrast agent. *Clin Radiol* 2007; **62**: 668-675

102 **Bartolozzi C**, Lencioni R, Ricci P, Paolicchi A, Rossi P, Passariello R. Hepatocellular carcinoma treatment with percutaneous ethanol injection: evaluation with contrast-enhanced color Doppler US. *Radiology* 1998; **209**: 387-393

103 **Wen YL**, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **181**: 57-63

104 **Meloni MF**, Goldberg SN, Livraghi T, Calliada F, Ricci P, Rossi M, Pallavicini D, Campani R. Hepatocellular carcinoma treated with radiofrequency ablation: comparison of pulse inversion contrast-enhanced harmonic sonography, contrast-enhanced power Doppler sonography, and helical CT. *AJR Am J Roentgenol* 2001; **177**: 375-380

105 **Choi D**, Lim HK, Kim SH, Lee WJ, Jang HJ, Lee JY, Paik SW, Koh KC, Lee JH. Hepatocellular carcinoma treated with percutaneous radio-frequency ablation: usefulness of power Doppler US with a microbubble contrast agent in evaluating therapeutic response-preliminary results. *Radiology* 2000; **217**: 558-563

106 **Kim CK**, Choi D, Lim HK, Kim SH, Lee WJ, Kim MJ, Lee JY, Jeon YH, Lee J, Lee SJ, Lim JH. Therapeutic response assessment of percutaneous radiofrequency ablation for hepatocellular carcinoma: utility of contrast-enhanced agent detection imaging. *Eur J Radiol* 2005; **56**: 66-73

107 **Solbiati L**, Goldberg SN, Ierace T, Dellanoce M, Livraghi T, Gazelle GS. Radio-frequency ablation of hepatic metastases: postprocedural assessment with a US microbubble contrast agent--early experience. *Radiology* 1999; **211**: 643-649

108 **Cioni D**, Lencioni R, Bartolozzi C. Therapeutic effect of transcatheter arterial chemoembolization on hepatocellular carcinoma: evaluation with contrast-enhanced harmonic power Doppler ultrasound. *Eur Radiol* 2000; **10**: 1570-1575

109 **Morimoto M**, Shirato K, Sugimori K, Kokawa A, Tomita N, Saito T, Imada T, Tanaka N, Nozawa A, Numata K, Tanaka K. Contrast-enhanced harmonic gray-scale sonographic-histologic correlation of the therapeutic effects of transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *AJR Am J Roentgenol* 2003; **181**: 65-69

110 **Pompili M**, Riccardi L, Covino M, Barbaro B, Di Stasi C, Orefice R, Gasbarrini G, Rapaccini GL. Contrast-enhanced gray-scale harmonic ultrasound in the efficacy assessment of ablation treatments for hepatocellular carcinoma. *Liver Int* 2005; **25**: 954-961

111 **Minami Y**, Kudo M, Kawasaki T, Kitano M, Chung H, Maekawa K, Shiozaki H. Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **180**: 703-708

112 **Lim HS**, Jeong YY, Kang HK, Kim JK, Park JG. Imaging features of hepatocellular carcinoma after transcatheter arterial chemoembolization and radiofrequency ablation. *AJR Am J Roentgenol* 2006; **187**: W341-W349

113 **Rankin RN**, Fenster A, Downey DB, Munk PL, Levin MF, Vellet AD. Three-dimensional sonographic reconstruction: techniques and diagnostic applications. *AJR Am J Roentgenol* 1993; **161**: 695-702

114 **Picot PA**, Rickey DW, Mitchell R, Rankin RN, Fenster A. Three-dimensional colour Doppler imaging. *Ultrasound Med Biol* 1993; **19**: 95-104

115 **Downey DB**, Fenster A. Vascular imaging with a three-dimensional power Doppler system. *AJR Am J Roentgenol* 1995; **165**: 665-668

116 **Ritchie CJ**, Edwards WS, Mack LA, Cyr DR, Kim Y. Three-dimensional ultrasonic angiography using power-mode Doppler. *Ultrasound Med Biol* 1996; **22**: 277-286
117 **Acar P**, Dulac Y, Taktak A, Abadir S. Real-time three-dimensional fetal echocardiography using matrix probe. *Prenat Diagn* 2005; **25**: 370-375
118 **Monaghan MJ**. Role of real time 3D echocardiography in evaluating the left ventricle. *Heart* 2006; **92**: 131-136
119 **Ohto M**, Kato H, Tsujii H, Maruyama H, Matsutani S, Yamagata H. Vascular flow patterns of hepatic tumors in contrast-enhanced 3-dimensional fusion ultrasonography using plane shift and opacity control modes. *J Ultrasound Med* 2005; **24**: 49-57
120 **Kennedy JE**, Wu F, ter Haar GR, Gleeson FV, Phillips RR, Middleton MR, Cranston D. High-intensity focused ultrasound for the treatment of liver tumours. *Ultrasonics* 2004; **42**: 931-935
121 **Li CX**, Xu GL, Jiang ZY, Li JJ, Luo GY, Shan HB, Zhang R, Li Y. Analysis of clinical effect of high-intensity focused ultrasound on liver cancer. *World J Gastroenterol* 2004; **10**: 2201-2204
122 **Wu F**, Wang ZB, Chen WZ, Zou JZ, Bai J, Zhu H, Li KQ, Jin CB, Xie FL, Su HB. Advanced hepatocellular carcinoma: treatment with high-intensity focused ultrasound ablation combined with transcatheter arterial embolization. *Radiology* 2005; **235**: 659-667

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CLINICAL STUDIES

Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients

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Abstract

Background/Aims: Our aim was to evaluate the relationship between hepatitis C virus (HCV) infection and development of diabetes mellitus (DM) or insulin resistance (IR) in comparison with hepatitis B virus (HBV) infection and eradication of HCV infection by interferon treatment. *Methods:* This study consisted of 952 outpatients, including 544 HCV-infected (HCV+chronic), 286 HBV-infected (HBV+chronic) and 122 patients whose HCV was cleared by interferon treatment (HCV+cleared) (diabetes study). Among 849 without overt DM, IR was assessed in 423 patients, including 232 HCV-infected (HCV+chronic), 135 HBV-infected (HBV+chronic) and 56 HCV-eradicated patients (HCV+cleared) (IR substudy). *Results:* The prevalence of DM in the HBV+chronic, HCV+chronic and HCV+cleared groups was 6.3, 13.6 and 9.0%, respectively (HBV+chronic vs HCV+chronic, $P < 0.005$), in the diabetes study, and the prevalence of IR in the HCV+chronic group (54.3%) was also higher than that in the HBV+chronic (36.3%) ($P < 0.005$) and HCV+cleared groups (35.7%) ($P < 0.05$) in the IR substudy. However, HCV infection was not shown to be independently associated with DM development [odds ratio (OR) 1.669; $P = 0.0936$] and with IR (OR 1.531; $P = 0.2154$) by multivariate analysis in comparison with HBV infection as control. *Conclusions:* HCV-infected patients showed a higher prevalence of DM and IR than those with HBV infection. However, in Japan, other confounding factors appeared to be more important risk factors for the development of disturbance in glucose metabolism.

Approximately 1.5 million people in Japan and 170 million people worldwide have been infected with the hepatitis C virus (HCV), and chronic HCV infection causes chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (1, 2). In most western countries, HCV infection is becoming a major cause of HCC and liver transplantation (3).

It is estimated that seven million people are affected by diabetes mellitus (DM) in Japan (4). Type 2 DM is a lifestyle-associated disease and is increasing worldwide, including in Japan (5). The risk factors associated with type 2 DM include family history, age, gender, obesity, smoking and physical activity (6). A close association between DM and insulin resistance (IR) is also reported (7).

The liver is crucial to carbohydrate metabolism and glucose homoeostasis, and hepatic dysfunction causes glucose abnormalities leading to DM, which is prevalent in chronic liver disease, and especially in liver cirrhosis (8,9). Cirrhotic patients have glucose intolerance attributable to IR, which is caused by a post-receptor defect, decreased binding of insulin to target tissues and inadequate response of β-cells to secrete insulin appropriately to overcome the defect in insulin action (10). It has also been reported that DM increases the risk of developing chronic liver disease and HCC (11), and DM has been associated with non-alcoholic fatty liver disease including non-alcoholic steatohepatitis (12). Thus, there is a close association between DM and liver diseases.

Recently, there has been growing evidence to suggest an association between HCV infection and DM. A high prevalence of DM has been reported among patients chronically infected with HCV in comparison with controls or other liver diseases (13–18), in addition to a high prevalence of HCV infection among

Table 1. Clinical characteristics of HBV+chronic, HCV+chronic and HCV+cleared patients in diabetes study and insulin resistance substudy

	HBV+ chronic	HCV+ chronic	HCV+ cleared	*P* value: HBV+ chronic vs HCV+ chronic	*P* value: HCV+ chronic vs HCV+ cleared	*P* value: HBV+ chronic vs HCV+ cleared
Diabetes study						
Number	286	544	122			
Age	45.1 ± 13.6	58.4 ± 13.0	53.2 ± 13.0	< 0.0001	< 0.0001	< 0.0001
Gender male (%)	164 (57.3)	257 (47.2)	82 (67.2)	< 0.01	< 0.0001	NS
Clinical stage (asymptomatic carrier/chronic hepatitis/ cirrhosis)	100/161/25	78/353/113	–/–/–	< 0.0001		
Insulin resistance substudy						
Number	135	232	56			
Age	44.5 ± 13.0	59.6 ± 13.1	53.5 ± 12.0	< 0.0001	< 0.005	< 0.005
Gender male (%)	77 (57.0%)	93 (40.1%)	38 (67.9%)	< 0.005	< 0.0005	NS
Hypertension	5 (3.7%)	49 (21.1%)	5 (9.1%)	< 0.0001	NS	NS
Hyperlipidaemia	32 (23.9%)	33 (14.2%)	17 (30.4%)	< 0.05	< 0.01	NS
Obesity	19 (17.4%)	40 (19.1%)	19 (38.8%)	NS	< 0.01	< 0.01
BMI (kg/m^2)	22.7 ± 3.3	22.8 ± 3.1	23.8 ± 3.3	NS	< 0.05	NS
Clinical stage (ASC/CH/cirrhosis)	40/77/18	37/140/55	–/–/–	< 0.005		
FPG (mg/dl)	96 ± 8	99 ± 10	100 ± 9	< 0.005	NS	< 0.01
IRI (μU/L)	8.7 ± 5.6	9.5 ± 5.3	7.9 ± 5.0	NS	< 0.05	NS
HOMA-IR	2.1 ± 1.4	2.4 ± 1.4	2.0 ± 1.3	NS	NS	NS
AST (IU/L)	42 ± 64	49 ± 27	23 ± 7	NS	< 0.0001	< 0.05
ALT (IU/L)	53 ± 121	49 ± 34	19 ± 9	NS	< 0.0001	< 0.05
γ-GTP (IU/L)	38 ± 68	40 ± 44	30 ± 22	NS	NS	NS
Platelet (× 10^9/L)	201 ± 62	168 ± 69	213 ± 61	< 0.0001	< 0.0001	NS
Total cholesterol (mg/dl)	192 ± 32	176 ± 34	197 ± 32	< 0.0001	< 0.0001	NS
Triglyceride (mg/dl)	92 ± 59	91 ± 46	103 ± 46	NS	NS	NS
HCV-RNA genotype 1/2	–	142/46	22/24		< 0.001	

ALT, alanine aminotransferase; ASC, asymptomatic carrier; AST, aspartate aminotransferase; BMI, body mass index; CH, chronic hepatitis; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus ribonucleic acid; HOMA-IR, homeostasis model of insulin resistance; IRI, immunoreactive insulin; NS, not significant.

patients with DM (19–21). Most previous studies have provided evidence of a positive association between them, with a few exceptions (22–24).

In this cross-sectional study, we investigated the prevalence of DM and IR in patients with chronic hepatitis C, and compared it with that in patients chronically infected with HBV and those who cleared HCV after interferon treatment as control.

Materials and methods

Diabetes study

Among the 1163 outpatients recruited from patients seropositive for the hepatitis B surface antigen (HBsAg) or the anti-hepatitis C virus antibody (HCV-Ab) who visited the First Department of Medicine, Chiba University Hospital, between January 2003 and December 2004, 87 patients were excluded because 60 patients had HCC and 27 were HCV ribonucleic acid (HCV-RNA) seronegative without previous interferon treatment. Among the remaining 1076 patients, the plasma glucose level and/or the HBA1C level were investigated retrospectively in 952 patients (88.5%), consisting of 544 patients chronically infected with HCV (HCV+chronic), 122 whose HCV had cleared after interferon treatment (HCV+cleared) and 286 chronically infected with HBV (HBV+chronic) (Table 1) (Fig. 1). One hundred and twenty-four patients whose plasma glucose level was not available were excluded: 50 patients chronically infected with HCV, 22 whose HCV had cleared after interferon treatment and 52 chronically infected with HBV. They were younger (45.0 ± 16.5 vs 53.8 ± 14.5, $P < 0.01$) with ASC more prevalent (42.9 vs 21.4%, $P < 0.05$) and cirrhosis less prevalent (3.8 vs 16.6%, $P < 0.05$), and the proportion of males was

Liver International (2008)

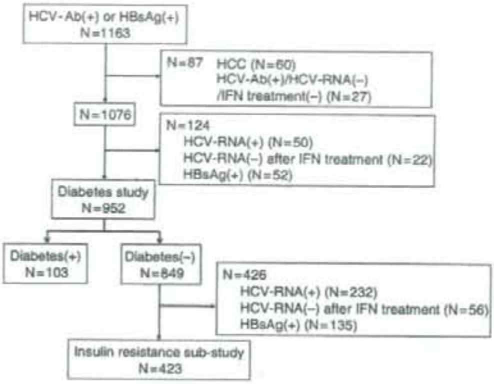

Fig. 1. Flow chart of patients analysed in diabetes study and insulin resistance substudy. IFN, interferon.

almost similar (44.4 vs 52.8%, $P=0.09$) compared with the 952 patients (Fig. 1). More in-depth data including body mass index (BMI), a key confounder, were not available in the diabetes study because it was a retrospective one.

Patients in the HCV+cleared group had completed interferon therapy at least more than 6 months before entry. Patients in the HCV+chronic group were seropositive for HCV-RNA and HCV-Ab, those in the HCV+cleared group were seropositive for HCV-Ab but seronegative for HCV-RNA, and those in the HBV+chronic group were seropositive for HBsAg. Patients seropositive for HBsAg and HCV-RNA were not included and those with autoimmune hepatitis, primary biliary cirrhosis, haemochromatosis, Wilson's disease, excessive alcohol intake of more than 50 g/day, HCC assessed by imaging examinations such as ultrasonography and computed tomography, and with a history of pancreatitis or pancreatic tumours were also excluded from this study. The definition of an asymptomatic carrier depends on normal alanine aminotransferase (ALT) levels in blood examinations at least two times per year for more than 3 years. The diagnosis of cirrhosis was based on histological findings by liver biopsy in 66 of 138 patients (48%) or on clinical features such as the presence of oesophageal varices, platelet counts $< 100 \times 10^9$/L because of hypersplenism and abdominal ultrasonographical findings (25) in the remaining patients. All cirrhotic patients were of Child-Pugh classification A (86%) or B (13%), except for one patient with HCV.

This study received ethics committee approval according to the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Insulin resistance substudy

Four hundred and twenty-three patients were enrolled, selected randomly from 849 patients diagnosed as non-diabetic among the above 952(Fig. 1). Among them, 232 patients were chronically infected with HCV (HCV+chronic), 56 patients had HCV cleared after interferon treatment (HCV+cleared) and 135 patients were chronically infected with HBV (HBV+chronic) (Table 1). The remaining 426 patients not recruited into this study, including 238 patients chronically infected with HCV, 55 patients whose HCV had cleared and 133 patients chronically infected with HBV, showed similar distribution in terms of male gender (52.3 vs 49.2%, $P=0.37$) and no difference in age distribution (52.3 ± 15.0 vs 54.0 ± 14.6, $P=0.10$), with cirrhosis being less prevalent (10.0 vs 19.9%, $P < 0.05$).

Presence of diabetes mellitus, hypertension, hyperlipidaemia and obesity and definition of insulin resistance

Patients were considered to have diabetes if they used insulin or hypoglycaemic drugs at the time of the survey or had a fasting plasma glucose level of 126 mg/dl or more, a non-fasting plasma glucose level of 200 mg/dl or more or a haemoglobin A1C level of 6.5% or more. IR was evaluated by the homoeostasis model of IR (HOMA-IR, fasting plasma glucose (mg/dl) $\times$ insulin (μU/ml) $\div$ 405) (26) in patients without overt diabetes and was defined as a HOMA-IR of 2.0 or more. The presence of hypertension was ascertained based on medication history or systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg. The diagnosis of hyperlipidaemia was made on the basis of the medication history and a total cholesterol level above 220 mg/dl or a triglyceride level above 150 mg/dl, and that of obesity was by a BMI (kg/m^2) of more than 25.0. The use of medications to establish diabetes, hyperlipidaemia and hypertension was not only based on patient self-report but was also confirmed by a medical record review.

Laboratory examination

Anti-hepatitis C virus antibody and HCV-RNA were determined by a second-generation enzyme-linked immunosorbent assay (Ortho Diagnostics, Tokyo, Japan) and the reverse-transcriptional polymerase chain reaction method (Amplicore HCV monitor assay version 2.0; Roche, Tokyo, Japan; lower detection limit, 500 copies/ml) (27). The HCV genotype was determined by serological grouping of serum antibody

(28), assuming that genotypes 1a and 1b correspond to group 1 and genotypes 2a and 2b correspond to group 2. Serum blood chemistries including haematological variables were obtained by a standard method using an autoanalyser. HBsAg was measured by an enzyme-linked immunosorbent assay (Abbott Laboratory, North Chicago, IL, USA).

Statistical analysis

Student's *t*-test and Fisher's exact test were used to analyse quantitative and qualitative data respectively. Multivariate logistic regression analysis was used to determine the adjusted odds ratios (ORs) of type 2 diabetes or IR with respect to HCV infection. Variables considered to be potential confounders in multivariate analysis were age, gender and clinical stage of liver disease for the development of diabetes, and age, gender, clinical stage of liver disease, hypertension, BMI, aspartate aminotransaminase (AST), ALT, γ-glutamyl transpeptidase and triglyceride for the development of IR. A *P*-value of < 0.05 was considered to indicate statistical significance.

Results

Diabetes study: prevalence of diabetes mellitus

Patient characteristics

The characteristics of the patients enrolled in this study are shown in Table 1. The mean age was statistically different among the three groups of patients with HBV infection (HBV+chronic), those with HCV infection (HCV+chronic) and those whose HCV had cleared after interferon treatment (HCV+cleared), and the proportion of male gender was also statistically different among the HCV+chronic and HBV+chronic, and HCV+chronic and HCV+cleared groups (Table 1). The clinical stages differed between the HBV+chronic and HCV+chronic groups, with more asymptomatic carriers in the HBV+chronic group and more cirrhotic patients in the HCV+chronic group (Table 1).

Prevalence of diabetes mellitus with various clinical backgrounds

The prevalence of DM was 18/286 (6.3%) in the HBV+chronic group, 74/544 (13.6%) in the HCV+chronic group and 11/122 (9.0%) in the HCV+cleared group, with the prevalence in the HCV+chronic group being significantly greater than that in the HBV+chronic group ($P < 0.005$) (Table 2). This result was also applicable when considering males and females separately. According to stratified age, the prevalence of DM was higher in the HCV+chronic group than in the HBV+chronic group without statistical significance. The prevalence of DM increased according to the progression of liver disease, but the prevalence was similar in asymptomatic carrier patients between the HBV+chronic and HCV+chronic groups (Table 2).

Table 2. Prevalence of diabetes mellitus with various clinical backgrounds in diabetes study

	HBV+chronic (Group B1)	HCV+chronic (Group C1)	HCV+cleared (Group CC1)
Number	286	544	122
Diabetes mellitus	18 (6.3%)***	74 (13.6%)***	11 (9.0%)
Gender			
Male	14 (8.5%)**	48 (18.7%)**	10 (12.2%)
Female	4 (3.3%)*	26 (9.1%)*	1 (2.5%)
Age			
≤ 49	4 (2.4%)	6 (4.8%)	3 (6.8%)
50–59	10 (11.8%)	23 (18.0%)	4 (12.1%)
≥60	4 (10.8%)	45 (15.4%)	4 (8.9%)
Clinical stage			
ASC	6 (6.0%)	5 (6.4%)	–
CH	9 (5.6%)*	44 (12.5%)*	–
Cirrhosis	3 (12%)	25 (22.1%)	–

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.005$.
ASC, asymptomatic carrier; CH, chronic hepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus.

Clinical factors associated with the development of diabetes mellitus in the HBV+chronic and HCV+chronic groups

Multivariate logistic regression analysis revealed male gender, older age and the presence of cirrhosis as independent risk factors for the development of DM, but not HCV infection (Table 3).

Clinical backgrounds and the prevalence of diabetes mellitus in asymptomatic carrier patients in the HCV+chronic and HCV+cleared groups

The prevalence of DM was 5/78 (6.4%) patients who were HCV-infected asymptomatic carriers and 11/122 (9.0%) patients with eradicated HCV after interferon treatment, showing no difference between them ($P = 0.6$). Multivariate logistic regression analysis with gender, age and HCV infection as confounding factors did not show ongoing HCV infection as an independent risk factor for developing DM (data not shown).