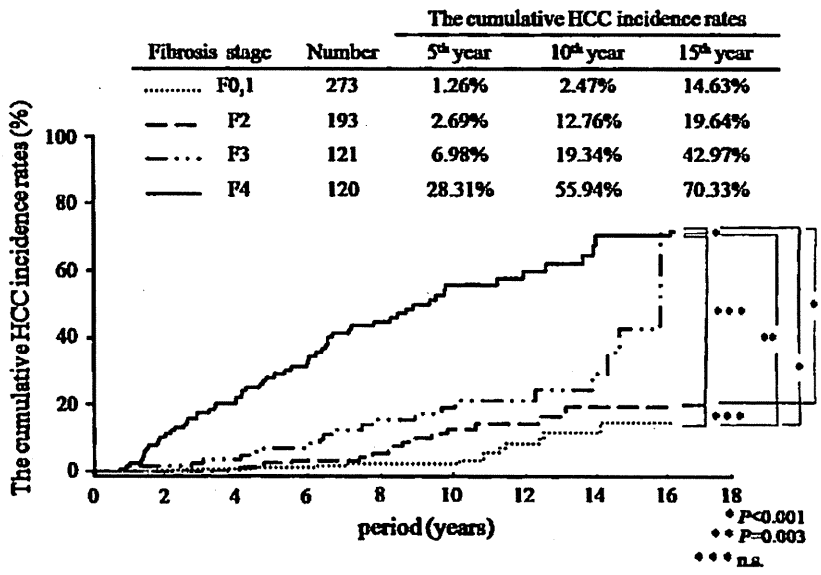

Fig. 1 Cumulative incidence of hepatocellular carcinoma (*HCC*) according to the fibrosis stage

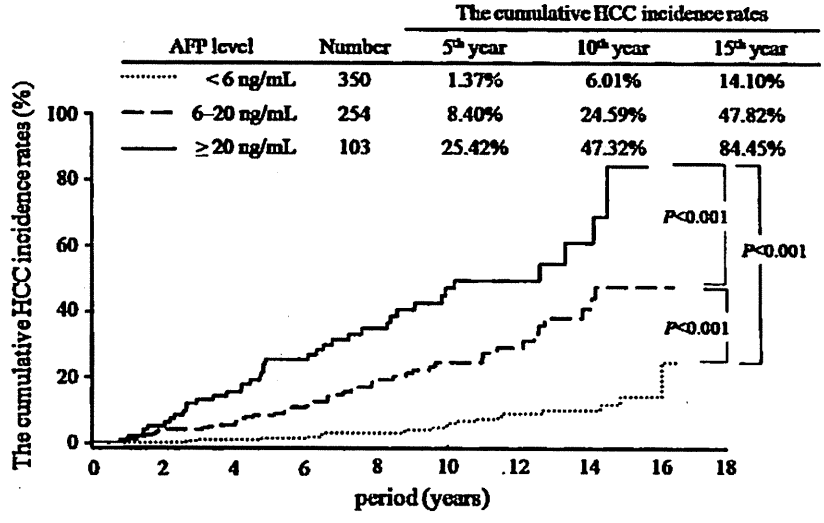

Fig. 2 Cumulative incidence of HCC according to alpha-fetoprotein (*AFP*) levels

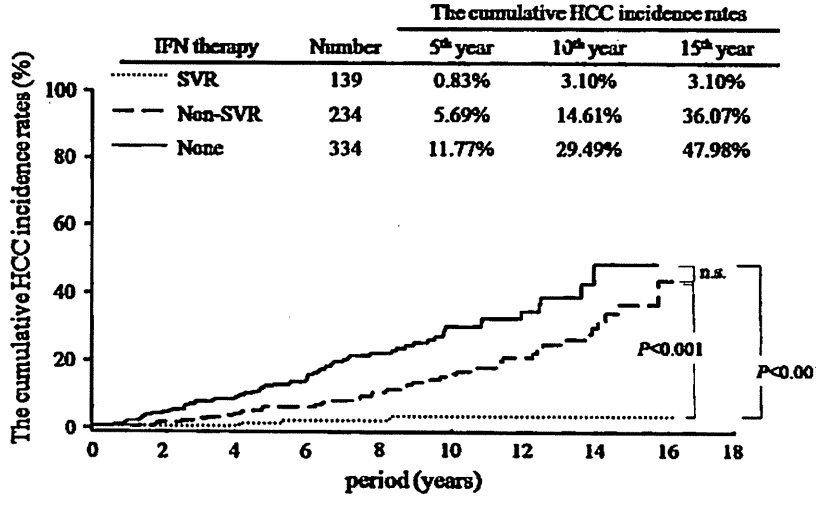

Fig. 3 Cumulative incidence of HCC according to interferon (*IFN*) therapy. *SVR* Sustained virological response

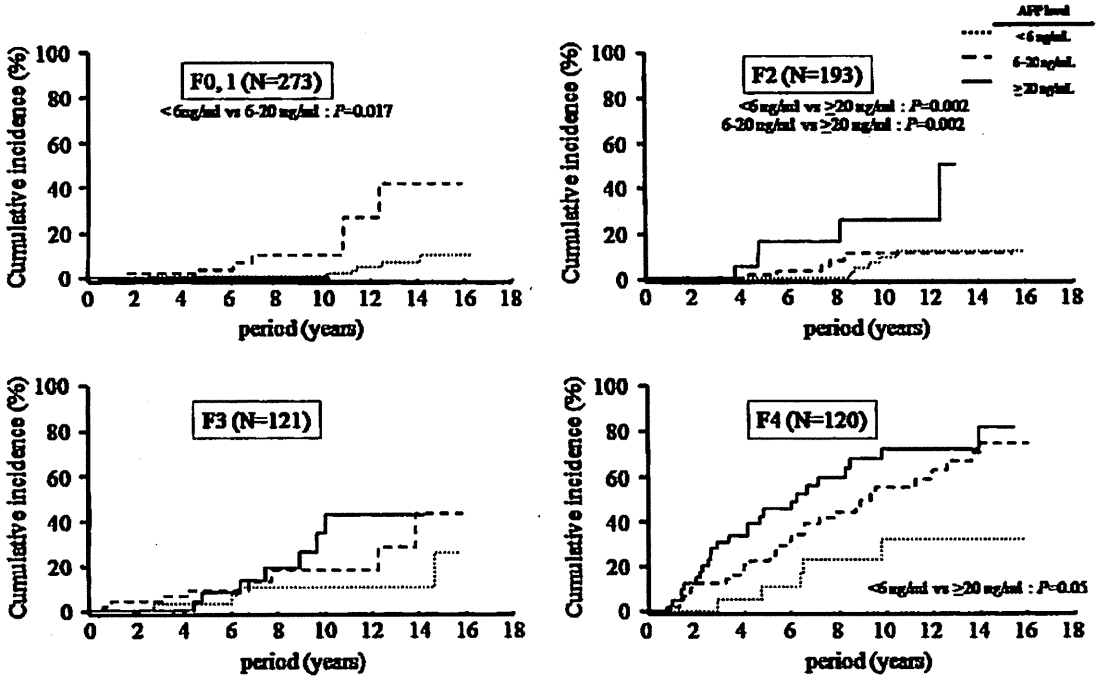

Fig. 4 Cumulative incidence of HCC according to AFP levels, stratified by the fibrosis stage

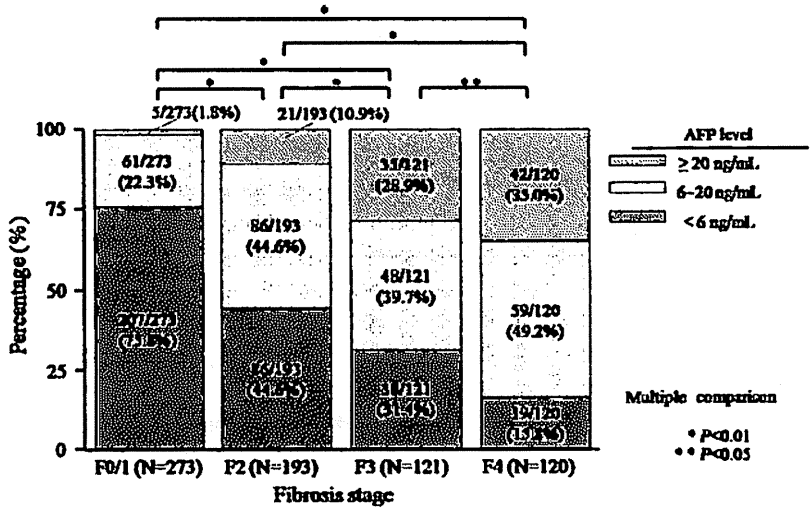

Fig. 5 Proportions of patients with three different AFP levels (<6 ng/mL, 6–20 ng/mL, and ≥20 ng/mL) at different fibrosis stages

among patients infected with HCV, including not only those with cirrhosis but also those with chronic hepatitis, we found AFP levels to be a dependable risk factor for HCC, in addition to the fibrosis stage. Of particular note, not only the patients with high AFP levels (≥20 ng/mL) but also those with even slightly elevated AFP levels (between 6 and 20 ng/mL) had increased risks for the development of HCC. In the patients in this study, the median AFP level was 6 ng/mL. It deviated slightly from serum levels of AFP in healthy adults that have been reported to range from 0.1 to 5.8 ng/mL [33]. Hence, we performed analyses by setting various AFP cutoff levels for evaluating their performance as risk factors. However, there were no significant differences in the analysis with the use of AFP cutoff levels exceeding 7 ng/mL. On the basis of these observations, an AFP cutoff level of 6 ng/mL was adopted in this study. In previous reports, AFP levels were associated with advanced fibrosis stage in patients infected with HCV in the absence of HCC [34–38]. In the present study, AFP levels were elevated in parallel with advanced fibrosis stages and correlated well with the fibrosis stage. As the patients with even slightly elevated AFP levels, between 6 and 20 ng/mL, had moderately advanced liver fibrosis stages, these AFP levels could

indicate an elevated risk for HCC in patients with chronic HCV infection.

Hu et al. [36] found that an AFP level of 15.0 mg/mL could detect severe fibrosis with a sensitivity of 22.8% and specificity of 94.5%. Moreover, they reported, during observation for 6 months of patients with chronic hepatitis C, that AFP levels stayed within the normal range (<10 ng/mL) in 60%, were persistently elevated in 24%, and fluctuated in the remaining 15%. By multivariate analysis, they identified AST, INR, and fibrosis as risk factors for AFP levels of >10 ng/mL. In view of the correlation between AFP levels and fibrosis stages, the AFP level at the time of liver biopsy was taken into account in the analysis in the present study; ALT levels are reported to be persistently elevated in the majority (60%) of patients with chronic hepatitis C.

Liver biopsy is the gold standard for assessing hepatic fibrosis [8, 9]. However, the needle liver biopsy has a sampling error and is too invasive as a routine procedure [10, 11]. Therefore, AFP levels may be used as a noninvasive and predictive marker in place of the fibrosis stage. The platelet count is known to reflect the severity of chronic hepatitis C [12, 13], and is used to estimate the degree of fibrosis without resort to liver biopsy [12–14]. Previous reports have shown low platelet counts to represent a risk factor for HCC in cirrhotic patients [13, 15, 16]. Matsumura et al. [13] reported that age and serum platelet count were significant risk factors for the development of HCC, and as such, they were a major clinico-laboratory means of evaluating the fibrosis stage. In the present study, however, the platelet count was not an independent risk factor for HCC development. When Cox regression analysis was performed on variables other than the fibrosis stage, platelet count and serum albumin levels were identified as independent risk factors for the development of HCC (data not shown).

IFN has been used to treat patients with HCV infection. Failure to achieve an SVR to IFN-based therapies, and preexisting advanced hepatic fibrosis and/or cirrhosis, are major predictors of HCC [6, 23, 25, 39, 40]. In the present study, SVR emerged as an independent risk factor for the development of HCC, while non-SVR was not. However, the cumulative incidence rate of HCC in patients with non-SVR was lower than that in those without IFN therapy. These results suggest that the use of IFN in patients with HCV-related liver disease may be beneficial in preventing the development of HCC. Several Japanese cohort studies have demonstrated that IFN therapy reduces the incidence of HCC, not only in sustained virological responders but also in transient responders who have failed to eliminate HCV [6, 41–45]. In cirrhotic patients, Nishiguchi et al. [39] reported that the relative risk of patients with IFN-alpha treatment developing HCC was 0.067 in comparison with the control group. In contrast, Valla et al. [46] could not prove any significant benefit for the prevention of HCC between patients with and without IFN treatment. Camma et al. [47] suggested a slight preventive effect of IFN on HCC development in patients with HCV-related cirrhosis. Shiffman et al. [48] have reported that continuous IFN therapy led to a decline in hepatic fibrosis despite the persistence of viremia. In addition, there are case reports that IFN therapy reduced AFP levels in virological nonresponders [49]. Murashima et al. [50] showed that IFN therapy, but not Strong Neo-Minophagen C (SNMC) (Glycyrrhizin, Tokyo, Japan), universally reduced basic AFP levels. In an in vitro study of the effects of IFN on an HCC cell line, IFN exhibited antitumor effects [51]. Taken together, these findings suggest that AFP levels may be useful for predicting the development of HCC during IFN-based treatments, including long-term low-dose IFN therapy.

There have been several reports on the relationship between chronological trends in platelet counts, AST or AFP levels, and the development of HCC [11, 26, 27, 52–54]. Tarao et al. [52, 53] showed that in patients with HCV-related cirrhosis, those with persistently high serum ALT levels had a high risk of developing HCC and multicentric carcinogenesis, whereas those with persistently low ALT levels faced a very low risk. Likewise, the dynamics of AFP levels in patients with chronic HCV infection may be useful to estimate the risk of developing HCC. Recently, Bruce et al. [32] found serial measurements of AFP helpful in identifying persons with advanced fibrosis. They used an AFP level of 8 ng/mL, the test manufacturer's upper limit of normal, as the evaluation of the risk of development of HCC. It is not certain whether or not AFP would be a risk factor of HCC development in patients with chronic liver disease of etiologies other than persistent HCV infection. Velazquez et al. [55] reported that an AFP level of >5 ng/mL at study entry was associated with the development of HCC in their univariate analysis but not in their multivariate analysis. They speculated that this could have been because the main causative factor of liver cirrhosis in their series was alcohol. Taken together, the findings of various studies suggest that the baseline AFP level may be more reliable as a predictive factor for the development of HCC in patients with HCV-related liver disease than in those with liver disease of other etiologies.

In conclusion, AFP is a noninvasive predictive marker for the development of HCC in patients infected with HCV. The present study indicates that not only high AFP levels (≥20 ng/mL) but also slightly elevated AFP levels, between 6 and 20 ng/mL, could indicate substantial risks for the development of HCC, complementing the fibrosis stage. In contrast, AFP levels of <6 ng/mL indicate a low risk of HCC development, irrespective of the liver fibrosis stage. IFN therapy significantly reduces the risk of the

development of HCC, especially in patients with an SVR to the therapy.

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References

1. Afdhal NH. The natural history of hepatitis C. Semin Liver Dis. 2004;24(Suppl 2):3–8.
2. Cohen J. Virology. Culture systems for hepatitis C virus in sight at last. Science. 2005;308:1539–41.
3. Sherman M. Hepatocellular carcinoma: epidemiology, risk factors, and screening. Semin Liver Dis. 2005;25:143–54.
4. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007;13:2436–41.
5. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132: 2557–76.
6. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med. 1999;131:174–81.
7. Liang TJ, Heller T. Pathogenesis of hepatitis C-associated hepatocellular carcinoma. Gastroenterology. 2004;127:S62–71.
8. Saadeh S, Cammell G, Carey WD, Younossi Z, Barnes D, Easley K. The role of liver biopsy in chronic hepatitis C. Hepatology. 2001;33:196–200.
9. Gebo KA, Herlong HF, Torbenson MS, Jenckes MW, Chander G, Ghanem KG, et al. Role of liver biopsy in management of chronic hepatitis C: a systematic review. Hepatology. 2002;36:S161–72.
10. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002;97:2614–8.
11. Yu ML, Lin SM, Lee CM, Dai CY, Chang WY, Chen SC, et al. A simple noninvasive index for predicting long-term outcome of chronic hepatitis C after interferon-based therapy. Hepatology. 2006;44:1086–97.
12. Ono E, Shiratori Y, Okudaira T, Imamura M, Teratani T, Kanai F, et al. Platelet count reflects stage of chronic hepatitis C. Hepatol Res. 1999;15:192–200.
13. Matsumura H, Moriyama M, Goto I, Tanaka N, Okubo H, Arakawa Y. Natural course of progression of liver fibrosis in Japanese patients with chronic liver disease type C—a study of 527 patients at one establishment. J Viral Hepat. 2000;7:268–75.
14. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol. 2001;96:3142–6.
15. Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, Guettier C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut. 2000;47:131–6.
16. Rodriguez-Diaz JL, Rosas-Camargo V, Vega-Vega O, Morales-Espinosa D, Mendez-Reguera A, Martinez-Tlahuel JL, et al. Clinical and pathological factors associated with the development of hepatocellular carcinoma in patients with hepatitis virus-related cirrhosis: a long-term follow-up study. Clin Oncol (R Coll Radiol). 2007;19:197–203.
17. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology. 1994;19:1513–20.
18. Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K, et al. Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol. 1999;37:1802–8.
19. Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, Kiyosawa K, et al. Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology. 2000;32:388–93.
20. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol. 1993;74(Pt 11):2391–9.
21. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol. 1997;35:201–7.
22. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. Cancer. 2000;89:53–9.
23. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:S35–50.
24. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology. 2004;127:S17–26.
25. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
26. Colombo M, de Franchis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. N Engl J Med. 1991;325:675–80.
27. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328:1797–801.
28. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology. 1994;19:61–6.
29. Ganne-Carrie N, Chastang C, Chapel F, Munz C, Pateron D, Sibony M, et al. Predictive score for the development of hepatocellular carcinoma and additional value of liver large cell dysplasia in Western patients with cirrhosis. Hepatology. 1996; 23:1112–8.
30. Sangiovanni A, Colombo E, Radaelli F, Bortoli A, Bovo G, Casiraghi MA, et al. Hepatocyte proliferation and risk of hepatocellular carcinoma in cirrhotic patients. Am J Gastroenterol. 2001;96:1575–80.
31. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Someya T, Hosaka T, et al. Prediction model of hepatocarcinogenesis for patients with hepatitis C virus-related cirrhosis. Validation with internal and external cohorts. J Hepatol. 2006;44:1089–97.
32. Bruce MG, Bruden D, McMahon BJ, Christensen C, Homan C, Sullivan D, et al. Clinical significance of elevated alpha-fetoprotein in Alaskan Native patients with chronic hepatitis C. J Viral Hepat. 2008;15:179–87.
33. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology. 1990;12:1420–32.
34. Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, et al. Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol. 2001;32:240–4.

35. Lu LG, Zeng MD, Wan MB, Li CZ, Mao YM, Li JQ, et al. Grading and staging of hepatic fibrosis, and its relationship with noninvasive diagnostic parameters. World J Gastroenterol. 2003;9:2574–8.
36. Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol. 2004;99:860–5.
37. Wilfredo Canchis P, Gonzalez SA, Isabel Fiel M, Chiriboga L, Yee H, Edlin BR, et al. Hepatocyte proliferation in chronic hepatitis C: correlation with degree of liver disease and serum alpha-fetoprotein. Liver Int. 2004;24:198–203.
38. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C trial. J Hepatol. 2005;43:434–41.
39. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346:1051–5.
40. Yu ML, Huang CF, Dai CY, Huang JF, Chuang WL. Long-term effects of interferon-based therapy for chronic hepatitis C. Oncology. 2007;72(Suppl 1):16–23.
41. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med. 1998;129:94–9.
42. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology. 1998;27:1394–402.
43. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology. 1999;29:1124–30.
44. Okanoue T, Itoh Y, Kirishima T, Daimon Y, Toyama T, Morita A, et al. Transient biochemical response in interferon therapy decreases the development of hepatocellular carcinoma for five years and improves the long-term survival of chronic hepatitis C patients. Hepatol Res. 2002;23:62–77.
45. Hino K, Okita K. Interferon therapy as chemoprevention of hepatocarcinogenesis in patients with chronic hepatitis C. J Antimicrob Chemother. 2004;53:19–22.
46. Valla DC, Chevallier M, Marcellin P, Payen JL, Trepo C, Fonck M, et al. Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. Hepatology. 1999;29:1870–5.
47. Camma C, Di Bona D, Craxi A. The impact of antiviral treatments on the course of chronic hepatitis C: an evidence-based approach. Curr Pharm Des. 2004;10:2123–30.
48. Shiffman ML, Hofmann CM, Contos MJ, Luketic VA, Sanyal AJ, Sterling RK, et al. A randomized, controlled trial of maintenance interferon therapy for patients with chronic hepatitis C virus and persistent viremia. Gastroenterology. 1999;117:1164–72.
49. Stein DF, Myaing M. Normalization of markedly elevated alpha-fetoprotein in a virologic nonresponder with HCV-related cirrhosis. Dig Dis Sci. 2002;47:2686–90.
50. Murashima S, Tanaka M, Haramaki M, Yutani S, Nakashima Y, Harada K, et al. A decrease in AFP level related to administration of interferon in patients with chronic hepatitis C and a high level of AFP. Dig Dis Sci. 2006;51:808–12.
51. Yano H, Iemura A, Haramaki M, Ogasawara S, Takayama A, Akiba J, et al. Interferon alfa receptor expression and growth inhibition by interferon alfa in human liver cancer cell lines. Hepatology. 1999;29:1708–17.
52. Tarao K, Rino Y, Ohkawa S, Shimizu A, Tamai S, Miyakawa K, et al. Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. Cancer. 1999;86:589–95.
53. Tarao K, Rino Y, Ohkawa S, Tamai S, Miyakawa K, Takakura H, et al. Close association between high serum alanine aminotransferase levels and multicentric hepatocarcinogenesis in patients with hepatitis C virus-associated cirrhosis. Cancer. 2002;94:1787–95.
54. Moriyama M, Matsumura H, Aoki H, Shimizu T, Nakai K, Saito T, et al. Long-term outcome, with monitoring of platelet counts, in patients with chronic hepatitis C and liver cirrhosis after interferon therapy. Intervirology. 2003;46:296–307.
55. Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology. 2003;37:520–7.

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G Funds Collection

The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area

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Summary

Background: The incidence of hepatocellular carcinoma (HCC) in Japan has still been increasing. The aim of the present study was to analyze the epidemiological trend of HCC in the western area of Japan, Kyushu.

Material/Methods: A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. Cohorts of patients with HCC were categorized into five year intervals. The etiology of HCC was categorized to four groups as follows; B: HBsAg positive, HCV-RNA negative, C: HCV-RNA positive, HBsAg negative, B+C: both of HBsAg and HCV-RNA positive, non-BC: both of HBsAg and HCV-RNA negative.

Results: B was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had C, and 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). The ratio of C cases decreased from 73.1% in 1996–2001 to 64.9% in 2002-2007. On the other hand, B and -nonBC cases increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively.

Conclusions: The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

key words: hepatitis virus • hepatocellular carcinoma • Japan

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Background

The three leading causes of death in Japan are malignancy neoplasms, cardiovascular diseases, and cerebrovascular diseases. Since 1981, malignant neoplasms have been the leading cause of death in Japan. For the last 30 years, liver cancer has been the third leading cause of death from malignant neoplasms in men. In women, liver cancer has ranked fifth during the past decade [1]. Hepatocellular carcinoma (HCC) accounts for 85% to 90% of primary liver cancers[2] and the age-adjusted HCC mortality rate has increased in recent decades in Japan [3]. Similarly, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [4,5]. HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, or nonalcoholic fatty liver disease. Of the hepatitis viruses which cause HCC, HCV is predominant in Japan [6–9].

Although the age-adjusted incidence of HCC has increased in Japan, sequential changes in etiology of HCC patients between 2001 and 2008 are not fully understood [10]. To clarify factors affecting epidemiological changes in Japanese HCC patients, especially the recent trend of HCC, we analyzed the epidemiological trend of HCC in the western area of Japan, Kyushu area.

Material and Methods

Patients

A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. The diagnosis of HCC was based on AFP levels and imaging techniques including ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI), hepatic angiography (HAG), and/or tumor biopsy. The diagnostic criteria for HCC were either a confirmative tumor biopsy or elevated AFP (>20 ng/mL) and neovascularization in HAG and/or CT.

Etiology of HCC

A diagnosis of chronic HCV infection was based on the presence of HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg). The etiology of HCC was categorized to four groups as follows; **B:** HBsAg positive, HCV-RNA negative, **C:** HCV-RNA positive, HBsAg negative, **B+C:** both of HBsAg and HCV-RNA positive, **nonBC:** both of HBsAg and HCV-RNA negative.

Statistical analysis

The data were analyzed by the Mann-Whitney test for the continuous ordinal data, the x2 test with Yates' correction and the Fisher exact test for the association between two qualitative variables. The standard deviation was calculated based on the binomial model for the response proportion. P<0.05 was considered statistically significant.

Results

Clinical features of the studied patients

A total of 10,010 patients with HCC were diagnosed at our study group from 1996 to 2008. Table 1 show that the proportion of patients diagnosed with B was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had C, and an additional 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. In analysis of patients in HCC by category, the median age of patients at diagnosis of **B** was 57 years old significant younger than other types HCC(**C: 69, nonBC: 70,.B+C 65 years old).**

As shown in Figures 1 and 2, the number and ratio of B cases remained unchanged from1996 to 2001 and thereafter increased and plateaued, whereas C rapidly increased from 1996 to 2000 and thereafter decreased and plateaued. In addition, the number and ratio of the **nonBC** cases has increased continued gradually and continued in this study period.

Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals

Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). Table 2 show that the incident rate of C decreased significantly from 73.1% in 1996-2001 to 64.9% in 2002-2007 (1996–2001 vs. 2002–2007, p=<0.001). On the other hand, the incident rate of **B** and **-nonBC** increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively. Not only the incident rate but also number of **B** and **-nonBC** became larger in same 6 years periods.

Table 3 shows that male/female ratio of **C** and **-nonBC** decreased significantly from 2.2 and 4.0 in 1996-2001 to 1.8 and 2.7 in 2002-2007, respectively (p<0.001). The ratio became clearly smaller, indicates an increase in female patients with **C** and **nonBC.** On the other hand, the male/female ratio of **B** patients did not significantly change during the period. The median age at diagnosis of **B, C,** and **nonBC** in six-year intervals were significant increase from 56 to 58, from 67 to 71 and from 68 to 71 years of age during the period.

Discussion

Our study was the twenty-three major liver center-based study designed to examine the sequential change in the background of HCC patients during the past 13 years, 1996–2008. More than 80% of our patients had chronic HBV or HCV infections. During this observation period, the number and proportion of HCC-C reached a peak in 2000 and thereafter decreased and became stabilized. Previous studies from Japan reported that the proportion of the HCC patients with HCV infection had been increased and reached a plateau in the period of 1981–2001 [1,3,10–12]. However, in our study, the number and proportion of the HCC patients with HCV infection cases decreased in 2001–2008. The reason may be explained as follows; interferon therapy for chronic hepatitis C may have been associated with a decreased incidence of HCC [13–17]. Oral supplementation with a oral branched-chain amino acids has been useful in the prevention HCC [18]. Finally, the chronically HCV-infected

Table 1. The characteristic of HCC patients during the period of 1996–2008.

Age (y.o.)	B		C		nonB		B+C		Total
	Male	Female	Male	Female	Male	Female	Male	Female	
0–	1	0	0	1	0	0	0	0	2
10–	4	1	0	0	0	2	0	0	7
20–	6	2	1	0	1	1	0	0	11
30–	31	5	4	0	11	3	2	0	56
40–	204	22	130	12	32	15	12	0	427
50–	507	66	728	145	167	32	31	6	1,682
60–	287	118	1836	741	411	102	35	13	3,543
70–	140	64	1775	947	483	133	22	14	3,578
80–	9	18	271	214	97	65	1	4	679
90–	0	0	9	5	9	2	0	0	58
Total	1,189	296	4,754	2,065	1,211	355	103	37	10,010
	1,485 (4.8%)		6,819 (68.1%)		1,566 (15.6%)		140 (1.4%)		
Median	57	63	67	70	68	70	61	68	67
	57		69		70		65		
Mean	56	64	68	71	69	71	62	68	67
	58		68		68		63		
Range	1–87	14–89	27–94	0–93	28–96	17–90	36–82	55–82	0–96
	1–89		0–94		17–96		36–82		

Age: B vs. C p≤0.001; B vs. B+C p≤0.001; B vs. nonBC p≤0.001; C vs. BC p≤0.001; C vs. nonBC p=0.043; BC vs. nonB+C p≤0.001. IQR – interquartile range; SD – standard deviation.

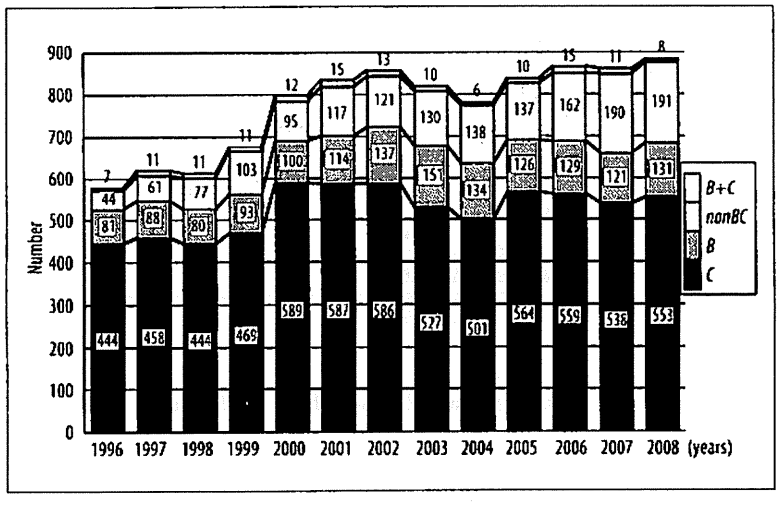

Figure 1. Sequential changes in the number of HCC patients categorized by etiology during the period 1996–2008.

population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence for the presence of HCVAb among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest proportion of 7% in individuals who were more than 70 years old [10,19]. In this study, the median age of the HCC patients with HCV infection steadily increased from 67 to 71 years of age during the studied period. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC.

The prevalence rate of HBV in Kyushu area has been reported to be higher than other area in Japan [1]. In Kyushu area, 95% of patients with chronic HBV infection had HBV genotype C except for Okinawa [20]. HBV genotype C is thought to be associated with higher incidence of HCC

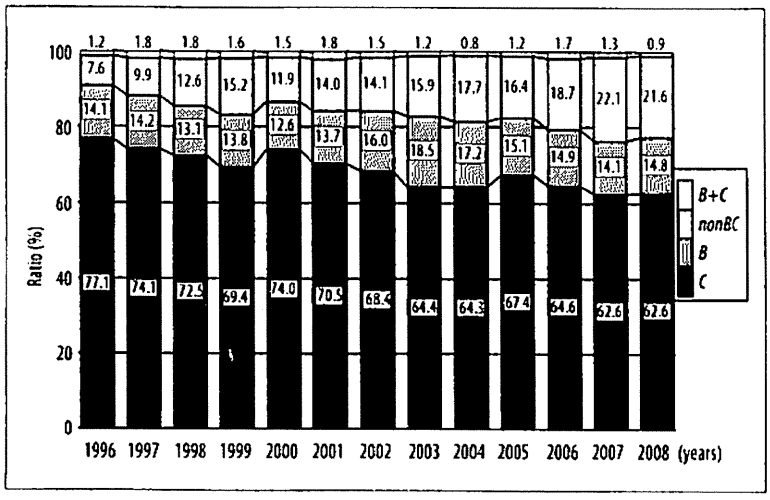

Figure 2. Sequential changes in the ratio of HCC patients categorized by etiology during the period 1996–2008.

Table 2. Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals.

Period		1996–2001	2002–2007	P value
Number		3,023	4,173	
Sex	Male	2,162	2,849	
	Female	861	1,324	
Ratio (male/female)		2.5	2.2	0.003
Age (y.o.) (IQR)		66 (14)	69 (12)	<0.001
Hepatitis virus (%)	B	13.9	16.2	
	C	73.1	64.9	
	B+C	1.7	1.3	
	nonBC	11.3	17.6	0.001

QR – interquartile range.

Table 3. The median age and male/female ratio of HCC patients during the period of 1996–2007.

Period		1996–2001	2002–2007	P value
B				
Age (y.o.) (IQR)		56 (14)	58 (15)	0.001
Sex	Male	331	519	
	Female	88	157	
Ratio (male/female)		3.8	3.3	0.391
C				
Age (y.o.) (IQR)		67 (9)	71 (11)	<0.001
Sex	Male	1,524	1,753	
	Female	687	955	
Ratio (male/female)		2.2	1.8	0.002
nonBC				
Age (y.o.) (IQR)		68 (12)	71 (13)	<0.001
Sex	Male	273	534	
	Female	69	201	
Ratio (male/female)		4.0	2.7	0.012

QR – interquartile range.

compared with other HBV genotypes [21]. In the present study, the incident rate of HCC patients with HBV infection became larger in this study period. To explain this change, we must consider from two viewpoints. The one is that the number of patients with HCC caused by HCV infection decreased, the other is that the proportion of chronic HBV infected patients who have reached the age of developing HCC is relatively high as described below.

Nationwide health survey for HBsAg in the over 40 years of age population had been done between 2002 and 2006 in Japan. This survey reports indicated that the average HBsAg prevalence was 1.2% in the total Japanese population patients with chronic HBV infection [10] and the age-specific prevalence of HBsAg was higher in the group aged between 50 (1.4%) and 55 years (1.5%). In the HCC patients with HBV genotype C, the mean age was 55 years in Japan [20]. This overlap between age-specific prevalence and hepatocellular carcinogenic age would be associated with the increase of HCC patients with HBV infection. Nucleoside analogue reverse transcriptase inhibitor (NARTI) therapy effectively reduces the incidence of HCC in chronic hepatitis B patients [22,23]. However, Interferon therapy for chronic hepatitis C started from 1992, whereas NARTI therapy for HBV started from 2000 in Japan [24,25]. Hence, HBV associated HCC will probably decrease in Japan during the next 10 to 20 years.

The survey of HCC patients associated with nonBC infection in Japan was conducted by Inuyama Hepatitis Research Group from 1995 to 2003. The ratio of HCC patients with nonBC accounted 9.3% [1]. In the present study, the ratio of HCC patients with nonBC was 14.1%. Furthermore, the number and the proportion of HCC patients with nonBC have been gradually increasing in the periods. The current two studies account for the increase in number and proportion of HCC patients with nonBC. First, Lai et al. reported

that type 2 diabetes increases the risk of developing HCC in those who are HCV negative or have a high level of total cholesterol [26]. Second, Nakano et al. reported that epidemiological studies on diabetes mellitus revealed that the number of patients with diabetes mellitus is gradually increasing in Japan along with development of car society and westernization of food intake. Since prevalence of diabetes mellitus increases with aging, proportion of individuals with diabetes mellitus aged over 60 has exceeded two-third of estimated total number of patients (7.40 million in 2002) in Japan where aging of society is rapidly progressing [27]. In a word, the number of type 2 diabetes people is increasing in Japan and they were regarded as a high risk for HCC. Then, the number and the proportion of HCC patients with nonBC have been increased recent twelve years in Japan.

It is known that 2 to 4 decades of chronic HCV infection are required to develop cirrhosis and subsequent HCC [28–31]. The number of HCC cases has increased in Japan, because individuals infected with HCV during the past have grown old and have reached the cancer-bearing age. The prevalence of HCV infection in young Japanese individuals is low and the incidence of HCVAb is very low because of preventative actions against HCV infection such as the screening of blood products for HCV and the use of sterile medical equipment [32]. Additionally, we showed that the number and proportion of patients with HCC-C cases decreased, whereas the number and ratio of HCC-nonBC steadily increased during the studied period. These findings may be expected that the incidence of HCC patients with nonBC in Japan may continue to increase even after the consequence of the HCV epidemic level off, a country that is far advanced with regard to HCC patients with HCV infection, in the near future.

Conclusions

In summary, HCC patients had increased from 1996 to 2000 and this increase was originated from HCC patients with HCV infection. The number and proportion of HCC patients with HCV infection reached a peak in 2000 and thereafter decreased and became stabilized. The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

References:

1. Umemura T, Kiyosawa K: Epidemiology of hepatocellular carcinoma in Japan. Hepatol Res, 2007; 37(Suppl.2): S95–100
2. El-Serag HB, Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology, 2007; 132: 2557–76
3. Kiyosawa K, Tanaka E: Characteristics of hepatocellular carcinoma in Japan. Oncology, 2002; 62: 5–7
4. McGlynn KA, Tsao L, Hsing AW et al: International trends and patterns of primary liver cancer. Int J Cancer, 2001; 94: 290–96
5. Bosch FX, Ribes J, Diaz M, Cleries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology, 2004; 127: S5–16
6. Hamasaki K, Nakata K, Tsutsumi T et al: Changes in the prevalence of hepatitis B and C infection in patients with hepatocellular carcinoma in the Nagasaki Prefecture, Japan. J Med Virol, 1993; 40: 146–49
7. Kato Y, Nakata K, Omagari K et al: Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Analysis of infectious hepatitis viruses. Cancer, 1994; 74: 2234–38
8. Shiratori Y, Shiina S, Imamura M et al: Characteristic difference of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan. Hepatology, 1995; 22: 1027–33
9. Shiratori Y, Shiina S, Zhang PY et al: Does dual infection by hepatitis B and C viruses play an important role in the pathogenesis of hepatocellular carcinoma in Japan? Cancer, 1997; 80: 2060–67
10. Kiyosawa K, Umemura T, Ichijo T et al: Hepatocellular carcinoma: recent trends in Japan. Gastroenterology, 2004; 127: S17–26
11. Taura N, Yatsuhashi H, Hamasaki K et al: Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: Long term trends in Japan. Hepatol Res, 2006; 34: 130–34
12. Taura N, Hamasaki K, Nakao K et al: Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. Oncol Rep, 2006; 16: 837–43
13. Nishiguchi S, Kuroki T, Nakatani S et al: Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet, 1995; 346: 1051–55
14. Nishiguchi S, Shiomi S, Nakatani S et al: Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. Lancet, 2001; 357: 196–97
15. Kasahara A, Hayashi N, Mochizuki K et al: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology, 1998; 27: 1394–402
16. Ikeda K, Saitoh S, Arase Y et al: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology, 1999; 29: 1124–30
17. Makiyama A, Itoh Y, Kasahara A et al: Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. Cancer, 2004; 101: 1616–22
18. Muto Y, Sato S, Watanabe A et al: Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol, 2005; 3: 705–13
19. Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology, 2002; 62: 8–17
20. Orito E, Mizokami M: Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Intervirology, 2003; 46: 408–12
21. Orito E, Mizokami M: Differences of HBV genotypes and hepatocellular carcinoma in Asian countries. Hepatol Res, 2007; 37: S33–35
22. Matsumoto A, Tanaka E, Rokuhara A et al: Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic. Hepatol Res, 2005; 32(3): 173–84
23. Xu J, Shi J, Wang YP et al: Milder liver cirrhosis and loss of serum HBeAg do not imply lower risk for. Med Sci Monit, 2009; 15(6): CR274–79
24. Hayashi N, Takehara T: Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol, 2006; 41(1): 17–27
25. Kumashiro R, Kuwahara R, Ide T et al: Subclones of drug-resistant hepatitis B virus mutants and the outcome of breakthrough hepatitis in patients treated with lamivudine. Intervirology, 2003; 46(6): 350–54
26. Lai MS, Hsieh MS, Chiu YH, Chen TH: Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. Hepatology, 2006; 43: 1295–302
27. Nakano T, Ito H: Epidemiology of diabetes mellitus in old age in Japan. Diabetes Res Clin Pract, 2007; 77(Suppl.1): S76–81
28. Deuffic S, Poynard T, Valleron AJ: Correlation between hepatitis C virus prevalence and hepatocellular carcinoma mortality in Europe. J Viral Hepat, 1999; 6: 411–13
29. El-Serag HB, Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med, 1999; 340: 745–50
30. Planas R, Balleste B, Antonio Alvarez M et al: Natural history of decompensated hepatitis C virus-related cirrhosis. A study of 200 patients. J Hepatol, 2004; 40: 823–30
31. Davila JA, Morgan RO, Shaib Y et al: Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology, 2004; 127: 1372–80
32. Sasaki F, Tanaka J, Moriya T et al: Very low incidence rates of community-acquired hepatitis C virus infection in company employees, long-term inpatients, and blood donors in Japan. J Epidemiol, 1996; 6: 198–203

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Original Article

Hepatic arterial infusion chemotherapy using 5-fluorouracil and systemic interferon-α for advanced hepatocellular carcinoma in combination with or without three-dimensional conformal radiotherapy to venous tumor thrombosis in hepatic vein or inferior vena cava

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Aim: We investigated the efficacy of hepatic arterial infusion chemotherapy (HAIC) using 5-fluorouracil (5-FU) and systemic interferon (IFN)-α (HAIC-5-FU/IFN) for advanced hepatocellular carcinoma (HCC) with venous tumor thrombosis (VTT) in the hepatic vein trunk (Vv2) or inferior vena cava (Vv3).

Methods: Thirty-three patients with HCC/Vv2/3 underwent HAIC with 5-FU (500 mg/body weight/day, into hepatic artery on days 1–5 on the first and second weeks) and IFN-α (recombinant IFN-α-2b 3 000 000 U or natural IFN-α 5 000 000 U, intramuscularly on days 1, 3 and 5 of each week). Three-dimensional conformal radiotherapy (3D-CRT) was used in combination with HAIC-5-FU/IFN in 14 of 33 patients to reduce VTT.

Result: The median survival time (MST) was 7.9 months, and 1- and 2-year survival rates were 30% and 20%, respectively. Evaluation of intrahepatic response after two cycles of HAIC-5-FU/IFN showed complete response (CR) in three (9%) and partial response (PR) in seven (21%), with an objective response rate of 30%. Multivariate analysis identified reduction of VTT ($P = 0.0006$), size of largest tumor ($P = 0.013$) and intrahepatic response CR/PR ($P = 0.030$) as determinants of survival. CR/PR correlated significantly with tumor liver occupying rate ($P = 0.016$) and hepatitis C virus Ab ($P = 0.010$). Reduction of VTT correlated significantly with radiotherapy ($P = 0.021$) and platelet count ($P = 0.015$). Radiotherapy-related reduction in VTT significantly improved survival of 16 patients with Vv3 and non-CR/PR response of HAIC-5-FU/IFN ($P = 0.028$).

Conclusion: As for advanced HCC with VTT of Vv2/3, HAIC-5-FU/IFN responsive patients could obtain favorable survival. Despite ineffective HAIC-5-FU/IFN, the combination with effective radiotherapy to VTT might improve patients' prognosis.

Key words: 5-fluorouracil, hepatocellular carcinoma, interferon, radiotherapy, venous tumor thrombosis

INTRODUCTION

THE PROGNOSIS OF patients with advanced hepatocellular carcinoma (HCC) remains poor,[1–3] although that of patients with HCC has gradually improved following the development of new diagnostic techniques and advancements in therapeutic modalities, such as surgical resection, radiofrequency ablation, percutaneous ethanol injection, transcatheter arterial chemoembolization (TACE), radiotherapy and hepatic arterial infusion chemotherapy (HAIC).[4–8] Recent advances in implantable drug delivery systems have facilitated repeated arterial infusions of anti-cancer agents to tumors in the corresponding arterial perfusion area. HAIC is considered suitable for HCC patients with poor hepatic reserve due to high drug concentrations in

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local tissue and late rates of adverse effects of anti-cancer agents. Among several anti-cancer agents, intra-arterial 5-fluorouracil (5-FU) and systemic interferon (IFN) have been reported as one of the most effective combination chemotherapies for HCC with portal vein tumor thrombus (PVTT).[9–13] Vascular invasion of HCC with PVTT, venous tumor thrombosis (VTT) and biliary thrombosis in the liver, represent the worst prognostic factors in patients with advanced HCC, especially PVTT.[14–19] On the other hand, VTT is less commonly recognized poor prognostic factor than PVTT. To define the therapeutic benefits of HAIC-5-FU/IFN for HCC with VTT in the hepatic vein trunk (Vv2), or inferior vena cava (Vv3), we retrospectively analyzed the treatment response, survival time and prognostic factors.

Three-dimensional conformal radiotherapy (3D-CRT) allows the delivery of higher radiation doses to tumors and low radiation dose to normal tissue. 3D-CRT improves the anti-tumor effect of radiotherapy and minimizes damage to normal tissue. This modality is probably suited as local radiotherapy for PVTT in patients with poor hepatic reserves.[20,21] The synergistic effects of the combination of chemotherapy and radiotherapy have been reported in various malignancies such as lung cancer and esophageal cancer.[22–25] Recently, Han *et al.*[26] reported a response rate of 45% in HCC patients with PVTT treated by HAIC with 5-FU/cisplatin and 3D-CRT. Furthermore, Katamura *et al.*[27] reported the efficacy of intra-arterial 5-FU/IFN combined with 3D-CRT for PVTT. To our knowledge, there are no studies on the therapeutic efficacy of radiotherapy for HCC with VTT. In addition, it is still unclear whether radiotherapy has any additional effects on HAIC-5-FU/IFN. Based on the above results, we retrospectively analyzed and compared differences in the clinical course and outcome of HCC patients treated by HAIC-5-FU/IFN with or without 3D-CRT.

METHODS

Study design and eligibility

THE FOLLOWING ENROLLMENT criteria were applied in the study: (i) HCC with VTT in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein (Vv2), or inferior vena cava (Vv3); (ii) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or PS 1; (iii) Child–Pugh stage A or B; (iv) serum total bilirubin <3.0 mg/dL; (v) leukocyte count >2000/mm^3; (vi) platelet count >50 000/mm^3; (vii) serum creatinine <1.5 mg/dL; (viii) at least a 4-week rest period of no treatment since any previous treatment for HCC; (ix) the initial administration of HAIC for HCC; and (x) no other serious medical condition that would interfere with HAIC. The presence of extrahepatic metastases was not an exclusion criterion when they were not considered prognostic factors. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Review Board of Hiroshima University. Written informed consent was obtained from each patient after detailed explanation about the therapy. From March 2004 to November 2010, 33 patients met the above criteria for HAIC-5-FU/IFN. The baseline characteristics of these patients are summarized in Table 1.

Treatment protocol

The patients received repeated arterial infusion chemotherapy via drug delivery systems implanted in the subcutaneous inguinal region. The arterial catheter was implanted using the method described previously by our group.[9] One course of chemotherapy represented 2 weeks. 5-FU (500 mg/body weight/day; Kyowa Hakko, Tokyo) was administered using a mechanical infusion pump from day 1 to day 5 on the first and second weeks. Recombinant IFN-α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan) at 3 000 000 U (3 MU) or natural IFN-α (OIF, Otsuka Pharmaceuticals, Tokyo) at 5 000 000 U (5 MU), was administered intramuscularly on days 1, 3 and 5 of each week (total dose: 18 and 30 MU, respectively). As for the two types of IFN, similar effects were reported previously between recombinant IFN-α-2b and natural IFN-α when combined with intra-arterial 5-FU for the treatment of advanced HCC.[10] After each treatment course, 2–4 weeks of rest/no treatment period was enforced. HAIC-5-FU/IFN was repeated several times during the treatment as much as possible, until we considered that it was impossible to continue further HAIC-5-FU/IFN based on the following criteria: (i) PS changed to 3 or 4; (ii) adverse events were estimated as grade 4 by Common Technology Criteria for Adverse Events (CTCAE) version 4.0; (iii) patients were evaluated clinically to have progressive disease; and (iv) patient requested termination of treatment. Fourteen out of 33 patients received 3D-CRT to VTT to control VTT progression. 3D-CRT was applied to objective progressive VTT of Vv2/3, which was shown in dynamic computed tomography (CT) before or during two courses of HAIC-5-FU/IFN. From March 2004 to July 2006, when the decision to introduce 3D-CRT was clinically left to the

Table 1 Clinical characteristics of 33 patients with hepatocellular carcinoma (HCC) and venous tumor thrombus (VTT)

Clinical characteristics	Category	
Sex	Male/female	30/3
Age	<65 years/≥65 years	15/18
ECOG PS	0/1	23/10
HCV Ab	+/−	16/17
HBs Ag	+/−	7/26
Child–Pugh stage	A/B	25/8
Previous treatment	Yes/no	9/24
α-fetoprotein (ng/mL)	<5 000/≥5 000	17/16
des-γ-carboxy prothrombin (mAU/mL)	<10 000/≥10 000	14/19
Platelet count (/mm^3)	<150 000/≥150 000	19/14
Size of largest tumor (mm)	<100/≥100	16/17
Tumor liver occupying rate (%)	<50/≥50	20/13
Tumor stage†	IVA/IVB	19/14
Grade of venous invasion‡	Vv 2/3	13/20
Grade of portal invasion§	Vp 0/1/2/3/4	7/1/4/8/13
Extrahepatic metastasis	Yes/no	16/17
Radiotherapy to venous tumor thrombus	Yes/no	14/19

†According to the Liver Cancer Group of Japan.
‡Venous invasion. Vv1, tumor thrombus in peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.
§Portal invasion. Vp1, tumor thrombus in a third or more peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.
ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBs Ag, hepatitis B virus surface antigen; HCV Ab, hepatitis C virus antibody.

attending physician, one patient of Vv2 and five patients of Vv3 were enrolled. Since August 2006, the indication of 3D-CRT was limited to objective progressive VTT of Vv3 in principle, eight patients of Vv3 were enrolled. Patients received 3D-CRT in the Division of Radiation Oncology at our hospital. They received high-energy photon beam irradiation using 18, 10 or 6 MV, delivered by a three-dimensional conformal technique (CLINAC 2300 C/D or CLINACiX linear accelerators, Varian Medical Systems Inc., Palo Alto, CA, USA). The planning CT determined the gross tumor volume (GTV) representing only the VTT. The clinical target volume (CTV) was also determined; which included GTV and intrahepatic tumor forming the basal part of VTT. The planning target volume (PTV) represented the CTV plus a 10–20-mm margin in all directions for internal motion and set-up error. Four to five portal fields were used. The outlined target volumes, total liver tissue and organs at risk, including the spinal cord, bilateral kidneys, esophagus, stomach and other nearby intestinal tract targets, were transferred to the treatment planning system (Pinnacle 3, Philips Medical Systems, Eindhoven, The Netherlands) with reference to the diagnostic enhanced CT images. The prescribed dose was 30, 39 or 45 Gy, based on the dose-volume constraint of normal tissues and liver function. Using this protocol, it was estimated that 95% of the PTV should receive at least 95% of the prescribed dose, 50% of the liver tissue should not receive more than 25 Gy, 50% of each kidney not more than 20 Gy and that the maximum dose to the spinal cord, intestinal tract and esophagus was not more than 40 Gy. Finally, five patients received a total dose of 30 Gy, five patients 39 Gy and four patients 45 Gy, in daily doses of 3 Gy per fraction.

Evaluation

Every patient underwent dynamic CT before and after two courses of HAIC-5-FU/IFN, and the therapeutic effect was classified according to Response Evaluation Criteria in Solid Tumor (RECIST) version 1.1[28] after completion of two cycles of the chemotherapy. A complete response (CR) was defined as disappearance of all target/non-target lesions, no appearance of any other lesion within 4 weeks, and normalization of α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP). CR was confirmed at 4 weeks after the first evalu-

ation of CR. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of target lesions with the baseline sum of the longest diameter of target lesions as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor PD criteria. We also evaluated the treatment effect of VTT by measuring the longest diameter to increase or decrease, the response of intrahepatic tumor to the therapy and overall systemic response. Adverse reactions were assessed every week during the treatment using the CTCAE. Radiotherapy-induced liver disease (RILD) exhibited the following criteria:[29] development of anicteric elevation of alkaline phosphatase level of at least twofold, nonmalignant ascites in the absence of documented progressive disease and increased transaminases levels of at least fivefold the upper limit of normal or of pretreatment level.

Statistical analysis

Data were analyzed statistically on 1 March 2011. Differences between background factors were examined for statistical significance using logistic regression test and Pearson's χ^2 test where appropriate. Consecutive data (e.g. α-fetoprotein) was classified by each median value referring to scatter diagram or histogram. Univariate analysis of predictors of survival was assessed by the cumulative survival rate, which was calculated from the initial date of HAIC-5-FU/IFN and assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Variables that achieved statistical significance ($P < 0.05$) or those with P-values of less than 0.10 on univariate analysis were entered into multivariate analysis. Multivariate analysis of predictors of survival was assessed by Cox proportional hazard model or Logistic regression analysis. All analyses were performed using the Statistical Package for Social Sciences (version 11, SPSS Inc., Chicago, IL). We assessed the survival benefits and safety of HAIC-5-FU/IFN combined with or without 3D-CRT to VTT.

RESULTS

Overall survival and response

FIGURE 1 SHOWS the cumulative survival rate of 33 patients who underwent HAIC using 5-FU/IFN. The median survival time (MST) was 7.9 months. The 1- and 2-year survival rates were 30% and 20%, respectively. The median observation time was 5.5 months (range, 0.7 to 54.9 months). Table 2 shows the response to therapy as evaluated by RECIST. In five patients (shown as NE), CT could not be performed after the HAIC. The systemic (i.e., whole body) response to the treatment was: CR in three cases, PR in five, SD in four and PD in 16, with an overall systemic response rate of 24%. We also defined intrahepatic (i.e., limited to the liver) response as one of the treatment factors in order to evaluate localized therapeutic effects in the liver. The intrahepatic response was: CR in three, PR in seven, SD in six and PD in 12, with an overall intrahepatic response rate of 30%, similar to the systemic response rate.

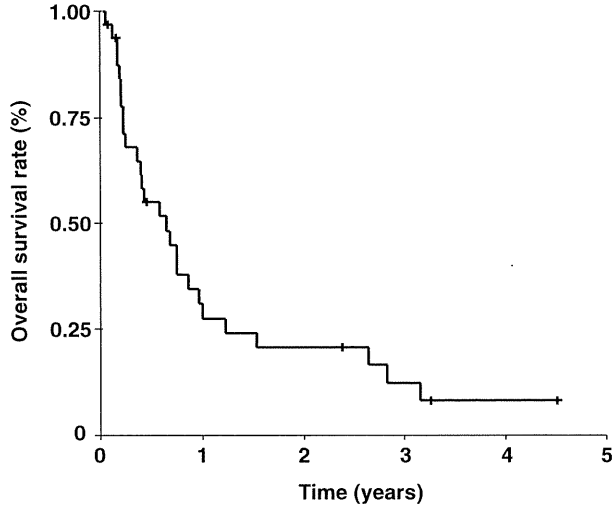

Figure 1 Overall survival of 33 patients with venous tumor thrombosis (VTT) treated by hepatic arterial infusion chemotherapy (HAIC). The median survival time (MST) was 7.9 months, and the 1- and 2-year survival rate were 30% and 20%, respectively. The median observation time was 5.5 months (range, 0.7–54.9 months).

Univariate analysis (Table 3) and multivariate analysis (Table 4) identified three factors that contributed to overall survival; treatment-related reduction in VTT

Table 2 Clinical response to the therapy to hepatocellular carcinoma (HCC) with venous tumor thrombus (VTT)

	CR	PR	SD	PD	NE	RR
Systemic evaluation of response: whole body	3	5	4	16	5	24%
Intrahepatic response: liver only	3	7	6	12	5	30%

CR, complete response; NE, not evaluable; PD, progressive disease; PR, partial response; RR, response rate for patients with CR and PR per entire group of patients; SD, stable disease.

Table 3 Univariate analysis of factors that contributed to overall survival (Log rank test)

	Category	*n*	*P*-value
Sex	Female vs. male	3/30	0.567
Age (years)	≥65 vs. <65	18/15	0.326
ECOG PS	0 vs. 1	23/10	0.324
HCV Ab	Presence vs. absence	16/17	0.215
HBs Ag	Absence vs. presence	26/7	0.023
Child–Pugh stage	A vs. B	25/8	0.004
Previous treatment	No vs. yes	24/9	0.414
α-fetoprotein (ng/mL)	<5 000 vs. ≥5 000	17/16	0.559
des-γ-carboxy prothrombin (mAU/mL)	<10 000 vs. ≥10 000	14/19	0.309
Platelet count (/mm^3)	<150 000 vs. ≥150 000	19/14	0.0008
Size of largest tumor (mm)	<100 vs. ≥100	16/17	0.0003
Tumor liver occupying rate (%)	<50 vs. ≥50	20/13	0.0013
Grade of venous invasion (Vv)†	2 vs. 3	13/20	0.274
Grade of portal invasion (Vp)‡	0, 1, 2 vs. 3, 4	12/21	0.224
Extrahepatic metastasis	No vs. yes	17/16	0.040
Radiotherapy to venous tumor thrombus	Yes vs. no	14/19	0.667
Intrahepatic response	CR, PR vs. Other	10/23	0.0029
Effect of treatment on venous tumor thrombus	Decrease vs. increase	18/15	0.0001

†Venous invasion. Vv1, tumor thrombus in a second branch or more of peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

‡Portal invasion. Vp1, tumor thrombus in a third or more peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.

ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBs Ag, hepatitis B virus surface antigen; HCV Ab, hepatitis C virus antibody.

($P = 0.0006$, hazard ratio, HR, 6.611, 95% confidence interval [CI] 2.262–19.322), largest tumor size <100 mm ($P = 0.013$, HR 3.896, 95%CI 1.328–11.432), and intrahepatic response of complete response/partial response (CR/PR) ($P = 0.030$, HR 2.968, 95%CI 1.108–7.951). Patients classified as CR/PR based on intrahepatic response had a significantly longer MST than the non-CR/PR group (18.7 vs. 4.4 months, respectively, $P = 0.0029$, log rank test) (Fig. 2). Univariate analysis (Table 5) and multivariate analysis (Table 6) identified two factors that contributed to intrahepatic response of CR/PR; tumor liver occupying rate of >50% ($P = 0.016$, OR 23.239, 95%CI 1.791–301.508) and positivity for hepatitis C virus antibody (HCV Ab) ($P = 0.010$, OR 16.886, 95%CI 1.969–144.774).

Effect of radiotherapy

The clinical characteristics of patients treated by HAIC-5-FU/IFN with and without radiotherapy to VTT are summarized in Table 7. Patients treated with radiotherapy had a tendency to be elderly, hepatitis B virus

Table 4 Multivariate analysis for factors that contribute to overall survival, Cox proportional hazards model with stepwise selection

	Category	HR	95% CI	*P*-value
Effect of treatment of venous tumor thrombus	Decrease	6.611	2.262–19.322	0.0006
	Increase	1		
Size of largest tumor (mm)	<100	3.896	1.328–11.432	0.013
	≥100	1		
Intrahepatic response	CR, PR	2.968	1.108–7.951	0.030
	Other	1		

95% CI, 95% confidence interval; CR, complete response; HR, Hazard ratio, PR, partial response.

(HBV) negative and HCV positive. About two-thirds of patients with VTT of Vv3 received 3D-CRT. Figure 3 shows the overall response to treatment of all patients classified according to the application of 3D-CRT. In patients who received HAIC alone, five out of 19 patients were classified as CR/PR based on intrahepatic response, with a response rate of 26%. Furthermore, VTT was considered to have decreased in seven out of 19 patients with a VTT-treatment effective rate of 37%. For patients who received HAIC combined with radiotherapy, CR/PR was achieved in five out of 14 patients, with a response rate of 36%, and VTT decreased in 11 out of 14 patients, with a treatment effective rate of up to 79%. Radiotherapy had no significant effect on the intrahepatic response ($P = 0.561$, Pearson's χ^2 test). The combination of HAIC and radiotherapy had a significant effect on VTT ($P = 0.017$, Pearson's χ^2 test). Table 8 shows the results of univariate analysis for factors that contributed to the effect of treatment on VTT. Multivariate analysis (Table 9) two factors that significantly and independently influenced the VTT; platelet count less than 150 000/mm^3 ($P = 0.015$, OR 16.087, 95%CI 1.704–151.861) and response of VTT to radiotherapy ($P = 0.021$, OR 14.982, 95%CI 1.508–148.827). Figure 4 shows the cumulative survival rates of 16 patients with VTT in the inferior vena cava (Vv3), based on the VTT response to 3D-CRT. The nine patients who received HAIC-5-FU/IFN and 3D-CRT to VTT and showed a decrease in VTT had a significantly longer MST of 9.2 months ($P = 0.028$, log rank test), compared with the seven patients who received HAIC-5-FU/IFN without or with ineffective 3D-CRT (these patients showed increases in VTT and MST of 3.1 months).

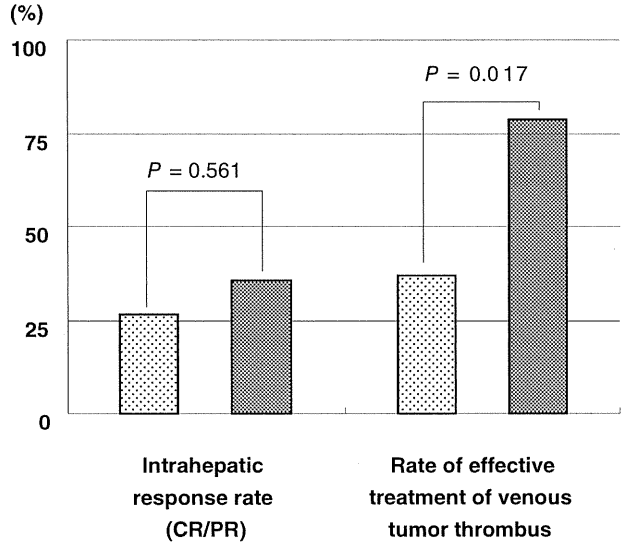

Figure 3 Overall treatment response according to the treatment regimen presence or absence of undergoing radiotherapy to venous tumor thrombus (VTT). ▨, HAIC alone; ▩, HAIC plus 3-D conformal radiotherapy (3D-CRT). HAIC, hepatic arterial infusion chemotherapy.

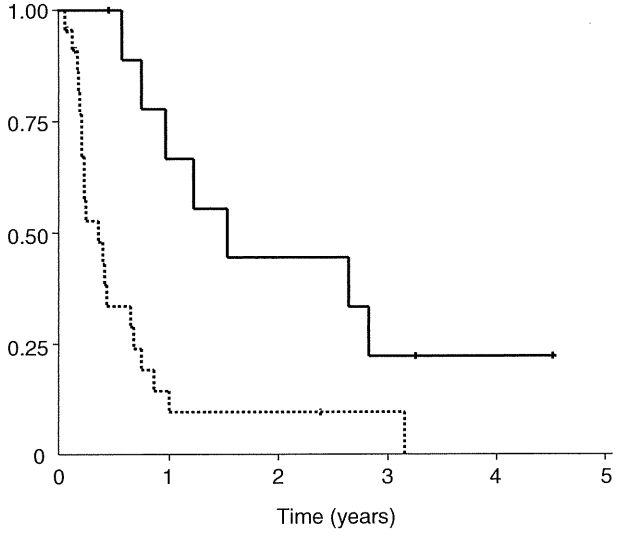

Figure 2 Cumulative survival rate of 33 patients with venous tumor thrombosis (VTT) treated by hepatic arterial infusion chemotherapy (HAIC). Solid line: 10 patients, who underwent HAIC and classified as complete response/partial response (CR/PR) based on intrahepatic response, had a significantly longer median survival time (MST) of 18.7 months ($P = 0.0029$, log rank test). Dashed line: 23 patients who were classified as non-CR/PR based on intrahepatic response resulting in MST of 4.4 months. ——, CR/PR of intrahepatic response; ·····, Non-CR/PR of intrahepatic response.

Incidence of extrahepatic metastasis

Figure 5 shows the cumulative rate of extrahepatic metastases in 17 patients who were negative for extrahepatic metastasis before HAIC-5-FU/IFN. Eight (47%) patients developed extrahepatic metastases after starting HAIC-5-FU/IFN, including seven with lung metastases and one with adrenal gland metastasis. The median time to the diagnosis of metastasis was 7.1 months. The 6- and 12-month cumulative incidence rates were 30% and 56%, respectively. The median survival time was 4.4 months after the diagnosis of extrahepatic metastasis.

Other anti-cancer treatments

Nine out of 33 (27%) patients received additional courses of HAIC with 5-FU/IFN after completing the two

Table 5 Univariate analysis for factors that contribute to intrahepatic response after two cycles of hepatic arterial infusion chemotherapy (HAIC) treatment, Pearson's χ^2 test

	Category	*n*	*P*-value
Sex	Female vs. male	3/30	0.905
Age (years)	≥65 vs. <65	18/15	0.103
ECOG PS	0 vs. 1	23/10	0.980
HCV Ab	Presence vs. absence	16/17	0.017
HBs Ag	Absence vs. presence	26/7	0.299
Child–Pugh stage	A vs. B	25/8	0.208
Previous treatment	No vs. yes	24/9	0.061
α-fetoprotein (ng/mL)	<5 000 vs. ≥5 000	17/16	0.520
des-γ-carboxy prothrombin (mAU/mL)	<10 000 vs. ≥10 000	14/19	0.561
Platelet count (/mm^3)	<150 000 vs. ≥150 000	19/14	0.086
Size of largest tumor (mm)	<100 vs. ≥100	16/17	0.103
Tumor liver occupying rate (%)	<50 vs. ≥50	20/13	0.023
Grade of venous invasion (Vv)†	2 vs. 3	13/20	0.020
Grade of portal invasion (Vp)‡	0, 1, 2 vs. 3, 4	12/21	0.963
Extrahepatic metastasis	No vs. yes	17/16	0.497
Radiotherapy to venous tumor thrombus	Yes vs. no	14/19	0.161

†Venous invasion. Vv1, tumor thrombus in a second branch or more of peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

‡Portal invasion. Vp1, tumor thrombus in a third or more peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.

ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBs Ag, hepatitis B virus surface antigen; HCV Ab, hepatitis C virus antibody.

recommended courses. On the other hand, 24 out of 33 (73%) patients did not complete HAIC-5-FU/IFN. Subsequently, 21 (64%) patients received other anti-cancer treatments, and 12 patients (36%) received best supportive care (BSC). The other treatments included TACE in 17 patients (MST 8.3 months), HAIC in six patients (MST 14.9 months) and systemic chemotherapy in six patients (MST 2.8 months). The MST of the additional treatment group was 11.8 months, which was significantly longer than the BSC group (with MST of 3.0 months, $P = 0.0078$, Log rank test). With regard to the regimens of other treatments, trans-arterial treatments tended to be associated with longer survival than systemic chemotherapy and BSC.

Adverse reactions and complications

Fever, fatigue, nausea and anorexia were the most common adverse events, but these were mostly CTCAE grade 1 or 2. CTCAE grade 3 or 4 adverse reactions included leukopenia in five patients (15%), thrombocytopenia in two (6%), anemia in three (9%) and anorexia in two (6%). Six patients required treatment with granulocyte colony-stimulating factor for leukopenia. Three patients required blood transfusion, but none

Table 6 Multivariate analysis for factors that influenced intrahepatic response to two cycles of hepatic arterial infusion chemotherapy (HAIC) treatment. Logistic regression analysis

Factors	Category	OR	95% CI	*P*-value
Tumor liver occupying rate (%)	<50	23.239	1.791–301.508	0.016
	≥50	1		
HCV Ab	Presence	16.886	1.969–144.774	0.010
	Absence	1		

95% CI, 95% confidence interval; HCV Ab, hepatitis C virus antibody; OR, odds ratio.

Table 7 Clinical characteristics of patients with venous tumor thrombus treated by hepatic arterial infusion chemotherapy (HAIC) using 5-fluorouracil (5-FU) and systemic interferon (IFN)-α (HAIC-5-FU/IFN) with and without radiotherapy to venous tumor thrombus, Pearson's χ^2 test

Clinical characteristics	Category	Total ($n = 33$)	HAIC alone ($n = 19$)	HAIC plus radio therapy ($n = 14$)	*P*-value
Sex	Male/female	30/3	18/1	12/2	0.373
Age (years)	<65/≥65	15/18	11/8	4/10	0.024
ECOG PS	0/1	23/10	14/5	9/5	0.561
HCV Ab	+/−	16/17	6/13	10/4	0.024
HBs Ag	+/−	7/26	7/12	0/14	0.011
Child–Pugh stage	A/B	25/8	16/3	9/5	0.187
Previous treatment	Yes/no	9/24	3/16	6/8	0.084
α-fetoprotein (ng/mL)	<5 000/≥5 000	17/16	8/11	9/5	0.208
des-γ-carboxy prothrombin (mAU/mL)	<10 000/≥10 000	14/19	9/10	5/9	0.503
Platelet count (/mm^3)	<150 000/≥150 000	19/14	11/8	8/6	0.966
size of largest tumor (mm)	<100/≥100	16/17	11/8	5/9	0.208
Tumor liver occupying rate (%)	<50/≥50	20/13	11/8	9/5	0.710
Grade of venous invasion (Vv)†	Vv 2/3	13/20	12/7	1/13	0.001
Grade of portal invasion (Vp)‡	Vp 0,1,2/3,4	12/21	6/13	6/8	0.506
Extrahepatic metastasis	Yes/no	16/17	9/10	7/7	0.881

†Venous invasion. Vv1, tumor thrombus in a second branch or more of peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

‡Portal invasion. Vp1, tumor thrombus in a third or more peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.

ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBs Ag, hepatitis B virus surface antigen; HCV Ab, hepatitis C virus antibody.

required platelet transfusion. Cutaneous ulcerations developed in the inguinal region in four patients, requiring implantation of reservoir system. Furthermore, two patients developed bleeding esophageal varices at one month after completion of HAIC-5-FU/IFN. Three patients developed radiation esophagitis, which required hospitalization as CTCAE grade 3. Of the latter group, one developed esophageal stenosis requiring endoscopic dilatation at 2 months after completion of radiotherapy. None of the patients who received the combination of HAIC-5-FU/IFN and radiotherapy developed hepatic failure that fulfilled the criteria of RILD.[29] On the other hand, three patients who did not receive 3D-CRT developed hepatic failure with hyperbilirubinemia; the cause of hepatic failure was considered to be the rapid progression of intrahepatic HCC.

Causes of death

At the time of analysis, six patients were still alive, whereas 27 patients had died. All 27 deaths were cancer-related, with the majority being due to progression of intrahepatic HCC. Among them, three patients died of HCC rupture and intra-abdominal bleeding. Two patients who did not receive 3D-CRT died of esophageal variceal bleeding. None died directly of extrahepatic metastases, and one patient died of septic necrotizing limb fasciitis. During the periods of treatment, we have no sudden death patient, which was clinically suspected to be due to pulmonary artery embolism.

DISCUSSION

INVASION OF A major vessel, especially the trunk of PVTT, is a poor prognostic factor in patients with advanced HCC.[14–19] Furthermore, the best available treatment for advanced HCC with PVTT is considered HAIC-5-FU/IFN.[9–13] Based on the lack of sufficient information on the efficacy of HAIC-5-FU/IFN for advanced HCC with VTT in the hepatic vein trunk (Vv2) or inferior vena cava (Vv3), we investigated the efficacy of HAIC-5-FU/IFN for HCC with VTT in this retrospective study. We also investigated the response to the combination of HAIC-5-FU/IFN and 3D-CRT to VTT of Vv3. In 33 patients, the intrahepatic response rate to HAIC-5-FU/IFN was 30%,

Table 8 Univariate analysis for determinants of effect of treatment on venous tumor thrombus after two cycles of hepatic arterial infusion chemotherapy (HAIC) treatment, Pearson's χ^2 test

Factors	Category	*n*	*P*-value
Sex	Female vs. male	3/30	0.658
Age (years)	≥65 vs. <65	18/15	0.112
ECOG PS	0 vs. 1	23/10	0.730
HCV Ab	Presence vs. absence	16/17	0.112
HBs Ag	Absence vs. presence	26/7	0.120
Child–Pugh stage	A vs. B	25/8	0.767
Previous treatment	No vs. yes	24/9	0.943
α-fetoprotein (ng/mL)	<5 000 vs. ≥5 000	17/16	0.611
des-γ-carboxy prothrombin (mAU/mL)	<10 000 vs. ≥10 000	14/19	0.335
Platelet count (/mm^3)	<150 000 vs. ≥150 000	19/14	0.010
Size of largest tumor (mm)	<100 vs. ≥100	16/17	0.112
Tumor liver occupying rate (%)	<50 vs. ≥50	20/13	0.027
Grade of venous invasion (Vv)†	2 vs. 3	13/20	0.135
Grade of portal invasion (Vp)‡	0, 1, 2 vs. 3, 4	12/21	0.290
Extrahepatic metastasis	No vs. yes	17/16	0.227
Radiotherapy to venous tumor thrombus	Yes vs. no	14/19	0.017

†Venous invasion. Vv1, tumor thrombus in a second branch or more of peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

‡Portal invasion. Vp1, tumor thrombus in a third or more peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein.

ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBs Ag, hepatitis B virus surface antigen; HCV Ab, hepatitis C virus antibody.

with MST of 7.9 months. Multivariate analysis (Table 6) identified two factors that influenced the intrahepatic response to HAIC-5-FU/IFN: tumor liver occupying rate of >50% ($P = 0.016$) and positivity for HCV Ab ($P = 0.010$). The combination of HAIC-5-FU/IFN with 3D-CRT to VTT had a significantly better treatment effective rate of VTT (79%) than HAIC-5-FU/IFN alone (37%). Multivariate analysis (Table 4) identified three independent factors that influenced survival: treatment-related reduction in VTT ($P = 0.0006$), largest tumor size <100 mm ($P = 0.013$), and CR/PR for intrahepatic response ($P = 0.030$). While 3D-CRT did not significantly improve the survival times, it significantly reduced VTT, thus indirectly contributing to the high intrahepatic response and presumably improving the survival rate. Among 16 patients with disadvantageous conditions (VTT-Vv3 and non-CR/PR), effective 3D-CRT resulted in significant prolongation of survival time compared with patients who did not receive or showed ineffective response to 3D-CRT ($P = 0.028$, Fig. 4). This result suggests the prognostic value of radiotherapy to VTT for advanced HCC patients treated by HAIC-5-FU/IFN.

The response rate to HAIC-5-FU/IFN in HCC with VTT (30%) was similar to the previously reported response

Table 9 Multivariate analysis for factors that contributed to the effect of treatment on venous tumor thrombus after two cycles of hepatic arterial infusion chemotherapy (HAIC) treatment. Logistic regression analysis

	Category	OR	95% CI	*P*-value
Platelet count (/mm^3)	<150 000	16.087	1.704–151.861	0.015
	≥150 000	1		
Radiotherapy to venous tumor thrombus	Yes	14.982	1.508–148.827	0.021
	No	1		

95% CI, 95% confidence interval; OR, odds ratio.

rate to HAIC-5-FU/IFN in HCC with PVTT (29–52%).[9,12,13] Multivariate analysis found that tumor liver occupying rate ($P = 0.016$) and positivity for HCV Ab ($P = 0.010$) contributed to intrahepatic response of CR/PR (Table 6). Two previous studies[9,13] also reported that positivity for HCV Ab was also a pretreatment predictive factor for response and survival of advanced HCC treated with HAIC-5-FU/IFN. The exact reason for the correlation between HCV positivity and the response to HAIC-5-FU/IFN is not clear. Several studies have investigated the differences between the HCV and HBV in relation to HCC, such as the mechanism of hepatocarcinogenesis[30,31] and cytokine pattern in hepatitis.[32] These factors could influence the tumor response to therapy.

Similar to a previous report on advanced HCC with PVTT treated by HAIC-5-FU/IFN,[9,12,13] patients classified as CR/PR based on intrahepatic response had a significantly longer MST than the non-CR/PR group (18.7 vs. 4.4 months, respectively, $P = 0.0029$, log rank test) (Fig. 2). Multivariate analysis showed that survival correlated with effect of treatment VTT ($P = 0.0006$), tumor size ($P = 0.013$) and CR/PR based on intrahepatic response ($P = 0.030$) (Table 4).

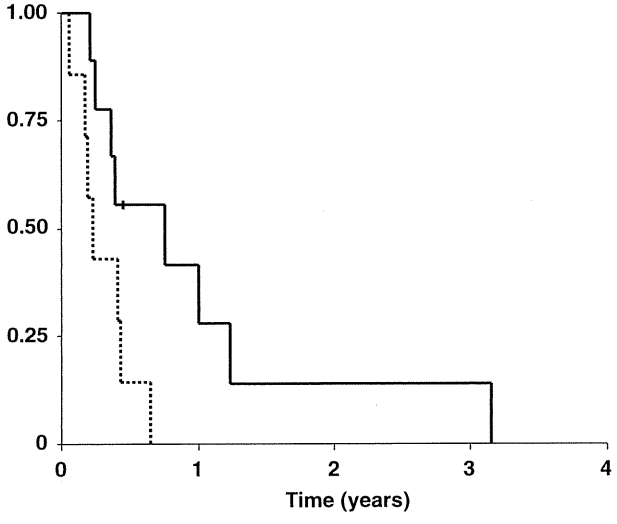

Figure 4 Cumulative survival rate of 16 patients with advanced hepatocellular carcinoma (HCC) and venous tumor thrombosis (VTT) of Vv3 who were not evaluated as complete or partial response (non-CR/PR). Solid line: nine patients who underwent arterial infusion chemotherapy (HAIC) and responded to 3-D conformal radiotherapy (3D-CRT), resulting in a decrease in VTT and significantly longer median survival time (MST) of 9.2 months ($P = 0.028$, log rank test). Dashed line: seven patients who underwent HAIC without or with ineffective radiotherapy, resulting in increase of VTT and MST of 3.1 months. ——, Effective 3D-CRT to VTT; ------, No or ineffective 3D-CRT to VTT.

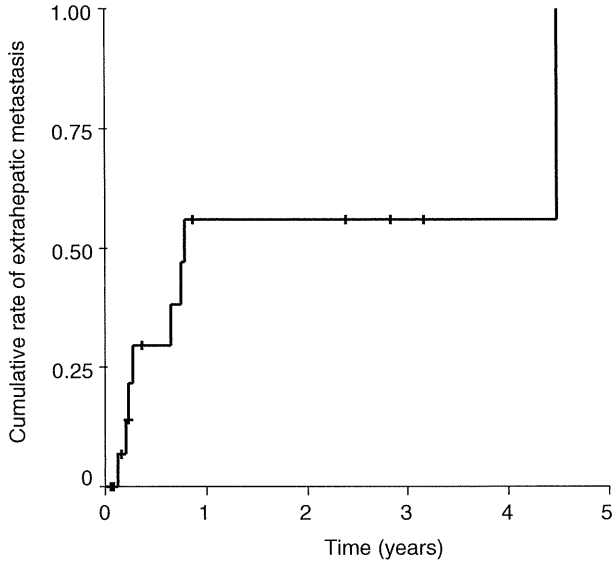

Figure 5 Cumulative rate of extrahepatic metastasis in 17 patients who were negative for extrahepatic metastasis at baseline (before treatment). The median time to metastasis was 7.1 months. The 6- and 12-month cumulative rate of metastasis was 30% and 56%, respectively. HAIC, hepatic arterial infusion chemotherapy; non-CR/PR, not evaluated complete or partial response on intrahepatic response evaluation; VTT, venous tumor thrombus; Vv3, tumor thrombus in the inferior vena cava.

The highest response rate was registered with the combination of HAIC-5-FU/IFN and 3D-CRT to VTT (79%, Fig. 3). This finding is similar to that reported by Katamura *et al.*[27] who reported a rate of 75% for HAIC-5-FU/IFN with radiotherapy to PVTT. Although 3D-CRT was considered, in general, tolerable to allow continuation of HAIC-5-FU/IFN without the development of RILD in our study, radiotherapy caused severe esophageal complications in three out of 14 patients (21%). This finding suggests that it is often difficult to avoid the harmful effect of irradiation to the radiosensitive esophagus, which is anatomically close to VTT of Vv3. Careful planning of 3D-CRT and reduction of radiation dose as much as possible might avoid esophageal complications associated with 3D-CRT of Vv3.

Although the response of VTT to radiotherapy was high in this study, the addition of 3D-CRT to the management of advanced HCC with VTT did not improve survival ($P = 0.667$, log rank test). This result could be causally related to the existence of five patients in HAIC-