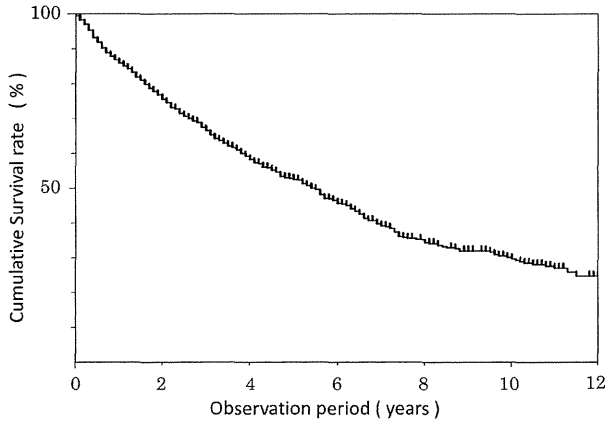

Fig. 1 Overall survival rates of entire patients at 5 and 10 years were 52.7 and 29.7 %, respectively

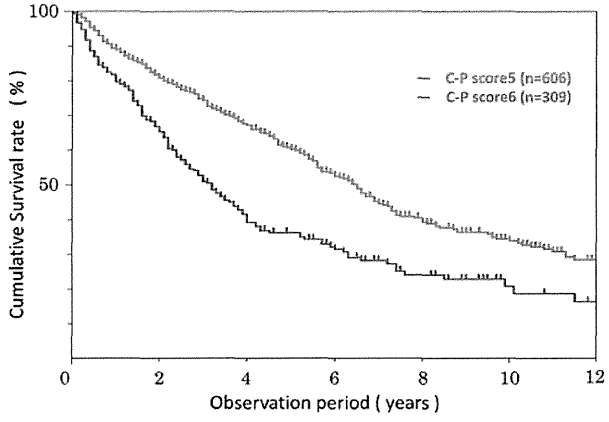

Fig. 2 Prognosis of the Child-Pugh score 5 group was significantly better compared to the Child-Pugh score 6 group

Overall survival rate of entire patient based on the FIB-4 index

When patients were categorized according to the FIB-4 index as <2.0 ($n = 93$), ≥2.0 and <4.0 ($n = 311$), and ≥4.0 ($n = 511$), the survival rates at were 70.5 % (95 % CI 59.0–79.9), 56.4 % (95 % CI 50.1–62.5), and 47.1 % (95 % CI 42.2–52.1) at 5 years, respectively, and 55.3 % (95 % CI 42.2–67.7), 32.2 % (95 % CI 25.5–39.6), and 23.7 % (95 % CI 18.6–29.7) at 10 years, respectively (Fig. 3). The FIB-4 index <2.0 group had a significantly better prognosis than the FIB-4 index ≥2.0 and <4.0 group ($p = 0.028$). The FIB-4 index ≥2.0 and <4.0 group had a significantly better prognosis than the FIB-4 index ≥4.0 group ($p = 0.010$).

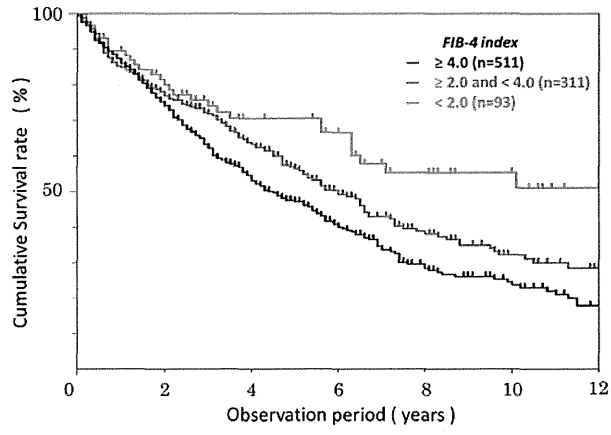

Fig. 3 Overall survival rate based on the FIB-4 index. The FIB-4 index of patients with Child-Pugh class A predicts outcomes with good discriminative ability

Overall survival and recurrence rate of patients who underwent hepatectomy/LAT based on the FIB-4 index

When focusing on HCC patients who underwent hepatectomy/LAT, FIB-4 index was <2.0 in 70 patients (10.8 %), ≥2.0 and <4.0 in 222 patients (34.2 %), and ≥4.0 in 357 patients (55.0 %). Figure 4a shows the overall survival rates of these three groups. The survival rates of the FIB-4 index <2.0, ≥2.0 and <4.0, and ≥4.0 groups were 80.0 % (95 % CI 67.1–88.7), 68.9 % (95 % CI 61.7–75.2), and 59.1 % (95 % CI 53.2–64.7) at 5 years, respectively, and 65.2 % (95 % CI 49.4–78.1), 40.9 % (95 % CI 32.6–49.9), and 28.6 % (95 % CI 22.1–36.1) at 10 years, respectively. The FIB-4 index <2.0 group had a significantly better prognosis than the FIB-4 index ≥2.0 and <4.0 group ($p = 0.047$). The FIB-4 index ≥2.0 and <4.0 group had a significantly better prognosis than the FIB-4 index ≥4.0 group ($p = 0.005$).

Figure 4b shows overall intrahepatic recurrence rates following the initial treatment in these three groups. The recurrence rates of patients with FIB-4 index <2.0, ≥2.0 and <4.0, and ≥4.0 were 54.3 % (95 % CI 52.4–79.5), 63.6 % (95 % CI 56.1–70.5) and 79.6 % (95 % CI 74.5–89.0), respectively, at 5 years and 67.4 % (95 % CI 52.4–79.5), 79.0 % (95 % CI 70.6–85.5) and 89.7 % (95 % CI 84.2–93.4), respectively, at 10 years. The FIB-4 index ≥4.0 group had a significantly higher recurrence rate than the other groups (FIB-4 index ≥4.0 vs ≥2.0 and <4.0, $p < 0.001$; ≥4.0 vs <2.0, $p = 0.001$). There were no significant differences in recurrence rate between the FIB-4 index <2.0 and ≥2.0 and <4.0 groups ($p = 0.465$).

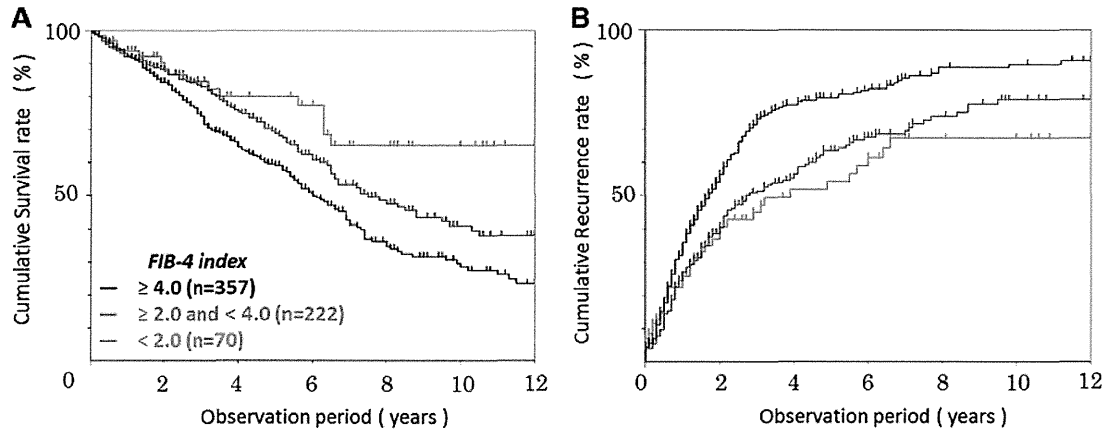

Fig. 4 Overall survival (**a**) and recurrence rate (**b**) based on the FIB-4 index in patients who underwent hepatectomy/LAT. The FIB-4 index <2.0 group had a significantly better prognosis than the other groups, and the recurrence rate of FIB-4 index ≥4.0 group was higher than that of the other groups

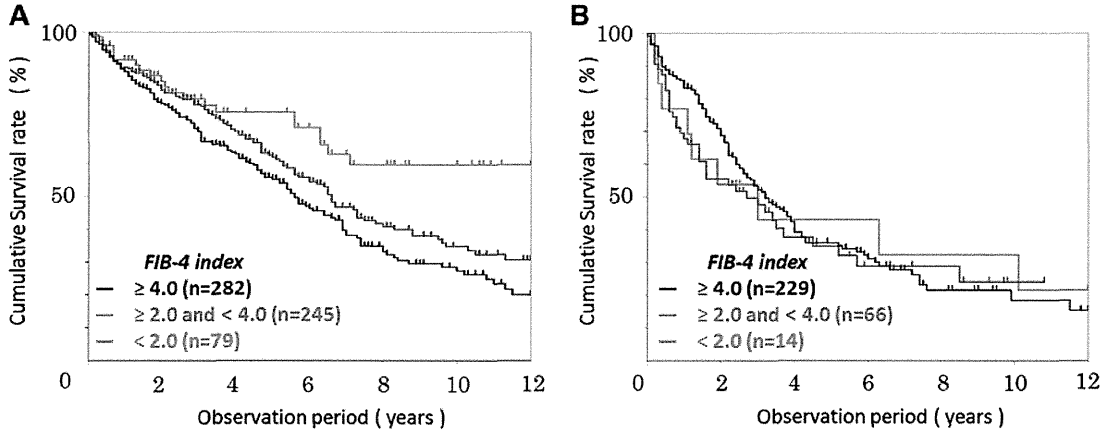

Fig. 5 Overall survival rates of patients with a Child-Pugh score of 5 (**a**) and 6 (**b**) based on the FIB-4 index. The FIB-4 index was useful for assessing mortality among patients with a Child-Pugh score of 5, but there were no significant difference among patients with a Child-Pugh score of 6

Overall survival rate of patients with C-P score 5 or 6 based on the FIB-4 index

When focusing on HCC patients with C-P score 5, FIB-4 index was <2.0 in 79 patients (13.0 %), ≥2.0 and <4.0 in 245 patients (40.4 %), and ≥4.0 in 282 patients (46.5 %). Figure 5a shows the overall survival rates of these three groups. The survival rates of the FIB-4 index <2.0, ≥2.0 and <4.0, and ≥4.0 groups were 75.7 % (95 % CI 63.2–84.9), 61.9 % (95 % CI 54.8–68.5), and 55.2 % (95 % CI 48.6–61.5) at 5 years, respectively, and 59.7 % (95 % CI 44.9–72.9), 34.7 % (95 % CI 27.1–43.2), and 27.3 % (95 % CI 20.6–35.3) at 10 years, respectively. The survival rate was highest in patients with FIB-4 index <2.0, followed by those with FIB-4 index ≥2.0 and <4.0, and those with FIB-4 index ≥4.0, although the difference between patients with FIB-4 index ≥2.0 and <4.0 and those with FIB-4 index ≥4.0 was not significant statistically (FIB-4 index <2.0 vs ≥2.0 and <4.0, $p = 0.028$, FIB-4 index <2.0 vs ≥4.0, $p < 0.001$, and FIB-4 index ≥2.0 and <4.0 vs ≥4.0, $p = 0.052$).

When focusing on HCC patients with C-P score 6, FIB-4 index was <2.0 in 14 patients (4.5 %), ≥2.0 and <4.0 in 66 patients (21.4 %), and ≥4.0 in 229 patients (74.1 %). Figure 5b shows overall survival rate of these three groups. The survival rate of patients with FIB-4 index <2.0, ≥2.0 and <4.0, and ≥4.0 were 43.1 % (95 % CI 19.0–71.0), 35.0 % (95 % CI 22.9–49.4) and 36.0 % (95 % CI 28.8–43.8), respectively, at 5 years and 32.3 % (95 % CI 11.6–63.5), 24.1 % (95 % CI 12.7–40.8) and 18.4 % (95 % CI

Table 2 Factors associated with patient survival (univariate analysis)

Factor	Hazard ratio	95 % CI	*p* value
Age (years)			
<65	1		
≥65	1.335	1.095–1.628	0.004
Sex			
Female	1		
Male	1.156	0.605–1.422	0.172
AST (IU/mL)			
≤40	1		
>40	1.518	1.235–1.860	0.001
ALT (IU/mL)			
≤35	1		
>35	1.158	0.954–1.407	0.139
Platelet count ($\times 10^4/m^3$)			
≥15	1		
<15	1.026	0.804–1.181	0.792
Total bilirubin (mg/dL)			
≤1.2	1		
>1.2	1.195	0.888–1.608	0.239
Albumin (g/dL)			
>3.5	1		
≤3.5	1.912	1.549–2.360	<0.001
Prothrombin time (%)			
>70	1		
≤70	1.086	0.519–1.634	0.921
Etiology (viral hepatitis)			
Present	1		
Absent	1.356	1.041–1.761	0.024
Child-Pugh score			
5	1		
6	1.845	1.528–2.227	<0.001
Alcohol abuse			
Absent	1		
Present	1.033	0.844-1.264	0.756
AFP (ng/mL)			
≤20	1		
>20	1.449	1.207–1.739	<0.001
AFP-L3 (%)			
≤10	1		
>10	1.904	1.549–2.342	<0.001
DCP (mAU/mL)			
≤40	1		
>40	1.614	1.339–1.945	<0.001
FIB-4 index			
<2.0	1		
≥2.0 and <4.0	1.550	1.048–2.292	0.028
≥4.0	2.001	5.761–2.917	<0.001
Tumor size (cm)			
<3	1		
≥3	2.220	1.849–2.665	<0.001
Tumor number			
Single	1		
Multiple	2.350	1.953–2.824	<0.001
Vascular invasion			
Absent	1		
Present	4.989	3.883–6.410	<0.001
Antiviral therapy			
−	1		
+	0.501	0.393–0.639	<0.001
Initial treatment			
Hepatectomy			
−	1		
+	0.438	0.368–0.531	<0.001
LAT			
−	1		
+	0.880	0.717–1.071	0.218

CI confidence interval, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *LAT* locoregional ablative therapy

11.1–28.9), respectively, at 10 years. There were no significant differences in survival rates among these three groups (FIB-4 index <2.0 vs ≥2.0 and <4.0, $p = 0.812$; FIB-4 index <2.0 vs ≥4.0, $p = 0.743$; FIB-4 index ≥2.0 and <4.0 vs ≥4.0, $p = 0.393$).

Factors associated with patient survival

Factors significantly associated with overall survival in the univariate analysis are listed in Table 2. The following associations were statistically significant: age, AST, albumin, HCC etiology, C-P score, AFP, AFP-L3, DCP, FIB-4 index, tumor size, number of tumors, vascular invasion, antiviral therapy, and hepatectomy/LAT as initial treatment. Factors that were significantly associated with overall survival in the multivariate analysis were C-P score 6 [HR 1.564 (95 % CI 1.257–1.946); $p < 0.001$], FIB-4 index ≥2.0 and <4.0 [HR 1.638 (95 % CI 1.084–2.474); $p = 0.019$] and FIB-4 index ≥4.0 [HR 1.828 (95 % CI 1.217–2.744); $p = 0.004$], AFP-L3 >10 % [HR 1.458 (95 % CI 1.163–1.829); $p = 0.001$], tumor size ≥3 cm [HR 1.718 (95 % CI 1.382–2.136); $p < 0.001$], number of tumors (multiple) [HR 1.464 (95 % CI 1.172–1.828); $p = 0.001$], vascular invasion (present) [HR 2.884 (95 % CI 2.102–3.957); $p < 0.001$], antiviral therapy [HR 0.761 (95 % CI 0.585–0.989); $p = 0.041$], and hepatectomy as initial treatment [HR 0.625 (95 % CI 0.497–0.786); $p < 0.001$] (Table 3).

Table 3 Factors associated with patient survival based on multivariable Cox proportional hazards modeling with forward selection

Factor	Hazard ratio	95 % CI	*p* value
Child-Pugh score			
5	1		
6	1.564	1.257–1.946	<0.001
FIB-4 index			
<2.0	1		
≥2.0 and <4.0	1.638	1.084–2.474	0.019
≥4.0	1.828	1.217–2.744	0.004
AFP-L3 (%)			
≤10	1		
>10	1.458	1.163–1.829	0.001
Tumor size (cm)			
<3	1		
≥3	1.718	1.382–2.136	<0.001
Tumor number			
Single	1		
Multiple	1.464	1.172–1.828	0.001
Vascular invasion			
Absent	1		
Present	2.884	2.102–3.957	<0.001
Antiviral therapy			
−	1		
+	0.761	0.585–0.989	0.041
Initial treatment			
Hepatectomy			
−	1		
+	0.625	0.497–0.786	<0.001

CI confidence interval, *AFP-L3 Lens culinaris* agglutinin-reactive α-fetoprotein

Discussion

C-P classification and tumor staging are both important factors for predicting mortality in HCC patients. Therefore, we have to assess both tumor factors and residual liver function when choosing the type of treatment for HCC. Recently, proposed staging system for HCC combined tumor factors and liver functional markers has been reported (the Cancer of the Liver Italian Program (CLIP) investigators 1998; Llovet et al. 1999; Kudo et al. 2004).

In patients with HCC who have C-P class A liver function at diagnosis, in the present study, there were significant differences in the survival rates between C-P score 5 and 6 groups. However, we are not able to further stratify HCC patients with C-P score 5 in terms of liver function, because the minimum score of C-P is 5 points.

It has been reported that the FIB-4 index is well correlated with liver fibrosis (Sterling et al. 2006; Vallet-Pichard et al. 2007; Shah et al. 2009). Although liver fibrosis is reportedly intertwined with hepatocarcinogenesis and the prognosis of chronic hepatitis C (Tamaki et al. 2013; Vergniol et al. 2014), there have been few reports on whether the FIB-4 index is associated with mortality in HCC patients. In this study, we investigated the impact of the FIB-4 index on prognosis of HCC with C-P class A.

When patients were categorized as <2.0, ≥2.0 and <4.0, and ≥4.0 by FIB-4 index, patients with FIB-4 index <2.0 had a highest survival rate, followed by those with FIB-4 index ≥2.0 and <4.0, and those with FIB-4 index ≥4.0. In addition, the FIB-4 index was also useful for predicting prognosis in patients who underwent curative treatment (hepatectomy/LAT), and the recurrence rate of FIB-4 index ≥4.0 group was higher than that of the other groups in this analysis. These results indicate that the calculation of the FIB-4 index at the start of follow-up is useful for predicting the outcome of HCC patients with C-P class A.

Whereas we found a significant difference in survival rates based on the FIB-4 index in patients with C-P score 5, we did not find a difference in survival rates in patients with C-P score 6. The C-P scoring system is considered to reflect remnant liver function. In contrast, the FIB-4 index is a marker of liver fibrosis. Although liver fibrosis is partly linked to remnant liver function, these are not completely coincident. Patients with C-P class A can have various degrees of liver fibrosis. Therefore, we were able to further stratify the prognosis of HCC patients with C-P score 5 using the FIB-4 index, a quantitative marker of liver fibrosis.

Both the FIB-4 index and C-P score were identified as independent risk factors for predicting the outcome of HCC along with tumor factors such as tumor size, number, and tumor marker levels, and initial treatment in the multivariate analysis. In addition, the hazard ratio of the FIB-4 index was higher than the HR for the C-P score. Therefore, FIB-4 index has a strong impact on the prognosis of patients with HCC when they have C-P class A liver function.

The utility of FIB-4 index is enhanced by the fact that it is calculated using age and general laboratory data, in terms of low cost, easy to calculate. In addition, the index can be monitored easily with repeated calculation. Nevertheless, the FIB-4 index has several limitations. The formula for the FIB-4 index includes platelet count. Therefore, caution is needed when a patient's platelet count is low due to extrahepatic causes, for example, idiopathic thrombocytopenic purpura and post-splenectomy.

There are several issues that should be further studied in the future. The FIB-4 index has been developed as a noninvasive marker of fibrosis in patients with chronic hepatitis C and non-alcoholic fatty liver disease (Sterling et al. 2006; Vallet-Pichard et al. 2007; Shah et al. 2009). Since the predominant etiology of HCC in the present study was HCV,

further studies are required for assessing the prognosis of HCC due to other etiologies (HBV and non-HBV + HCV). Additionally, we did not investigate other serum liver fibrosis markers including hyaluronic acid and type IV collagen 7s. Hence, it is necessary to assess the utility of these values for the prediction of prognosis in HCC patients and their relationship to the FIB-4.

In conclusion, the FIB-4 index was closely associated with mortality in HCC patients with C-P class A, especially those with C-P score 5. The FIB-4 index was identified as an independent predictive factor for HCC prognosis from a set of tumor and therapy-related factors. Therefore, the FIB-4 index is very useful to clinicians when predicting mortality and determining treatment strategies for HCC patients with C-P score 5.

Acknowledgments There is no grant or other financial support for this study.

Conflict of interest The authors declare no conflicts of interests.

References

A new prognostic system for hepatocellular carcinoma (1998) A retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology 28:751–755

Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M et al (2014a) The ION-1 investigators. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 370:1889–1898

Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E et al (2014b) Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. ION-2 investigators. N Engl J Med 370:1483–1493

Buster EH, Hansen BE, Buti M, Delwaide J, Niederau C, Michielsen PP et al (2007) HBV 99-01 Study Group. Peginterferon alpha-2b is safe and effective in HBeAg-positive chronic hepatitis B patients with advanced fibrosis. Hepatology 46:388–394

Clinical Practice Guidelines for Hepatocellular Carcinoma (2009) The Japan Society of Hepatology update. Hepatol Res 2010(40):2–144

de Lope CR, Tremosini S, Forner A, Reig M, Bruix J (2012) Management of HCC. J Hepatol 56:75–87

El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132:2557–2576

European Association for the Study of the Liver (2012) European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 56:908–943

Fernández-Esparrach G, Sánchez-Fueyo A, Ginès P, Uriz J, Quintó L, Ventura PJ et al (2001) A prognostic model for predicting survival in cirrhosis with ascites. J Hepatol 34:46–52

Izumi R, Shimizu K, Ii T, Yagi M, Matsui O, Nonomura A et al (1994) Prognostic factors of hepatocellular carcinoma in patients undergoing hepatic resection. Gastroenterology 106:720–727

Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS et al (2013) POSITRON Study; FUSION Study; Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 368:1867–1877

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90

Kudo M, Chung H, Haji S, Osaki Y, Oka H, Seki T et al (2004) Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. Hepatology 40:1396–1405

Kumada T, Toyoda H, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A et al (2013a) Characteristics of elderly hepatitis C virus-associated hepatocellular carcinoma patients. J Gastroenterol Hepatol 28:357–364

Kumada T, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y et al (2013b) Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: a propensity score analysis. J Hepatol 58:427–433

Leroy V, Hilleret MN, Sturm N, Trocme C, Renversez JC, Faure P et al (2007) Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. J Hepatol 46:775–782

Llovet JM, Brú C, Bruix J (1999) Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 19:329–338

Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, Reindollar R, Rustgi V, McPhee F, Wind-Rotolo M, Persson A, Zhu K, Dimitrova DI, Eley T, Guo T, Grasela DM, Pasquinelli C et al (2012) Preliminary study of two antiviral agents for hepatitis C genotype 1. N Engl J Med 366:216–224

Merkel C, Bolognesi M, Sacerdoti D, Bombonato G, Bellini B, Bighin R et al (2000) The hemodynamic response to medical treatment of portal hypertension as a predictor of clinical effectiveness in the primary prophylaxis of variceal bleeding in cirrhosis. Hepatology 32:930–934

Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60:646–649

Schiff E, Simsek H, Lee WM, Chao YC, Sette H Jr, Janssen HL et al (2008) Efficacy and safety of entecavir in patients with chronic hepatitis B and advanced hepatic fibrosis or cirrhosis. Am J Gastroenterol 103:2776–2783

Sebastiani G, Vario A, Guido M, Noventa F, Plebani M, Pistis R et al (2006) Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. J Hepatol 44:686–693

Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ (2009) Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 7:1104–1112

Shim JH, Lee HC, Kim KM, Lim YS, Chung YH, Lee YS et al (2010) Efficacy of entecavir in treatment-naïve patients with hepatitis B virus-related decompensated cirrhosis. J Hepatol 52:176–182

Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J et al (2006) APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 43:1317–1725

Tamaki N, Kurosaki M, Matsuda S, Muraoka M, Yasui Y, Suzuki S et al (2013) Non-invasive prediction of hepatocellular carcinoma development using serum fibrosis marker in chronic hepatitis C patients. J Gastroenterol. doi:10.1007/s00535-013-0914-y

Toyoda H, Kumada T, Tada T, Sone Y, Kaneoka Y, Maeda A (2011) Characteristics and prognosis of patients with hepatocellular carcinoma after the year 2000 in Japan. J Gastroenterol Hepatol 26:1765–1771

Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K (2009) Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 44:102–107

Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V et al (2007) FIB-4: an inexpensive and accurate marker

of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. Hepatology 46:32–36

Vergniol J, Boursier J, Coutzac C, Bertrais S, Foucher J, Angel C et al (2014) The evolution of non-invasive tests of liver fibrosis is associated with prognosis in patients with chronic hepatitis C. Hepatology 60:65–76

Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS et al (2003) A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic heparins C. Hepatology 38:518–526

J Gastroenterol (2015) 50:795–804
DOI 10.1007/s00535-014-1011-6

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term prognosis of patients with hepatitis B infection: causes of death and utility of nucleos(t)ide analogue therapy

Toshifumi Tada · Takashi Kumada · Hidenori Toyoda · Seiki Kiriyama · Makoto Tanikawa · Yasuhiro Hisanaga · Akira Kanamori · Shusuke Kitabtake · Takanori Ito

Received: 17 September 2014 / Accepted: 21 October 2014 / Published online: 7 November 2014

Abstract
Background Long-term nucleos(t)ide analogue (NA) therapy for chronic hepatitis B (CHB) patients has been reported to reduce the risk of hepatocellular carcinoma (HCC) development. However, survival rates and causes of death in CHB patients either treated or not treated with NA therapy are unclear. Therefore, we investigated the prognosis of CHB in both of these groups.
Methods A total of 919 CHB patients who were treated ($n = 189$) or not treated ($n = 730$) with NA therapy were enrolled; of these, 135 were selected from each group by propensity score matching. Survival, mortality from both HCC and non-liver related diseases, and causes of death were analyzed.
Results In all patients ($n = 919$), cumulative survival and mortality from both HCC and non-liver related diseases did not differ significantly according to NA therapy status. Of 66 patients who died during the follow-up period, 59.1 % died due to liver-related diseases (including HCC); of the remainder, 48.1 % died of non-liver related malignancies. In patients selected by propensity score matching ($n = 270$), cumulative survival and mortality from HCC were significantly improved in those who received NA therapy compared with those who did not ($p = 0.015$ and 0.018, respectively). Cox proportional hazards models indicated that NA therapy was independently associated with survival of CHB patients (hazard ratio, 0.286; 95 % confidence interval, 0.122–0.668; $p = 0.004$).
Conclusions Approximately 40 % of CHB patients died of non-liver-related diseases. Additionally, in patients who required anti-viral therapy for CHB, NA therapy improved survival and mortality from HCC.

Keywords Hepatitis B · Nucleos(t)ide analogue · Hepatocellular carcinoma · Survival · Mortality

Abbreviations

CHB	Chronic hepatitis B
HCC	Hepatocellular carcinoma
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
NA	Nucleos(t)ide analogue
HBeAg	Hepatitis B e antigen
ALT	Alanine aminotransferase
AUC	Area under the curve
ROC	Receiver operating characteristic
CI	Confidence interval
γ-GTP	Gamma-glutamyl transpeptidase
ALP	Alkaline phosphatase
AFP	Alphafetoprotein
HBcrAg	Hepatitis B virus core-related antigen
BCP	Basal core promoter
US	Ultrasound
CT	Computed tomography
MRI	Magnetic resonance imaging
IFN	Interferon
RR	Relative risk
RFA	Radiofrequency ablation
PEI	Percutaneous ethanol injection
TACE	Trancecatheter arterial chemoembolization
HAIC	Hepatic arterial infusion chemotherapy

T. Tada (✉) · T. Kumada · H. Toyoda · S. Kiriyama · M. Tanikawa · Y. Hisanaga · A. Kanamori · S. Kitabtake · T. Ito
Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502, Japan
e-mail: tadat0627@gmail.com

Introduction

Chronic hepatitis B (CHB) affects over 350 million people worldwide. Long-term complications of infection include cirrhosis and hepatocellular carcinoma (HCC), which together cause over 500,000 deaths annually [1, 2]. Hepatitis B surface antigen (HBsAg)-positive patients have a 70-fold increased risk of developing HCC compared to their HBsAg-seronegative counterparts [3, 4]. Hepatitis B virus (HBV) infection is endemic in Southeast Asia, China, Taiwan, Korea, and sub-Saharan Africa, where up to 85–95 % of patients with HCC are HBsAg positive [5]. HCC is the third and fifth leading cause of cancer deaths in men and women, respectively, and the number of deaths and the mortality rate from HCC have greatly increased in Japan since 1975 [6]. Hepatitis C virus–related HCC accounts for 75 % of all HCC in Japan, while HBV-related HCC accounts for 15 % [6].

Nucleos(t)ide analogues (NAs) are an established treatment for CHB [7–9]. Between 2000 and 2006, lamivudine, adefovir dipivoxil, and entecavir were approved in Japan as NA therapies for CHB, and in 2014 tenofovir disoproxil fumarate was also approved. NAs have a powerful inhibitory effect on HBV DNA proliferation, regardless of genotype, and act as antiviral agents and promote quiescence of hepatitis in nearly all patients, including those of more advanced age with little prospect of spontaneous remission. NA therapy for CHB has been reported to not only prevent the progression of hepatitis, but to also reduce the risk of development of HCC [10, 11]. However, survival rates and causes of death, including those that are non-liver-related, have not been sufficiently investigated in CHB patients receiving or not receiving long-term NA therapy.

In the present study, we clarified these issues and also confirmed the impact of NA therapy on decreasing mortality in patients with CHB, using propensity score analysis to reduce biases associated with the selection of study patients [12–15].

Materials and methods

Patients

The study protocol was approved by the Institutional Ethics Committee of Ogaki Municipal Hospital in January 2011, and was in compliance with the Declaration of Helsinki. Written informed consent for the use of stored serum samples was obtained from all patients.

Between 1991 and 2010, 2220 consecutive HBsAg-positive patients who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital were prospectively enrolled in our HCC surveillance program. These patients included 1220 patients (55.0 %) in whom hepatocarcinogenesis was investigated in our previous study [11]. Of these, 919 met the following inclusion criteria: HBsAg-positive for more than 6 months; no evidence of HCV co-infection; no other causes of chronic liver disease (alcohol consumption >80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); no incomplete clinical data or missing serum samples; follow-up duration of greater than 3 years; no evidence of malignancies, including HCC, for at least 1 year from the start of the follow-up period; and receiving NA therapy for more than 1 year before the detection of HCC. In patients on NA therapy, the date of NA therapy initiation was considered the start of the follow-up period. In the non-NA group (controls), the date of the first visit was defined as the start of follow-up. The end of follow-up was defined as the final visit for patients who had not died, and as the date of death for patients who died during follow-up.

Of the 919 eligible patients, 189 received NA therapy during the follow-up period (NA group) and 730 patients did not (non-NA group). We first compared the survival rates between the two groups and determined the causes of death in all patients. Then, to reduce the confounding effects of covariates, we used propensity scores to match NA patients to unique non-NA patients. Eight covariates, including age, sex, HBV DNA concentration, HBsAg, hepatitis B e antigen (HBeAg), genotype, platelet count, and alanine aminotransferase (ALT) activity were taken into account at the start of follow-up. Based on previously reported cut-off values for NA therapy indications or relation to the progression of HBV patients [16–19], we computed the propensity scores using logistic regression with the following independent variables: age (≤40 years or >40 years), sex (female or male), HBV DNA concentration (≤5.0 log copies/ml or >5.0 log copies/ml), HBsAg concentration (≤3.0 log copies/ml or >3.0 log copies/ml), HBeAg (negative or positive), genotype (genotype C or non-genotype C), platelet count ($>150 \times 10^3/m^3$ or $\leq 150 \times 10^3/m^3$), and ALT activity (≤35 IU/ml or >35 IU/ml). The calculated propensity scores of the NA and non-NA groups were 0.22079–0.98208 (median, 0.5072) and 0.22079–0.99659 (median, 0.9206), respectively; these scores were then rounded to two decimal places. We conducted one-to-one matching of patients based on consistency of propensity scores to the second decimal place. Propensity score matching resulted in the selection of 270 patients (NA group, 135 patients; non-NA group, 135 patients) (Fig. 1). The p value of the calculated propensity score was 0.372 based on the Hosmer–Lemeshow test [20]. The area under the curve (AUC) of the receiver operating characteristic (ROC)-calculated

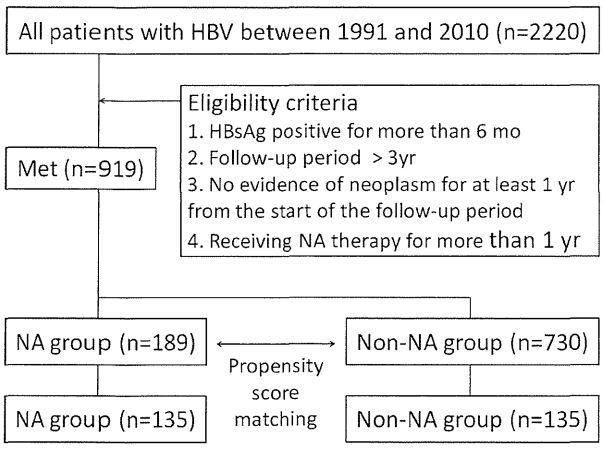

Fig. 1 Flowchart of the patient selection process

propensity score was 0.862 [95 % confidence interval (CI), 0.834–0.891] [21].

Surveillance, diagnosis, and causes of death

All patients were followed up at our hospital at least every 6 months. During each follow-up examination, we measured platelet counts and levels of ALT, gamma-glutamyl transpeptidase (γ-GTP), total bilirubin, alkaline phosphatase (ALP), albumin, and alphafetoprotein (AFP). We used commercially available kits to test blood samples for HBsAg, HBeAg, and anti-HBe (Abbott Japan, Tokyo, Japan). After December 2007, which was the start of the follow-up period for the CHB patients, serum HBV DNA concentrations were monitored by polymerase chain reaction assay (COBAS AmpliPrep-COBAS TaqMan HBV Test v2.0, Roche Diagnostics), with a lower detection limit of approximately 2.1 log copies/ml. Before November 2007, these concentrations were measured once at the start of the follow-up period using patients' stored frozen serum (80 °C) with the COBAS TaqMan HBV Test v2.0. HBV genotyping was performed as previously described [22]. Serum levels of HBV core-related antigen (HBcrAg) were measured using a chemiluminescence enzyme immunoassay (CLEIA) as previously described [23, 24]. Precore nucleotide 1896 and basal core promoter (BCP) dinucleotide 1762/1764 were determined using the line probe assay (INNO-LiPA HBV PreCore assay; Innogenetics NV) [25, 26]. The probes were designed to determine the nucleotides at position 1896 (G vs. A) in the precore region and positions 1762 (A vs. T) and 1764 (G vs. A and G vs. T) in the BCP region. A line probe assay was used to identify any emergence of YMDD mutations (INNO-LiPA HBV DR assay; Innogenetics NV).

In accordance with the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [27], cirrhotic patients under surveillance underwent ultrasound (US) and monitoring of tumor markers every 3–4 months, and dynamic computed tomography (CT) or magnetic resonance imaging (MRI) every 12 months. For patients with chronic hepatitis, we performed US and monitoring of tumor markers every 6 months. The diagnosis of cirrhosis was made based on histological examination or typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [28–30]. Patients who did not satisfy these criteria were classified as having chronic hepatitis. As recommended by the diagnosis algorithm of the Japan Society of Hepatology [27], HCC was diagnosed principally based on the results from ultrasonography and dynamic CT (hyperattenuation during the arterial phase in all or part of the tumor, and hypoattenuation in the portal venous phase) and/or MRI.

Diseases other than HCC were initially detected based on clinical symptoms and/or abnormal surveillance data, medical check-ups (in community or workplace), or assessment of physicians. These conditions were then diagnosed based on disease-specific criteria by the appropriate specialists in our hospital. Causes of death data were defined by these specialists using the International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-9 codes for deaths occurring prior to 1 January 2003, and thereafter ICD-10 codes) [31]. All of the studies were performed retrospectively by collecting and analyzing data from the patient records.

Ogaki Municipal Hospital is located in a region of 400,000 inhabitants and is the only general hospital in the region employing ten or more gastroenterologists. Therefore, a large number of CHB patients requiring HCC surveillance visit regularly as outpatients. Additionally, there is close contact, including sharing of patient mortality data (if the patients died other than in our hospital), between family physician clinic or care hospital in community and our hospital.

Treatments

The 135 patients in the NA group received the following NA therapies: lamivudine (17 patients), lamivudine and adefovir dipivoxil (26 patients), and entecavir (92 patients). The indications for NA therapy in each patient were determined according to the guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), or the Asian Pacific Association for the Study of the Liver (APASL) [7–9]. Of the 135 patients in the non-NA group, 103 did not receive treatment because at the time of their enrollment NA had not yet been approved in Japan, while the remaining 32 patients declined NA therapy.

Statistical analysis

Continuous variables are expressed as medians (range). The Mann–Whitney *U* test was used for continuous variables, and the Chi square test with Yates' correction or Fisher's exact test was used for categorical variables. Actuarial analysis of cumulative survival and mortality was performed using the Kaplan–Meier method, and differences were tested with the log-rank test. Cox proportional hazards models with forward selection were used for multivariate analysis of factors related to survival.

Discrimination of the propensity score model was assessed using the area under the ROC curve [21], with higher values indicating better discrimination. Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test [20]. The Hosmer–Lemeshow test compares model performance (observed versus expected) across deciles of risk to test whether the model is biased (i.e., performs differently at the extremes of risk). A non-significant value for the Hosmer–Lemeshow test suggests an absence of such bias.

We considered *p* values of 0.05 or less to be significant. Statistical analysis was performed with SPSS, version 18.0 for Windows (IBM Japan, Tokyo, Japan).

Results

Patient characteristics and causes of death in all patients

Table 1 shows baseline characteristics of all 919 patients before propensity matching. There were significant differences in age, HBV genotype, HBsAg concentration, HBV DNA concentration, HBcrAg concentration, presence of HBeAg, BCP mutations, platelet count, ALT level, γ-GTP level, and history of interferon (IFN) therapy. HCC developed in 24 of 189 patients (12.7 %) in the NA group and 66 of 730 patients (9.0 %) in the non-NA-

Table 1 Characteristics of all patients

	NA group (n = 189)	Non-NA group (n = 730)	p value
Age (year)[a]	53 (27–81)	48 (0–84)	<0.001
Sex (female/male)	77/112	331/399	0.256
Genotype (A/B/C/D/F/n.d.)	2/6/172/0/1/8	32/70/474/3/0/151	<0.001
HBsAg ($\log_{10}$ IU/ml)[a]	3.5 (0.6–5.5)	3.3 (0.1–7.9)	<0.001
HBV DNA ($\log_{10}$ copies/ml)[a]	6.8 (2.1–9.9)	3.7 (2.1–9.9)	<0.001
HBcrAg ($\log_{10}$ IU/ml)[a]	5.6 (2.9–7.0)	2.9 (2.9–7.0)	<0.001
HBeAg (positive/negative)	93/96	161/569	<0.001
Precore region (W/M/n.d.)	40/127/22	109/416/205	0.382
BCP (W/M/n.d.)	34/125/30	155/323/252	0.008
Platelet count ($\times 10^3/m^3$)[a]	15.2 (3.2–38.8)	19.5 (3.7–55.1)	<0.001
ALT (IU/ml)[a]	69 (7–1088)	25 (5–3410)	<0.001
γ-GTP (IU/ml)[a]	44 (7–530)	22 (5–797)	<0.001
History of IFN therapy (yes/no)	17/172	37/693	0.041
Cirrhosis (absence/presence)	119/70	639/91	<0.001
Development of HCC	24	66	0.171
Initial treatments of HCCs			
Resection	13	35	
RFA	5	7	
PEI	0	2	
TACE	4	9	
HAIC	1	3	
None	1	10	
Follow-up duration (year)[a]	10.9 (3.1–20.9)	10.8 (3.0–20.9)	0.988
Propensity score[a]	0.5072 (0.22079–0.98208)	0.9206 (0.22079–0.99659)	<0.001

[a] Data expressed as medians (range)

NA Nucleos(t)ide analogue, *n.d* Not done, *HBsAg* hepatitis B surface antigen, *HBV* Hepatitis B virus, *HBcrAg* Hepatitis B core-related antigen, *HBeAg* Hepatitis B e antigen, *W* Wild type, *M* Mutant type, *BCP* Basal core promoter, *ALT* Alanine aminotransferase, *γ-GTP* Gamma-glutamyl transpeptidase, *IFN*, Interferon; *HCC*, Hepatocellular carcinoma; *RFA*, Radiofrequency ablation; *PEI* Percutaneous ethanol injection, *TACE* Trancecatheter arterial chemoembolization, *HAIC* Hepatic arterial infusion chemotherapy

Table 2 Causes of death in all patients with HBV ($n = 66$)

ICD code Disease	NA group ($n = 13$)	Non-NA group ($n = 53$)	p value
Liver-related diseases	8/13 (61.5 %)	31/53 (58.5 %)	0.909
C22 HCC	6	26	
I85 Esophageal varices with bleeding	0	1	
K72 Hepatic failure	2	4	
Non-liver-related diseases	5/13 (38.5 %)	22/53 (41.5 %)	
Malignancies			
C18 Colorectal cancer	0	2	
C25 Pancreatic cancer	0	1	
C31 Maxillary cancer	1	0	
C34 Lung cancer	0	3	
C43 Malignant melanoma of skin	0	1	
C50 Breast cancer	0	2	
C85 Malignant lymphoma	1	0	
C92 Acute myeloblastic leukemia	1	1	
Diseases other than malignancies			
J18 Pneumonia	0	2	
J80 Acute respiratory distress syndrome	0	1	
J44 Chronic obstructive pulmonary disease	1	1	
J84 Interstitial pulmonary diseases	0	1	
I46 Cardiopulmonary arrest on arrival	1	1	
I63 Cerebral infarction	0	1	
M32 Systemic lupus erythematosus	0	1	
V03 Injured in transport accident	0	4	

NA Nucleos(t)ide analogue, *HBV* Hepatitis B virus, *ICD* International Statistical Classification of Diseases and Related Health Problems, *HCC* Hepatocellular carcinoma

group during the follow-up period, respectively. Initial treatments of the HCCs are also shown in Table 1. Of the 919 patients, 66 died during follow-up; causes of death are shown in Table 2. Mortality was due to liver-related diseases in 59.1 % (39/66) of patients, with HCC responsible in 82.1 % (32/39) of these. Conversely, in 48.1 % (13/27) of patients who died of non-liver-related diseases, the causes of death were a variety of malignancies other than HCC, including hematological diseases. In non-liver-related diseases other than malignancies, the feature of the causes of death was also not found. There were no significant differences between NA and non-NA groups in terms of causes of death, whether liver-related or non-liver-related.

Patient characteristics and causes of death determined after propensity score matching

The baseline characteristics of the 270 study patients after propensity score matching are summarized in Table 3. There were no significant differences in age, sex, HBV genotype, HBsAg concentration, HBV DNA concentration, HBcrAg concentration, presence of HBeAg, precore region mutations, BCP mutations, platelet count, ALT level, γ-GTP level, history of IFN therapy, or follow-up duration. NA was administered for a median of 5.5 years (range 1.0–10.0 years). HCC developed in 19 of 135 patients (14.1 %) in the NA group and 37 of 135 patients (27.4 %) in the non-NA-group during the follow-up period, respectively. Initial treatments of the HCCs are also shown in Table 3. In the NA group, eight of the 135 patients died during follow-up, and of these 62.5 % (5/8) died due to HCC. Conversely, 23 of 135 patients in the non-NA group died during follow-up, and of these 73.9 % (17/23) patients died due to HCC. Only three patients (13.0 %) died due to malignancies other than HCC.

Cumulative survival and mortality analysis

Figure 2a shows the survival curves for all 919 patients. The respective 5-, 10-, and 15-year cumulative survival rates were 97.7, 94.6, and 91.0 % in the NA-group patients ($n = 189$), and 99.6, 94.2, and 89.1 % in the non-NA-group patients ($n = 730$) ($p = 0.868$). In the survival analysis of the absence or presence of cirrhosis, there were no differences between the NA-group and the non-NA-group in the status of cirrhosis. Additionally, the respective 5-, 10-, and 15-year cumulative mortality rates from HCC were 0.6, 3.1, and 4.7 % in NA-group patients, and 0.2, 1.0, and 5.5 % in non-NA-group patients ($p = 0.788$) (Fig. 2b). In the mortality from HCC analysis of the absence or presence of cirrhosis, there was no difference between the NA-group and the non-NA-group in the non-cirrhotic patients. Conversely, in the cirrhotic patients, the respective 5-, 10-, and 15-year cumulative mortality rates from HCC were 1.5, 5.3, and 5.3 % in the NA- group ($n = 70$), and 1.2, 8.7, and 18.5 % in the non-NA-group ($n = 91$) ($p = 0.047$).

Figure 3a shows the survival curves for 270 patients after propensity score matching. The respective 5, 10, and 15-year cumulative survival rates were 99.2, 94.8, and 91.3 % in NA-group patients ($n = 135$), and 100, 89.4, and 75.4 % in non-NA-group patients ($n = 135$) ($p = 0.015$). In the survival analysis of the absence or presence of cirrhosis, there were no differences between the NA-group and the non-NA-group in the status of cirrhosis. Additionally, the respective 5, 10, and 15-year cumulative mortality rates from HCC were 0.0, 3.5, and 5.8 % in NA-group patients, and 0.0, 7.1, and 19.2 % in non-NA-group patients, ($p = 0.018$) (Fig. 3b). In the mortality from HCC analysis of the absence or presence of

Table 3 Characteristics of patients after propensity score matching

	NA group (n = 135)	Non-NA group (n = 135)	p value
Age (year)[a]	53 (27–81)	51 (15–79)	0.098
Sex (female/male)	57/78	50/85	0.384
Genotype (A/B/C/D/F/n.d.)	2/6/119/0/1/7	4/3/118/1/0/9	0.561
HBsAg ($\log_{10}$ IU/ml)[a]	3.5 (0.6–5.5)	3.4 (0.1–7.9)	0.541
HBV DNA ($\log_{10}$ copies/ml)[a]	6.6 (2.1–9.7)	6.6 (2.1–9.9)	0.963
HBcrAg ($\log_{10}$ IU/ml)[a]	5.4 (2.9–7.0)	5.1 (2.9–7.0)	0.319
HBeAg (positive/negative)	62/73	70/65	0.330
Precore region (W/M/n.d.)	31/88/16	25/94/16	0.657
BCP (W/M/n.d.)	25/86/24	22/92/21	0.743
Platelet count ($\times 10^3/m^3$)[a]	15.7 (3.2–38.8)	15.8 (3.7–47.0)	0.365
ALT (IU/ml)[a]	64 (7–1088)	51 (12–3410)	0.091
γ-GTP (IU/ml)[a]	43 (7–530)	33 (10–797)	0.056
History of IFN therapy (yes/no)	10/125	11/124	0.820
Follow-up duration (year)[a]	10.7 (3.1–20.5)	11.6 (3.0–18.5)	0.281
Cirrhosis (absence/presence)	84/51	94/41	0.248
Development of HCC	19	37	0.011
Initial treatments of HCCs			
Resection	10	15	
RFA	4	6	
PEI	0	2	
TACE	4	8	
HAIC	1	2	
None	0	4	
Mortality	8	23	
Causes			
Liver-related diseases			
HCC	5	17	
Hepatic failure	1	1	
Non-liver-related diseases			
Malignancies	0	3	
Diseases other than malignancies	2	2	
Propensity score[a]	0.6347 (0.22079–0.98208)	0.6347 (0.22079–0.98208)	0.986

[a] Data expressed as medians (range)

NA Nucleos(t)ide analogue, *n.d* Not done, *HBsAg* Hepatitis B surface antigen, *HBV* Hepatitis B virus, *HBcrAg* Hepatitis B core-related antigen, *HBeAg* Hepatitis B e antigen, *W* Wild type, *M* Mutant type, *BCP* Basal core promoter, *ALT* Alanine aminotransferase, *γ-GTP* Gamma-glutamyl transpeptidase, *IFN* Interferon, *HCC* Hepatocellular carcinoma, *RFA* Radiofrequency ablation, *PEI* Percutaneous ethanol injection, *TACE* Trancecatheter arterial chemoembolization, *HAIC* Hepatic arterial infusion chemotherapy

cirrhosis, there was no difference between the NA-group and the non-NA-group in the non-cirrhotic patients. Conversely, in the cirrhotic patients, the respective 5, 10, and 15-year cumulative mortality rates from HCC were 0.0, 5.2, and 5.2 % in the NA- group (n = 51), and 0.0, 11.2, and 30.3 % in the non-NA-group (n = 41) (p = 0.017). In the survival and mortality from HCC analysis of three types of NAs therapies, there were no differences among them.

Figure 4 shows the cumulative mortality from non-liver-related diseases. There were no significant differences between the NA and non-NA groups (a, all 919 patients; b, 270 propensity score–matched patients).

Factors associated with patient survival determined after propensity score matching

Multivariate analysis with Cox proportional hazards modeling using the covariates of age (≤40 years or >40 years), sex (female or male), treatment (NA or non-

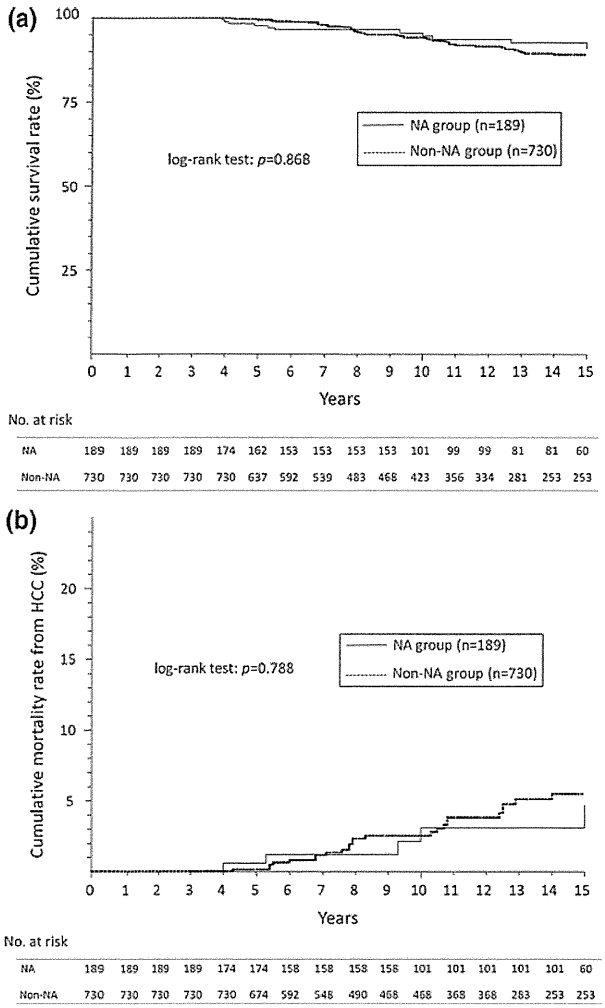

Fig. 2 **a** Cumulative survival in all chronic hepatitis B (CHB) patients (before propensity score matching) according to nucleos(t)ide analogue (NA) treatment status. **b** Cumulative mortality from hepatocellular carcinoma (HCC) in all CHB patients (before propensity score matching) according to NA treatment status. There are no significant differences between NA and non-NA groups in either cumulative survival or mortality from HCC

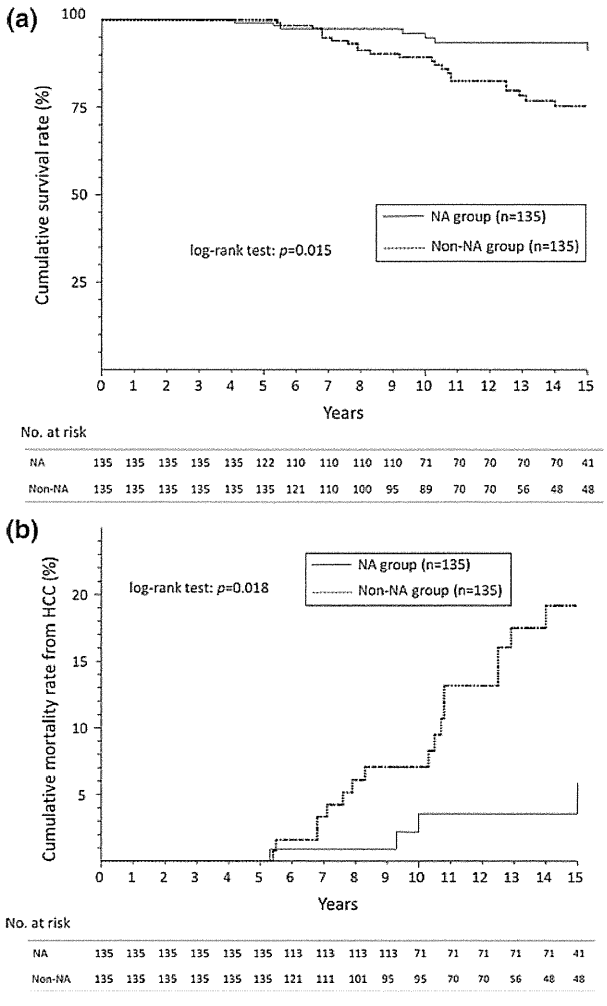

Fig. 3 **a** Cumulative survival in chronic hepatitis B (CHB) patients (after propensity score matching) according to nucleos(t)ide analogue (NA) treatment status. **b** Cumulative mortality from hepatocellular carcinoma (HCC) in all CHB patients (after propensity score matching) according to NA treatment status. There are significant differences between NA and non-NA groups in both cumulative survival ($p = 0.015$) and mortality from HCC ($p = 0.018$)

NA), HBsAg (≤ 3.0 log IU/ml or >3.0 log IU/ml), HBV DNA level (≤5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), precore region (wild type or mutant), BCP (wild type or mutant type), HBcrAg (≤ 3.0 log U/ml or > 3.0 log U/ml), genotype (genotype C or non-genotype C), platelet count (>150 × $10^3/m^3$ or ≤150 × $10^3/m^3$), ALT (≤35 IU/ml or >35 IU/ml), and γ-GTP (≤56 IU/ml or >56 IU/ml) showed that NA therapy was an independent factor associated with improved patient survival (hazard ratio [HR], 0.286; 95 % confidence interval [CI], 0.122–0.668; $p = 0.004$).

Discussion

In the present study, which used propensity score analysis to reduce biases associated with the selection of study patients, long-term NA therapy significantly reduced the cumulative mortality from HCC in CHB patients. In addition, there was no significant difference in non-liver-related mortality between the NA and non-NA groups. These results demonstrated that NA therapy improved the survival of patients who required anti-viral therapy for CHB. Moreover, multivariate analysis with Cox proportional hazards models showed that NA therapy was an

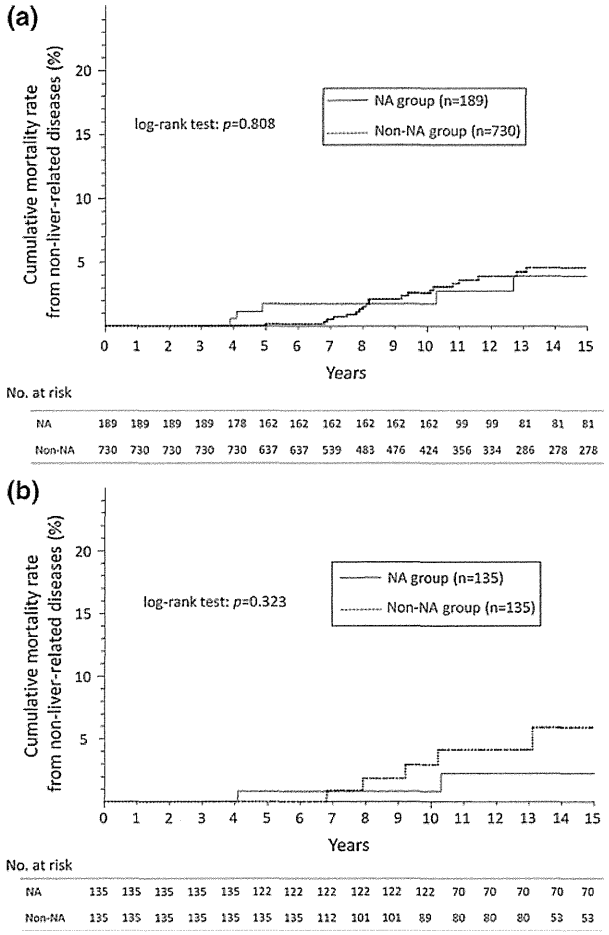

Fig. 4 Cumulative mortality from non-liver-related diseases. **a** Chronic hepatitis B (CHB) patients before propensity score matching. **b** CHB patients after propensity score matching. There are no significant differences between NA and non-NA groups either before or after propensity score matching

independent factor associated with improved survival of CHB patients.

We recently reported that NA therapy reduced the risk of HCC in patients with CHB [11]. In that study, which also used propensity score analysis, the respective 5-, 7-, and 10-year cumulative incidences of HCC were 2.7, 3.3, and 3.3 % in patients on NA therapy ($n = 117$) and 11.3, 26.0, and 40.0 % in patients not on NA therapy ($n = 117$). Further, multivariate analysis with Cox proportional hazards models showed that NA therapy significantly reduced the risk of hepatocarcinogenesis in CHB patients (HR, 0.28; 95 % CI, 0.13–0.62). In the present study, we further assessed the survival and the mortality from both HCC and non-liver related diseases, expanding the number of study patients compared with our previous study for hepatocarcinogenesis. The present study, which demonstrated improved survival of CHB patients on NA therapy, supports our previous results that showed a reduction in hepatocarcinogenesis by NAs. Conversely, other factors that were associated with the development of HCC in that study, including higher age, BCP mutations, and high HBcrAg and γ-GTP concentrations, were not identified in this study as independent factors influencing survival of CHB patients. It was considered that these factors associated with hepatocarcinogenesis [11] did not influence the survival of CHB patients, especially, after HCC development. NA therapy for CHB patients has been reported to not only prevent disease progression from advanced liver disease but also to reverse decompensated cirrhosis [32–34]. Thus even if HCC has developed in patients receiving NA, it is assumed that treatment of recurrent HCC is possible while maintaining liver function. In the present study, particularly, in the analysis of cirrhotic patients, the cumulative mortality rates from HCC in the NA-group were significantly lower than in the non-NA-group in both all and propensity score matched patients.

Chen et al. [35] used community cohort data to analyze mortality from non-liver-related causes of death in patients with CHB. They reported that the relative risks (RRs) and 95 % CIs for all non-liver-related deaths among HBsAg-positive subjects were 1.2 (1.1–1.3) in males and 1.4 (1.1–1.7) in females. Non-liver-related causes were further subdivided into cancer and non-cancer groups. For all non-liver cancers, the RRs were 1.2 (1.0–1.4) for males and 1.7 (1.2–2.3) for females. Non-cancer deaths that were non-liver-related had RRs of 1.2 (1.1–1.4) and 1.2 (0.9–1.6) in males and females, respectively. They concluded that HBV-infected individuals may be at increased mortality risk from non-liver-related causes; possible reasons include the direct effect of HBV infection, changes in the host immune system as a cause or effect of chronic infection, and behavioral factors associated with HBV infection.

In the present study, 66 of all 919 CHB patients died during follow-up; in approximately 40 % (27/66) of cases the causes of death were non-liver-related diseases, of which about 50 % (13/27) were malignancies other than HCC. Although this study was based on hospital-based subjects, we performed detailed analysis to categorize NA administration status in CHB patients compared with Chen et al.'s study. Additionally, our study revealed no significant difference in cumulative mortality between the NA and non-NA groups before and after propensity score matching. Further, malignancies arose from a variety of organs, and thus we recommend that CHB patients be monitored not only for the development of liver-related diseases but non-liver-related disease as well, particularly malignancies.

Since the present study was retrospective in nature, we used propensity score analysis to reduce the selection bias associated with indications for NA therapy. The p value of

0.372 by the Hosmer–Lemeshow test, which evaluates the goodness-of-fit for the calculated propensity score, was considered reassuring [20]. Additionally, the AUC of 0.862 (95 % CI, 0.834–0.891) in the ROC analysis suggested excellent discrimination for the calculated propensity score [21]. Consequently, the backgrounds and clinical data of propensity score–matched patients did not differ significantly between the NA and control groups.

The main limitations of this study include the hospital-based population and its retrospective nature. Although our hospital is located in a region of 400,000 inhabitants and is the only general hospital visited by a large number of CHB patients, further prospective studies with community-based subjects are warranted. Another limitation was that the propensity score analysis results may be limited by biases related to unmeasured and hidden covariates. Finally, one-to-one matching based on propensity scores resulted in a reduction in the number of patients included.

In conclusion, the survival of patients who received antiviral NA therapy for CHB was improved compared with that of untreated controls, and NA therapy specifically reduced the risk of HCC mortality. In addition, the causes of death of approximately 40 % of CHB patients who died during follow-up were non-liver-related. Further studies are warranted to confirm these findings in other populations.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lai CL, Ratziu V, Yuen MF, et al. Viral hepatitis B. Lancet. 2003;362:2089–94.
2. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11:97–107.
3. Beasley RP, Hwang LY, Lin CC, et al. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet. 1981;2:1129–33.
4. Szmuness W. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. Prog Med Virol. 1978;24:40–69.
5. Rustgi VK. Epidemiology of hepatocellular carcinoma. Gastroenterol Clin North Am. 1987;16:545–51.
6. Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology. 2004;127(Suppl 1):S17–26.
7. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661–2.
8. European Association For The Study Of The Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167–85.
9. Liaw Yun-Fan, Kao Jia-Horng, Piratvisuth Teerha, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int. 2012;6:531–61.
10. Hosaka T, Suzuki F, Kobayashi M, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology. 2013;58:98–107.
11. Kumada T, Toyoda H, Tada T, et al. Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: a propensity score analysis. J Hepatol. 2013;58:427–33.
12. Rosenbaum PR, Rubin DB. The Central Role of the Propensity Score in Observational Studies for Causal Effects. Biometrika. 1983;70:41–55.
13. Rosenbaum PR, Rubin DB. Reducing bias in observational studies using subclassification on the propensity score. J Am Stat Assoc. 1984;79:516–24.
14. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. Am Stat. 1985;39:33–8.
15. Joffe MM, Rosenbaum PR. Invited commentary: propensity scores. Am J Epidemiol. 1999;150:327–33.
16. Liaw YF. Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. Liver Int. 2009;29(Suppl 1):100–7.
17. Chen CJ, Yang HI, Su J, et al. REVEAL-HBV study group risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.
18. Yuen MF, Yuan HJ, Wong DK, et al. Prognostic determinants for chronic hepatitis B in asians: therapeutic implications. Gut. 2005;54:1610–4.
19. Kumada T, Toyoda H, Kiriyama S, et al. Incidence of hepatocellular carcinoma in patients with chronic hepatitis B virus infection who have normal alanine aminotransferase values. J Med Virol. 2010;82:539–45.
20. Hosmer DW, Lemeshow S. Applied logistic regression. New York: John Wiley & Sons; 2000.
21. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology. 1982;143:29–36.
22. Kato H, Orito E, Sugauchi F, et al. Determination of hepatitis B virus genotype G by polymerase chain reaction with hemi-nested primers. J Virol Methods. 2001;98:153–9.
23. Kimura T, Rokuhara A, Matsumoto A, et al. New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and relation between levels of HBcAg and HBV DNA. J Clin Microbiol. 2003;41:1901–6.
24. Wong DK, Tanaka Y, Lai CL, et al. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol. 2007;45:3942–7.
25. Liu CJ, Chen PJ, Lai MY, et al. Evolution of precore/core promoter mutations in hepatitis B carriers with hepatitis B e antigen seroreversion. J Med Virol. 2004;74:237–45.
26. Kao JH, Wu NH, Chen PJ, et al. Hepatitis B genotypes and the response to interferon therapy. J Hepatol. 2000;33:998–1002.
27. The Japan Society of Hepatology. Surveillance algorithm and diagnostic algorithm for hepatocellular carcinoma : Clinical Practice Guidelines for Hepatocellular Carcinoma. Hepatology Res. 2010;40(Supplement s1):6–7.
28. Shen L, Li JQ, Zeng MD, et al. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. World J Gastroenterol. 2006;12:1292–5.
29. Iacobellis A, Fusilli S, Mangia A, et al. Ultrasonographic and biochemical parameters in the non-invasive evaluation of liver fibrosis in hepatitis C virus chronic hepatitis. Aliment Pharmacol Ther. 2005;22:769–74.
30. Caturelli E, Castellano L, Fusilli S, et al. Coarse nodular US pattern in hepatic cirrhosis: risk for hepatocellular carcinoma. Radiology. 2003;226:691–7.
31. World Health Organization, The ICD-10 classification of mental and behavioural disorders : clinical descriptions and diagnostic guidelines.(1992).

32. Lai CL. Therapeutic advances in chronic hepatitis B. J Gastroenterol Hepatol. 2004;19(Suppl):S5–9.
33. Leung N. Chronic hepatitis B-treatment with nucleoside analogues. Med J Malaysia. 2005;60(Suppl):22–7.
34. Takeda A, Jones J, Shepherd J, et al. A systematic review and economic evaluation of adefovir dipivoxil and pegylated interferon-alpha-2a for the treatment of chronic hepatitis B. J Viral Hepat. 2007;14:75–88.
35. Chen G, Lin W, Shen F, et al. Chronic hepatitis B virus infection and mortality from non-liver causes: results from the Haimen City cohort study. Int J Epidemiol. 2005;34:132–7.

Journal of Gastroenterology and Hepatology JGHF

doi:10.1111/jgh.12915

HEPATOLOGY

Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection

Hidenori Toyoda, Takashi Kumada, Toshifumi Tada, Seiki Kiriyama, Makoto Tanikawa, Yasuhiro Hisanaga, Akira Kanamori, Shusuke Kitabatake and Takanori Ito

Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

Key words
antiviral therapy, chronic hepatitis C, hepatocellular carcinoma, risk factors, sustained virologic response.

Accepted for publication 25 January 2015.

Correspondence
Dr Hidenori Toyoda, Department of Gastroenterology, Ogaki Municipal Hospital, 4-86, Minaminokawa, Ogaki, Gifu 503-8502, Japan. Email, hmtoyoda@spice.ocn.ne.jp

Conflict of interest: There is no conflict of interest on this study.

Financial support: There is no grant or other financial support on this study.

Abstract

Background and Aim: Hepatocellular carcinoma (HCC) can develop in patients with chronic hepatitis C after they have achieved a sustained virologic response (SVR) to antiviral therapy, that is eradication of hepatitis C virus (HCV). Thus, surveillance for HCC remains necessary after SVR. We investigated factors that are predictive of HCC in HCV-infected patients who achieved SVR.

Methods: The incidence and risk factors for HCC were evaluated in 522 patients who achieved SVR with interferon-based antiviral therapy for HCV. Patients maintained regular follow-up every 6 months for HCC surveillance. The FIB-4 index and aspartate aminotransferase to platelet count ratio index were calculated based on laboratory data at the time that SVR was documented (SVR24).

Results: Patients continued follow-up visits for 1.0–22.9 years (median, 7.2 years) after SVR. HCC developed in 18 patients. The incidence of HCC was 1.2% at 5 years and 4.3% at 10 years. The use of peginterferon or ribavirin for treatment and a history of antiviral therapy prior to the course when SVR was achieved were not associated with the incidence of HCC after SVR. The presence of diabetes mellitus (risk ratio 2.08; $P = 0.0451$) and FIB-4 index calculated at the time of SVR24 (risk ratio 1.73; $P = 0.0198$) were associated with a higher likelihood of HCC after SVR by multivariate analysis.

Conclusions: Patients with diabetes mellitus and patients with the elevation of FIB-4 index at SVR24 are at higher risk of HCC after SVR. Surveillance for HCC should be continued in this patient subpopulation.

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide,[1] and its incidence is predicted to increase in Western countries.[2,3] Chronic hepatitis C virus (HCV) infection is a major cause of HCC,[3–5] and the prevention of HCC is a major goal of antiviral therapy in patients with chronic hepatitis C.

Sustained virologic response (SVR) is defined as the eradication of HCV with antiviral therapy. The benefit of HCV eradication is the prevention of the progression of chronic hepatitis and associated complications.[6] Several studies have confirmed that achievement of SVR results in the resolution of liver fibrosis[7–9] and a decreased incidence of HCC.[10–14] However, the development of HCC is sometimes observed in patients who achieve SVR.[15–19] Some cases involved very advanced HCC with very poor prognosis at the time of detection. Due to the decreased risk of HCC development in patients with SVR, they are less likely to participate in surveillance for HCC after SVR.[20] This may result in a failure to detect small HCCs, allowing the tumor to grow to a large size before detection and diagnosis at an advanced stage.

The emergence of new direct-acting antiviral drugs (DAAs) against HCV will dramatically increase the number of patients who achieve SVR.[21–24] Given the marked increase in the number of patients who achieve SVR, there will be an increase in the number of patients who develop HCC after SVR in the near future. Therefore, understanding the incidence and risk factors for the development of HCC in patients after SVR will be important for the management of this patient subpopulation.

In the present study, we investigated the incidence and risk factors for HCC in 522 patients with chronic HCV infection who achieved SVR with interferon (IFN)-based antiviral therapy.

Methods

Patients and follow-up. Between 1990 and 2012, a total of 1285 patients with chronic HCV infection underwent IFN-based

antiviral therapy at our institution. Patients were excluded if they had antibodies against human immunodeficiency virus or hepatitis B virus surface antigen or other forms of liver disease (e.g. autoimmune hepatitis, alcoholic liver disease, or hemochromatosis). Patients with cirrhosis were not included because IFN-based antiviral therapy is not permitted by the Japanese National Medical Insurance System for patients who had cirrhosis at the start of the antiviral therapy. Of these, 522 patients achieved SVR. HCV infection was confirmed by positive HCV antibody titers and the presence of serum HCV-RNA before treatment. SVR was confirmed by the absence of serum HCV-RNA 24 weeks after the end of treatment (SVR24). No patient had a history of HCC. The absence of HCC was confirmed by imaging studies in all patients at the start of antiviral therapy and when SVR was documented (i.e. SVR24). Liver biopsy was performed in 494 patients prior to the start of antiviral therapy. Liver histology was classified according to the METAVIR score.[25]

Patients continued to follow-up every 6 months after SVR with laboratory testing and ultrasonography at every visit. The absence of serum HCV-RNA was reconfirmed annually (at every two visits). Laboratory tests included complete blood cell count, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and tumor markers for HCC (alpha fetoprotein [AFP] and des-gamma-carboxy prothrombin). If a nodular lesion was detected by ultrasonography or an elevation in a tumor marker was observed, additional imaging studies (computed tomography, magnetic resonance imaging, or both) were performed. The diagnosis of HCC was based on appropriate imaging characteristics according to the criteria in the guidelines of the American Association for the Study of Liver Diseases,[26,27] with the findings of arterial hypervascularity and venous or delayed phase washout by contrast-enhanced dynamic computed tomography or magnetic resonance imaging. In addition, HCC was confirmed histologically based on the resected specimen when patients underwent surgical resection as a treatment.

The entire protocol was approved by the hospital institutional review board and carried out in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participating patients before the enrollment of the study.

Estimation of liver fibrosis when SVR was achieved with FIB-4 index and with the aspartate aminotransferase to platelet count ratio index (APRI). Liver fibrosis was estimated at SVR24 using the FIB-4 index[28] calculated as AST (IU/L) × age (years)/platelet count (10^9/L) × ALT (IU/L)$^{1/2}$, and using the APRI[29] calculated as AST (IU/L) (/upper limit of normal AST [IU/L]) × 100/platelet count (10^9/L).

Statistical analysis. SVR24 was defined as time zero for calculating the incidence of HCC. In the analysis of HCC incidence, patients who developed HCC were non-censored and those who did not were censored. The Kaplan–Meier method was used to calculate the rate of HCC development, and the log–rank test was used to analyze differences in incidence. A Cox proportional hazards model was used for univariate and multivariate analyses of factors related to the development of HCC. Univariate analysis was first performed for all variables analyzed. Variables that reached statistical significance ($P < 0.05$) in the univariate analysis were subsequently included in the multivariate analysis. Data analysis was performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC, USA). All P values were derived from two-tailed tests, with $P < 0.05$ accepted as statistically significant.

Results

Patients characteristics. Table 1 shows the baseline patient characteristics before antiviral therapy. Patients consisted of 292 (55.9%) males and 230 (44.1%) females, with a mean age of 50.6 ± 11.8 years. Approximately 20% of patients reported habitual alcohol intake (more than 50 g per day for more than 5 years) and 8.2% of patients had type 2 diabetes mellitus, defined based on the American Diabetes Association revised criteria.[30] Less than half of the patients achieved SVR with use of peginterferon (PEG-IFN) or use of ribavirin for therapy. More than half of the patients had been infected with HCV genotype 2 (2a or 2b) before the eradication, although genotype 1b is predominant in Japan. This is because patients with genotype 2 were more likely to achieve SVR than those with genotype 1. No patients had F4 fibrosis due to the disability for patients with cirrhosis to undergo IFN-based antiviral therapy by National Insurance System. The FIB-4 index and APRI at SVR24 was 1.99 ± 1.52 and 0.45 ± 0.52, respectively. The FIB-4 index at SVR24 was increased with the increase of pretreatment liver fibrosis grade as assessed in liver biopsy specimens despite the normalization of serum transaminase activity (Fig. 1).

Table 1 Baseline characteristics of the study patients before antiviral therapy (n = 522)

Age (years)†	50.6 ± 11.8
Sex (female/male)	230 (44.1)/292 (55.9)
Habitual alcohol intake (no/yes)	322 (79.9)/81 (20.1)
Diabetes mellitus (no/yes)	448 (91.8)/40 (8.2)
Treatment when SVR was achieved (naïve/retreatment)	410 (78.5)/112 (21.5)
Ribavirin use (no/yes)	267 (51.1)/255 (48.9)
Peginterferon use (no/yes)	282 (54.0)/240 (46.0)
Body mass index	23.2 ± 9.2
Baseline ALT (IU/L)	96.5 ± 93.8
Baseline AST (IU/L)	65.2 ± 57.3
Baseline GGTP (IU/L)	54.8 ± 73.5
Baseline albumin (g/dL)	4.16 ± 0.33
Baseline total bilirubin (mg/dL)	0.72 ± 0.65
Baseline AFP (ng/dL)	4.16 ± 6.68
Baseline platelet counts (× 10^3/μL)	187 ± 58
HCV genotype (1b/2a or 2b)	227 (44.6)/282 (55.4)
Pretreatment HCV-RNA levels (log_{10}IU/mL)	5.25 ± 1.47
Pretreatment liver activity (A0/A1/A2/A3)	31 (6.3)/296 (59.9)/150 (30.4) 17 (3.4)
Pretreatment liver fibrosis (F0/F1/F2/F3)	83 (16.8)/263 (53.2)/121 (24.5)/27 (5.5)

†At 24weeks after the end of antiviral therapy when SVR was documented (i.e. SVR24).

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; SVR, sustained virologic response.

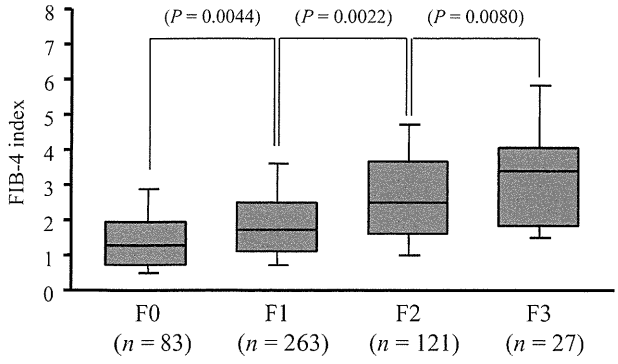

Figure 1 Association between pretreatment liver fibrosis as assessed by pathological evaluation of liver biopsy specimens and FIB-4 index at SVR24. The liver fibrosis grade was based on the METAVIR score.[25] FIB-4 index at SVR24 was 1.52 ± 1.10 in patients with F0, 1.81 ± 1.17 in F1, 2.36 ± 1.89 in F2, and 3.59 ± 2.66 in F3. SVR, sustained virologic response.

Incidence of HCC and risk factors associated with the development of HCC after SVR. During a median follow-up of 7.2 years (range, 1.0–22.9 years) in patients with SVR, HCC was diagnosed through screening in 18 patients with the median interval of 6.6 years (range, 1.5–17.1 years). Fifty-one of 522 patients (9.8%) were lost for follow-up during the study period after 2.3–18.2 years' follow-up, who were treated as censored cases. HCC was treated by surgical resection according to treatment guidelines for HCC in Japan,[31] and diagnosis of HCC was confirmed also histologically based on the resected specimen after treatment in 15 patients. The incidence of HCC at 5 and 10 years was 1.2% and 4.3%, respectively.

Risk factors associated with the development of HCC after SVR were investigated. Based on the univariate analysis, patient age (risk ratio [RR] 1.06; $P = 0.0228$), sex (RR 1.87; $P = 0.0250$), habitual alcohol intake (RR 1.72; $P = 0.0484$), diabetes mellitus (RR 1.91; $P = 0.0277$), baseline albumin (RR 0.12; $P = 0.0045$), baseline platelet counts (RR 0.82; $P = 0.0002$), liver fibrosis grade before treatment based on percutaneous liver biopsy (RR 2.35; $P = 0.0005$), FIB-4 index at SVR24 (RR 1.51; $P < 0.0001$), and APRI at SVR24 (RR 2.04; $P = 0.0031$) were identified as factors significantly associated with a likelihood of HCC after SVR (Table 2a). Pretreatment HCV-RNA levels, HCV genotype, past history of antiviral treatment, and use of PEG-IFN or use of ribavirin in the treatment regimen that achieved SVR were not associated with HCC development after SVR. Presence of diabetes mellitus (RR 2.08; $P = 0.0453$) and higher FIB-4 index at SVR24 (RR 1.73; $P = 0.0198$) were selected as a factor significantly associated with a higher likelihood of HCC according to the multivariate analysis (Table 2b).

Figure 2 shows the cumulative incidence of HCC after SVR based on the FIB-4 index at SVR24. The optimal cut-off point for the FIB-4 index was determined with a Cox proportional hazards model and the distribution of the FIB-4 index in the study patients. Patients were classified as having a FIB-4 index of < 2.0 or ≥ 2.0. The incidence of HCC in patients with a FIB-4 index ≥ 2.0 was significantly higher than that of patients with FIB-4 index < 2.0 ($P = 0.0001$).

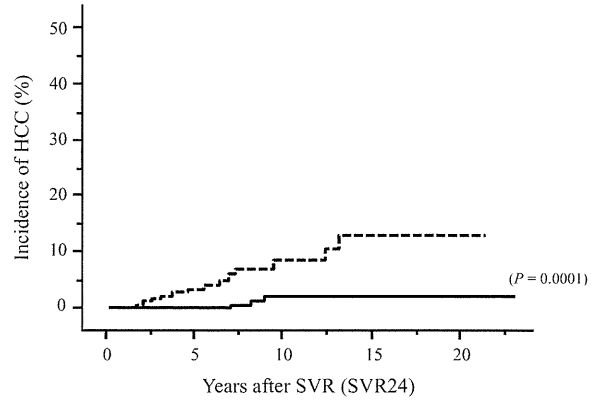

Figure 2 The incidence of hepatocellular carcinoma after SVR according to the FIB-4 index at SVR24. The incidence of HCC at 5 and 10 years was 0% and 2.0%, respectively, in patients with FIB-4 index at SVR24 < 2.0, and was 3.4% and 9.2%, respectively, in patients with FIB-4 index at SVR24 ≥ 2.0. —, FIB-4: < 2.0 ($n = 334$); - - -, FIB-4: ≥ 2.0 ($n = 188$). HCC, hepatocellular carcinoma; SVR, sustained virologic response.

Characteristics of patients who developed HCC after SVR. Table 3 summarizes the characteristics of 18 patients who developed HCC after SVR. The interval between SVR24 and the diagnosis of HCC was 6.76 ± 4.19 years. The percentages of patients who achieved SVR with the use of PEG-IFN or use of ribavirin was smaller comparing the entire study patients. AFP level increased significantly at HCC development, compared with the baseline AFP level ($P = 0.0437$). Whereas APRI at HCC development was significantly lower than that at SVR24 ($P = 0.0424$), no significant decrease was observed in FIB-4 index between at SVR24 and at HCC development ($P = 0.1750$). Liver fibrosis progressed to cirrhosis at the development of HCC in 6 of 15 patients (40.0%) who underwent surgical resection as a treatment of HCC and non-cancerous liver tissue at HCC development was available (Table S1).

Discussion

Some HCCs detected and diagnosed after the achievement of SVR could have been minute tumors that were undetectable by imaging studies during or even before antiviral therapy that grew to a detectable size after SVR.[15,32,33] However, our previous analysis of tumor volume doubling time of HCC detected after SVR suggests that HCC does develop after the eradication of HCV.[34] Indeed, there are several case reports of small HCC tumors diagnosed more than 5 years after SVR.[16,17] These findings highlight the importance of understanding the incidence and risk factors for HCC development after SVR.

In the present study, we investigated the incidence and risk factors for HCC diagnosed after the eradication of HCV in more than 500 patients who underwent surveillance for median 7.2 years. The incidence of HCC was 1.2% at 5 years and 4.3% at 10 years after SVR, which was significantly lower than the reported

Table 2 Univariate and multivariate analysis of factors associated with the development of HCC after SVR (n = 522)

		Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*P*-value
(a) *Univariate analysis*						
Age (years) at SVR24		0.0591	0.0280	5.19	1.0609 (1.0077–1.1247)	0.0228
Sex	Female				1	
	Male	0.6271	0.3165	5.02	1.8721 (1.0747–3.8910)	0.0250
Habitual alcohol intake	No				1	
	Yes	0.5450	0.2593	3.90	1.7246 (1.0042–2.8366)	0.0484
Diabetes mellitus	No				1	
	Yes	0.6488	0.2635	4.85	1.9132 (1.0830–3.1181)	0.0277
Treatment that achieved SVR	Naïve				1	
	Retreatment	0.1985	0.2642	0.53	1.2195 (0.6897–1.9906)	0.4664
Ribavirin use	No				1	
	Yes	−0.0444	0.2800	0.03	0.9565 (0.5284–1.6235)	0.8734
Peginterferon use	No				1	
	Yes	−0.0837	0.3024	0.08	0.9197 (0.4766–1.6090)	0.7797
Body mass index		−0.0547	0.0736	0.60	0.9468 (0.8384–1.0184)	0.4377
Baseline ALT (IU/L)		−0.0009	0.0029	0.12	0.9991 (0.9902–1.0022)	0.7312
Baseline AST (IU/L)		−0.0033	0.0035	1.45	0.9967 (0.9882–1.0010)	0.2287
Baseline GGTP (IU/L)		0.0032	0.0021	1.62	1.0033 (0.9977–1.0066)	0.2033
Baseline albumin (g/dL)		−2.0868	0.7299	8.05	0.1241 (0.0295–0.5220)	0.0045
Baseline total bilirubin (mg/dL)		−0.1743	0.5316	0.14	0.8400 (0.1760–1.5222)	0.7116
Baseline AFP (ng/dL)		−0.0031	0.0215	0.03	0.9969 (0.8847–1.0113)	0.8641
Baseline platelet count ($\times 10^3/\mu L$)		−0.1953	0.0589	13.53	0.8226 (0.7281–0.9176)	0.0002
HCV genotype	2a/2b				1	
	1b	−0.2449	0.2539	0.97	0.7828 (0.4594–1.2694)	0.3254
Pretreatment HCV level		−0.1841	0.1436	1.56	0.8319 (0.6328–1.1153)	0.2114
Pretreatment liver activity	A0/A1				1	
	A2/A3	0.1766	0.2521	0.48	1.1931 (0.7132–1.9549)	0.4879
Pretreatment liver fibrosis	F0/F1				1	
	F2/F3	0.8549	0.2634	12.18	2.3511 (1.4429–4.1526)	0.0005
FIB-4 index at SVR24		0.4095	0.0728	18.21	1.5060 (1.2841–1.7207)	< 0.0001
APRI at SVR24		0.7139	0.1832	8.76	2.0419 (1.3326–2.8052)	0.0031
(b) *Multivariate analysis*						
Age (years) at SVR24		0.0031	0.0448	0.005	1.0031 (0.9209–1.0992)	0.9442
Sex	Female				1	
	Male	0.6372	0.4373	2.48	1.8911 (0.8657–5.0473)	0.1151
Habitual alcohol intake	No				1	
	Yes	0.4572	0.3038	2.18	1.5797 (0.8543–2.8814)	0.1402
Diabetes mellitus	No				1	
	Yes	0.7318	0.3418	4.01	2.0788 (1.0170–4.0133)	0.0453
Baseline albumin (g/dL)		−1.7984	0.9374	3.70	0.1656 (0.0252–1.0343)	0.0544
Baseline platelet counts ($\times 10^3/\mu L$)		−0.0108	0.0084	1.90	0.9893 (0.9712–1.0041)	0.1679
Liver fibrosis	F0/F1				1	
	F2/F3	−0.1384	0.3373	0.17	0.8708 (0.4494–1.7262)	0.6829
FIB-4 index at SVR24		0.5474	0.2387	5.43	1.7288 (1.0927–2.8570)	0.0198
APRI at SVR24		0.0318	0.6396	0.002	1.0323 (0.2436–3.2694)	0.9604

AFP, alpha fetoprotein; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet count ratio index; AST, aspartate aminotransferase; GGTP, gamma-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR, sustained virologic response.

incidence of HCC in patients who underwent IFN-based antiviral therapy but failed SVR.[10–14] The incidence of HCC development in non-cirrhotic patients who achieved SVR in this study was higher than the incidence of HCC in non-cirrhotic patients with SVR in Western countries and similar to those with advanced fibrosis.[35] This is partly because the age of patients with chronic hepatitis C in Japan is older than those in Western countries.[36,37] The use of PEG-IFN or the use of ribavirin did not affect the incidence of HCC after SVR. This means that the risk of HCC development did not differ according to the antiviral treatment regimen used if HCV was eradicated.

Although univariate analysis identified patient age, sex, habitual alcohol intake, diabetes mellitus, baseline serum albumin and platelet count, pretreatment liver fibrosis, FIB-4 index at SVR24, and APRI at SVR24 as factors associated with HCC development after SVR, only the presence of diabetes mellitus and the FIB-4