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Utility of the FIB-4 Index for hepatocarcinogenesis in hepatitis C virus carriers with normal alanine aminotransferase levels

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SUMMARY. The FIB-4 index is a simple formula using age, aspartate aminotransferase, alanine aminotransferase (ALT) and platelet count to evaluate liver fibrosis. We investigated the ability of the FIB-4 index for hepatocarcinogenesis in hepatitis C virus (HCV) carriers with normal ALT levels. A total of 516 patients with ALT levels persistently at or below 40 IU/L during an observation period of over 3 years were included. Factors associated with the development of HCC were determined. Hepatocellular carcinoma (HCC) developed in 60 of 516 patients (11.6%). The incidence rate of HCC at 5 and 10 years was 2.6% and 17.6%, respectively. When patients were categorized according to the FIB-4 index as ≤2.0 (n = 226), >2.0 and ≤4.0 (n = 169), and >4.0 (n = 121), the cumulative incidence of HCC at 5 years was 0.5%, 1.3% and 8.0%, respectively, and 2.8%, 25.6% and 37.1% at 10 years, respectively. Patients with FIB-4 index >4.0 were at the highest risk ($P < 0.001$). Factors that were significantly associated with HCC in the multivariate analysis were FIB-4 index >2.0 (hazard ratio (HR), 7.690), FIB-4 index >4.0 (HR, 8.991), α-fetoprotein (AFP) >5 ng/mL (HR, 2.742), AFP >10 ng/mL (HR, 4.915) and total bilirubin >1.2 mg/dL (HR, 2.142). A scoring system for hepatocarcinogenesis that combines the FIB-4 index and AFP predicted patient outcomes with excellent discriminative ability. The FIB-4 index is strongly associated with the risk of HCC in HCV carriers with normal ALT levels.

Keywords: FIB-4 index, hepatitis C, hepatocellular carcinoma, α-fetoprotein.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide [1]. Hepatitis C virus (HCV) infection accounts for approximately 75–80% of HCC cases in Japan. It has been reported that liver fibrosis is an important factor in hepatocarcinogenesis [2]; therefore, making a precise evaluation of liver fibrosis is essential for predicting the incidence of HCC in HCV patients.

Liver biopsy is the gold standard for assessing liver fibrosis [3]; however, it is associated with rare but lethal complications such as haemorrhage [4]. Furthermore, pathological assessment is not always objective [5]. Recently, several noninvasive methods for assessing liver fibrosis have been reported [6–9]. With regards to laboratory data, the aminotransferase (AST) to platelet ratio index (APRI) [10] and FibroTest (Biopredictive SAS, Paris, France) [11] have been reported as tools for evaluating liver fibrosis. The FIB-4 index was developed as another noninvasive marker of fibrosis in patients with chronic hepatitis C and nonalcoholic fatty liver disease [12–14].

It has been reported that HCC occurs not only in HCV carriers with high levels of alanine aminotransferase (ALT), but also in those with persistently normal ALT (PNALT) levels [15,16]. Therefore, the risk of HCC in HCV patients with PNALT requires assessment. In this study, we evaluated the utility of the FIB-4 index for predicting the incidence of HCC in hepatitis C carriers with normal ALT levels.

Abbreviations: AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, the aminotransferase to platelet ratio index; AST, asparate aminotransferase; ChE, cholinesterase; CI, confidence interval; CT, computed tomography; DSA, digital subtraction angiography; EASL, European Association for the Study of the Liver; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PNALT, persistently normal ALT; US, ultrasonography; γ-GTP, γ-glutamyl transpeptidase.

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MATERIALS AND METHODS

Patient selection

A total of 4620 patients who tested positive for HCV visited the Department of Gastroenterology at Ogaki Municipal Hospital, Japan, between September 1995 and August 2004.

Among these patients, we identified 516 patients who were confirmed to be positive for HCV RNA for at least 2 time points with a >6 month interval, had no evidence of hepatitis B virus (HBV) infection, had no other potential causes of chronic liver disease (i.e. alcohol consumption < 80 g/day, no history of hepatotoxic drug use and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis and Wilson's disease), had a follow-up period >3 years, had no evidence of HCC at study entry and for at least 3 years from the start of the follow-up period, had no antiviral therapy involving interferon and/or ribavirin, had ALT measurements taken more than twice annually and had ALT values < 40 IU/L.

Previously, we suggested that the time integral of the ALT level ('integration value') would be more useful for predicting the incidence of HCC. The average ALT integration value was well correlated with the cumulative incidence of hepatocarcinogenesis [16, 17]. Therefore, we adopted average ALT integration values as the metric in this study. HCV genotype was determined by PCR using genotype-specific primers [18], and HCV RNA was quantitated (Amplicor 2; Diagnostics K.K., Tokyo, Japan) [19]. Blood biochemistry data except for ALT were obtained at the start of the follow-up period.

HCC surveillance and diagnosis

Ultrasonography (US) and blood tests including the tumour marker AFP were performed every 3 to 6 months for HCC surveillance. When abdominal US suggested a lesion suspicious for HCC or there was an abnormal rise in tumour marker levels, additional imaging such as contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI) or angiography was performed. The diagnosis of HCC was confirmed through histological examination or via typical radiological findings such as hypervascularity with digital subtraction angiography (DSA) or hyperattenuation with CT during hepatic arteriography in addition to US, CT and MRI. HCC was diagnosed according to European Association for the Study of the Liver (EASL) guidelines [20].

Calculation of the FIB-4 index

The FIB-4 index was calculated at the start of follow-up using the following formula: FIB-4 index = AST [IU/L] × age [years]/platelet count [10^9/L] × ALT [IU/L]$^{1/2}$. The optimal FIB-4 index cut-off point was determined using a Cox proportional hazards model and the distribution of patients. Patients were grouped according to their FIB-4 index score as follows: ≤2.0 (n = 226), >2.0 and ≤4.0 (n = 169), and >4.0 (n = 121).

Calculation of the score that combines the FIB-4 index and AFP

As AFP is reportedly a factor strongly associated with HCC development [21], we established a new scoring system that combines the FIB-4 index and AFP. AFP was measured in 477 patients at the start of follow-up period. AFP levels categorized as ≤5.0 ng/mL, >5.0 and ≤10.0 ng/mL, and >10.0 ng/mL were scored as 1, 2 and 3, respectively. The FIB-4 index, categorized as ≤2.0, >2.0 and ≤4.0, and >4.0, were scored as 1, 2 and 3, respectively. The total score was the sum of the FIB-4 index and AFP scores (Table 1). We estimated the incidence of hepatocarcinogenesis with this new scoring system.

Statistical analysis

The SPSS software package, version 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables are expressed as medians (quartile points). Associations between the FIB-4 index and serum AFP levels were analysed using Spearman's rank correlation. An actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed using the Kaplan–Meier method, and differences across groups were compared using a log-rank test. Cox proportional hazards modelling with the forward selection method was used to estimate the hazard ratio of HCC development associated with the following parameters: sex (female or male), FIB-4 index (≤2.0, >2.0 and ≤4.0, or >4.0), γ-glutamyl transpeptidase (γ-GTP) (≤56 IU/L or >56 IU/L), total bilirubin (≤1.2 mg/dL or >1.2 mg/dL), alkaline phosphatase (ALP) (≤338 IU/mL or >338 IU/mL), lactate dehydrogenase (LDH) (≤251 IU/L or >251 IU/L), albumin (< 3.5 g/dL or ≥3.5 g/dL), cholinesterase (≤431 IU/L or >431 IU/L) and AFP (≤5.0 ng/mL, >5.0 and ≤10.0 ng/mL, or >10.0 ng/mL). We used the lower or upper limit of the reference values at our institution as cut-off values for laboratory data. Statistical significance was defined as $P < 0.05$. The study protocol was approved by the institutional review board of Ogaki Municipal Hospital and conducted in compliance with the Helsinki Declaration.

Table 1 Calculation of the combined FIB-4 index and AFP score. It is the sum of the scores based on the FIB-4 index and AFP as follows

	Score		
	1	2	3
FIB-4 index	≤2.0	>2.0 and ≤4.0	>4.0
AFP (ng/mL)	≤5.0	>5.0 and ≤10.0	>10.0

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RESULTS

Patient characteristics

The clinical background of the study patients is shown in Table 2. The median age was 66 years, and there was a predominance of men (55.8%). The median FIB-4 index and serum AFP levels were 2.2 and 3.2 ng/mL, respectively. The median follow-up period was 11.3 years.

Overall incidence of hepatocarcinogenesis

HCC developed in 60 of 516 patients (11.6%). The incidence rate of HCC at 5 and 10 years were 2.6% and 17.6%, respectively (Figure S1).

Factors associated with the incidence of hepatocarcinogenesis

Factors significantly associated with the incidence of HCC in the univariate analysis are listed in Table 3. The following associations were statistically significant: age, AST, ALT, γ-GTP, platelet count, ALP, total bilirubin, albumin, AFP and the FIB-4 index. Factors that were significantly associated with the incidence of HCC in the multivariate analysis were FIB-4 index >2.0 (hazard ratio (HR), 7.690

Table 2 Clinical background of the study patients

	(*n* = 516)
Age (years)	66 (57–71)
Sex (female/male)	228 (44.2%)/288 (55.8%)
HCV genotype (1/2/unknown)	238 (46%)/128 (25%)/150 (29%)
Viral concentration (KIU/mL)	270 (9–803)
ALT (IU/mL)	28 (19–42)
Mean ALT integration value (IU/mL)	27 (21–33)
AST (IU/mL)	31 (22–46)
Platelet count ($\times 10^4/m^3$)	17 (13–21)
γ-GTP (IU/mL)	23 (18–41)
ALP (IU/mL)	247 (203–309)
Prothrombin time (%)	96 (82–107)
Total bilirubin (mg/dL)	0.5 (0.4–0.7)
Albumin (g/dL)	4.1 (3.9–4.3)
LDH (IU/L)	183 (160–212)
ChE (IU/L)	265 (211–308)
AFP (ng/mL)	3.2 (2.1–4.9)
Observation period (years)	11.3 (6.8–15.0)
FIB-4 index	2.2 (1.4–3.8)
Hepatocarcinogenesis (+/-)	60 (11.6%)/456 (88.4%)

Values are expressed as medians (first quartile – third quartile). HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, asparate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ChE, cholinesterase; AFP, α-fetoprotein.

Table 3 Factors associated with hepatocarcinogenesis (univariate analysis)

	Hazard ratio	95% CI	*P* value
Age (years)			
≤65	1		
>65	2.189	1.266–3.785	0.005
Sex			
Female	1		
Male	1.448	0.868–2.414	0.157
AST (IU/mL)			
≤40	1		
>40	2.988	1.772–5.039	0.000
ALT (IU/mL)			
≤35	1		
>35	1.916	1.147–3.199	0.013
γ-GTP (IU/mL)			
≤56	1		
>56	2.339	1.341–4.078	0.003
Platelet count ($\times 10^4/m^3$)			
≥15	1		
< 15	4.069	2.424–6.830	0.000
ALP (IU/mL)			
≤338	1		
>338	2.616	1.482–4.617	0.001
Total bilirubin (mg/dL)			
≤1.2	1		
>1.2	4.535	2.431–8.458	0.000
Albumin (g/dL)			
≥3.5	1		
< 3.5	3.385	1.746–6.56	0.000
LDH (IU/L)			
≤251	1		
>251	1.878	0.458–7.704	0.382
ChE (IU/L)			
≤431	1		
>431	1.739	0.241–12.576	0.584
AFP (ng/mL)			
≤5	1		
5 < and ≤10	3.760	2.094–6.751	0.000
>10	9.474	4.775–18.796	0.000
FIB-4 index			
≤2.0	1		
2.0 < and ≤4.0	8.017	2.751–23.365	0.000
>4.0	16.283	5.761–46.023	0.000

CI, confidence interval; AST, asparate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ChE, cholinesterase; AFP, α-fetoprotein.

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[95% confidence interval (CI), 2.636–22.438]; $P < 0.001$) and FIB-4 index >4.0 (HR, 8.991 [95% CI, 3.088–26.178]; $P < 0.001$), AFP >5 ng/mL (HR, 2.742 [95% CI, 1.497–5.023]; $P < 0.001$) and AFP >10 ng/mL (HR, 4.915 [95% CI, 2.353–10.267]; $P < 0.001$) and total bilirubin >1.2 mg/dL (HR, 2.142 [95% CI, 1.115–4.117]; $P = 0.022$) (Table 4).

Incidence of hepatocarcinogenesis based on the FIB-4 index and AFP

When patients were categorized based on the FIB-4 index as ≤2.0 ($n = 226$), >2.0 and ≤4.0 ($n = 169$), and >4.0 ($n = 121$), the cumulative incidence of HCC at 5 years was 0.5%, 1.3% and 8.0%, respectively, and 2.8%, 25.6% and 37.1% at 10 years, respectively (Fig. 1a). The FIB-4 index >4.0 group was at a significantly higher risk for HCC development than the FIB-4 index >2.0 and ≤4.0 group ($P = 0.011$). The FIB-4 index >2.0 and ≤4.0 group had a significantly higher risk than the FIB-4 index ≤2.0 group ($P < 0.001$).

When patients were categorized based on AFP as ≤5.0 ng/mL, >5.0 and ≤10.0 ng/mL, and >10.0 ng/mL, the cumulative incidence of HCC at 5 years was 0.93%, 5.4% and 21.7%, and 11.9%, 30.3% and 75.1% at 10 years, respectively (Fig. 1b). There were significant differences between the AFP ≤5.0 ng/mL and the AFP >5.0 and ≤10.0 ng/mL groups ($P < 0.001$), and between the AFP >5.0 and ≤10.0 ng/mL and the AFP >10.0 ng/mL groups ($P = 0.014$). The group with AFP >10.0 ng/mL was at the highest risk for HCC development.

Relationship between the FIB-4 index and AFP

There were no significant correlation between the FIB-4 index and AFP based on Spearman's rank correlation ($r = -0.023$, $P = 0.63$) (Figure S2).

Table 4 Factors associated with hepatocarcinogenesis (multivariate analysis) based on Cox proportional hazards modelling with forward selection

	Hazard ratio	95% CI	*P* value
FIB-4 index			
≤2.0	1		
2.0 < and ≤4.0	7.690	2.636–22.438	0.000
>4.0	8.991	3.088–26.178	0.000
AFP (ng/mL)			
≤5	1		
5< and ≤10	2.742	1.497–5.023	0.001
>10	4.915	2.353–10.267	0.000
Total bilirubin (mg/dL)			
≤1.2	1		
>1.2	2.142	1.115–4.117	0.022

CI, confidence interval; AFP, α-fetoprotein.

Incidence of hepatocarcinogenesis based on the combined FIB-4 index and AFP score

There were 5 categories in the scoring system that combines the FIB-4 index and AFP level (2–6 points). Figure 2 shows the incidence of HCC according to this scoring system. The distribution of patients in this scoring system was as follows: 180 (37.7%) patients with score 2, 145 (30.3%) with score 3, 100 (21.0%) with score 4, 33 (6.9%) with score 5 and 19 (4.1%) with score 6. The incidence rate of HCC increased as the score increased (2 *vs* 3, $P < 0.001$; 3 *vs* 4 points, $P = 0.070$; 4 *vs* 5 points,

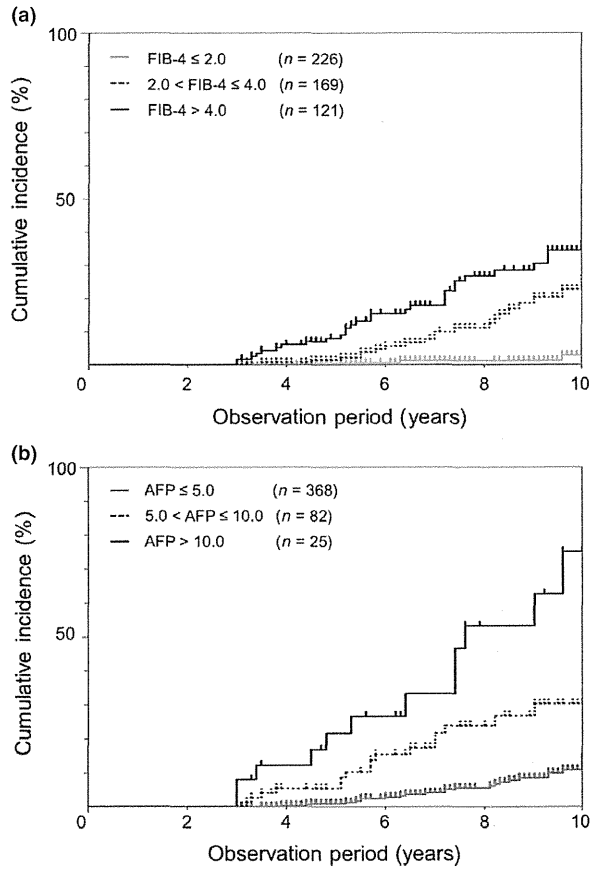

Fig. 1 (a) The cumulative incidence of hepatocellular carcinoma (HCC) at 5 years was 0.5% in patients with a FIB-4 index ≤2.0 ($n = 226$), 1.3% in patients with a FIB-4 index >2.0 and ≤4.0 ($n = 169$) and 8.0% in patients with a FIB-4 index >4.0 ($n = 121$). At 10 years, the cumulative incidence of HCC was 2.8%, 25.6% and 37.1%, respectively. (b) When patients were categorized based on α-fetoprotein (AFP) ≤5.0 ng/mL ($n = 368$), >5.0 and ≤10.0 ng/mL ($n = 82$) and >10.0 ng/mL ($n = 25$), the cumulative incidence rates for HCC at 5 years were 0.93%, 5.4% and 21.7%, respectively, and 11.9%, 30.3% and 75.1% at 10 years, respectively.

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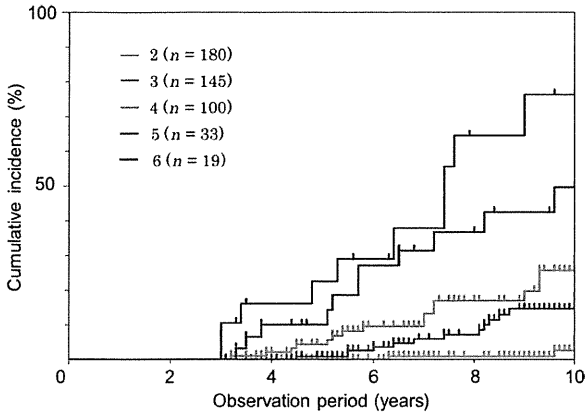

Fig. 2 Incidence of hepatocarcinogenesis based on the combined FIB-4 index and α-fetoprotein (AFP) level scoring system. This scoring system predicts patient outcomes with excellent discriminative ability.

$P = 0.011$; and 5 *vs* 6 points; $P = 0.270$). This scoring system reflected patient survival well.

When focusing on patients who persistently showed below 30 IU/L of ALT ($n = 306$), we got similar discrimination for hepatocarcinogenesis based on FIB-4 index, AFP or combination of both factors (data not shown).

DISCUSSION

Several noninvasive biomarkers and imaging modalities of liver fibrosis that may replace liver biopsy in the diagnosis of fibrosis have been described. The FIB-4 index is noninvasive liver fibrosis evaluation method calculated using age, AST, ALT and platelet count. Previous reports have shown that lower platelet count, older age and elevated AST or ALT are significant risk factors for HCC [22–25]. In other words, the FIB-4 index is a more complex marker for assessing liver fibrosis and the development of HCC.

We believe that the data are precious and important because we followed 516 PNALT patients with HCV for undergoing HCC screening and surveillance without antiviral therapy for median 11.3 years. When patients were categorized based on the FIB-4 index, the FIB-4 index >4.0 group was at the highest risk for HCC development, and the FIB-4 index >2.0 and ≤4.0 group was at higher risk than the ≤2.0 group. These results indicate that calculation of the FIB-4 index at the start of follow-up is useful for predicting the risk of HCC.

AFP is reportedly a good marker for distinguishing patients at high risk for future HCC development [26–29], and it is often used in combination with US for HCC screening. In a recent study, Tateyama *et al.* [30] found that slightly elevated AFP in HCV patients indicated a substantial risk for the development of HCC. In the present study, the group with slightly elevated AFP levels (>5.0 and ≤10.0 ng/mL) also had a significantly higher risk of HCC development than the group with low AFP levels (≤5.0 ng/mL).

As the FIB-4 index was not correlated with AFP, the FIB-4 index is a prognostic marker independent of AFP with regards to HCC development. Therefore, we developed a scoring system for hepatocarcinogenesis that combines the FIB-4 index and AFP. This scoring system showed good predictive power and discriminative ability. We observed a higher prevalence of HCC in patients with a higher score, especially scores of 5 and 6.

Recently, interferon therapy was shown to improve liver fibrosis and reduce the risk of HCC and liver failure by HCV eradication or prolonged normalization of ALT levels [31,32]. In addition, it has been reported that AFP levels decrease in response to interferon therapy in patients with chronic HCV[33,34]. For these reasons, patients with a higher FIB-4 index and elevated AFP should be treated with antiviral therapy as early as possible and be closely monitored for the occurrence of HCC with extensive surveillance consisting of US, CT, MRI and tumour markers.

The present study has several limitations. Firstly, histological confirmations for liver fibrosis were obtained in partial patients. Therefore, we did not confirm the correlation between FIB-4 index and pathological liver fibrosis in the present study, although several studies reported the correlation.

Secondly, although it was reported that utility of ΔFIB-4 for liver fibrosis [35], we did not investigate the changes of FIB-4 index over the years. Further examination with a larger number of patients is required to validate the significance of these findings.

In conclusion, the FIB-4 index was closely associated with the risk of HCC in hepatitis C virus carriers with normal ALT levels. Furthermore, we showed that the risk of HCC could be well stratified according to a scoring system that combines the FIB-4 index and AFP.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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

REFERENCES

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

2 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and

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molecular carcinogenesis. *Gastroenterology* 2007; 132: 2557–2576.
3 Gebo KA, Herlong HF, Torbenson MS *et al.* Role of liver biopsy in management of chronic hepatitis C. *Hepatology* 2002; 36(5 Suppl 1): S161–S172.
4 Bravo AA, Shcth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; 344(7): 495–500.
5 The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; 20: 15–20.
6 Friedrich-Rust M, Wunder K, Kriener S *et al.* Liver fibrosis in viral hepatitis: non invasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; 252: 595–604.
7 Fraquelli M, Rigamonti C, Casazza G *et al.* Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; 56: 968–973.
8 Nightingale K, McAleavey S, Trahey G. Shear–wave generation using acoustic radiation force :in vivo and ex vivo results. *Ultrasound Med Biol* 2003; 29: 1715–1723.
9 Huwart L, Sempoux C, Vicaut E *et al.* Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; 135: 32–40.
10 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic heparins C. *Hepatology* 2003; 38: 518–526.
11 Sebastiani G, Vario A, Guido M *et al.* Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol* 2006; 44: 686–693.
12 Sterling RK, Lissen E, Clumeck N *et al.* APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43: 1317–1725.
13 Vallet-Pichard A, Mallet V, Nalpas B *et al.* FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. *Hepatology* 2007; 46: 32–36.
14 Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ; Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; 7: 1104–1112.
15 Okanoue T, Itoh Y, Minami M *et al.* Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts. *Hepatol Res.* 2008; 38: 27–36.
16 Kumada T, Toyoda H, Kiriyama S *et al.* Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. *J Hepatol* 2009; 50: 729–735.
17 Kumada T, Toyoda H, Kiriyama S *et al.* Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. *Gut* 2007; 56: 738–739.
18 Okamoto H, Mishiro S, Tokita H, Tsuaa F, Miyakawa Y, Mayumi M. Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/la. *Hepatology* 1994; 20: 1131–1136.
19 Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. *Hepatology* 2002; 36: S65–S73.
20 European Association For The Study Of The Liver, European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; 56: 908–943.
21 Toyoda H, Kumada T, Osaki Y, Oka H, Kudo M. Role of tumor markers in assessment of tumor progression and prediction of outcomes in patients with hepatocellular carcinoma. *Hepatol Res.* 2007; 37: 166–171.
22 Ono E, Shiratori Y, Okudaira T *et al.* Platelet count reflects stage of chronic hepatitis C. *Hepatol Res.* 1999; 15: 192–200.
23 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: 268–275.
24 Lok AS, Seeff LB, Morgan TR *et al.* Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease.; HALT-C Trial Group. *Gastroenterology* 2009; 136: 138–148.
25 Kurosaki M, Matsunaga K, Hirayama I *et al.* The presence of steatosis and elevation of alanine aminotransferase levels are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy. *J Hepatol* 2008; 48: 736–742.
26 Kumada T, Toyoda H, Kiriyama S *et al.* Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus. *J Gastroenterol* 2011; 46: 536–544.
27 Sherman M. Hepatocellular carcinoma: epidermiology, risk factors, and screening. *Semin Liver Dis* 2005; 25: 143–154.
28 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; 328: 1797–1801.
29 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: 61–66.
30 Tateyama M, Yatsuhashi H, Taura N *et al.* Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus. *J Gastroenterol* 2011; 46: 92–100.
31 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol* 1999; 30: 653–659.

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32 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: 1051–1055.
33 Murashima S, Tanaka M, Haramaki M *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: 808–812.
34 Tamura Y, Yamagiwa S, Aoki Y *et al.* Serum alpha-fetoprotein levels during and after interferon therapy and the development of hepatocellular carcinoma in patients with chronic hepatitis C. *Dig Dis Sci* 2009; 54: 2530–2537.
35 Tamaki N, Kurosaki M, Tanaka K *et al.* Noninvasive estimation of fibrosis progression overtime using the FIB-4 index in chronic hepatitis C. *J Viral Hepat* 2013; 20: 72–76.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Incidence of hepatocarcinogenesis in whole patients.

Figure S2. There was no significant correlation between the FIB-4 index and α-fetoprotein (AFP).

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term prognosis of patients with chronic hepatitis C who did not receive interferon-based therapy: causes of death and analysis based on the FIB-4 index

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Abstract

Background Interferon (IFN)-based therapy has been reported to reduce the liver-related mortality rate in patients with chronic hepatitis C virus (HCV) infection. However, predictors of survival and causes of death, including non-liver-related causes, have not been sufficiently investigated in chronic HCV patients who have not received IFN-based therapy.

Methods A total of 1723 patients with chronic HCV infection who were not treated with IFN-based therapy were enrolled. Survival from liver-related diseases and non-liver-related diseases and causes of death were analyzed on the basis of the fibrosis-4 (FIB-4) index, an index of liver fibrosis.

Results The median follow-up duration was 10.3 years. Of 465 patients who died during the follow-up period, 48.4 % died of liver-related diseases; of the remainder, 51.6 % died of non-liver-related diseases. On the basis of FIB-4 index, the liver-related mortality rate increased as the FIB-4 index increased: 16.1 % in the FIB-4 index < 1.45 group, 36.7 % in the $1.45 \leq$ FIB-4 index ≤ 3.25 group, and 58.7 % in the FIB-4 index > 3.25 group ($p < 0.001$). Conversely, the non-liver-related mortality rate decreased as the FIB-4 index increased: 83.9, 63.3, and 41.3 %, respectively ($p = 0.001$). In the multivariate analysis, a FIB-4 index greater than 3.25 was identified as a risk factor independently associated with both liver-related death (hazard ratio 13.020; 95 % confidence interval 4.155–40.770) and non-liver-related death (hazard ratio 1.667; 95 % confidence interval 1.188–2.340).

Conclusions Patients with chronic HCV infection and an elevated FIB-4 index may benefit from monitoring not only for the development of liver-related diseases but also for the development of non-liver-related diseases.

Keywords Hepatitis C · FIB-4 index · Survival · Causes of death

Abbreviations

AFP	α-Fetoprotein
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
FIB-4	Fibrosis 4
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
ICD	International Statistical Classification of Diseases and Related Health Problems
IFN	Interferon

Introduction

Chronic hepatitis C virus (HCV) infection affects approximately 180 million individuals worldwide and is a common cause of chronic liver disease and hepatocellular carcinoma (HCC) in Japan, the USA, and many European countries [1, 2]. Chronic infection with HCV induces the

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progression of liver fibrosis, which results in the development of cirrhosis and HCC. Interferon (IFN)-based therapy has been used to treat patients with chronic HCV. Many investigators have reported that IFN-based therapy is effective in reducing serum levels of alanine aminotransferase (ALT), eliminating circulating HCV RNA, and decreasing the degree of liver fibrosis in chronic HCV patients [3–7]. Eradication of HCV has also been reported to reduce the incidence of HCC [6, 8]. Taken together, the findings indicate that IFN-based therapy clearly reduces the rate of liver-disease-related death and improves life expectancy [9–11]. However, predictors of survival and causes of death, including non-liver-related causes, have not been sufficiently investigated in chronic HCV patients who have not received IFN-based therapy.

Advanced liver fibrosis in the context of chronic HCV is one of the strongest indications for antiviral therapy, including IFN-based therapy; therefore, making a precise evaluation of the extent of liver fibrosis is essential in the treatment of chronic HCV patients. Liver biopsy is still considered the gold standard for the evaluation of liver fibrosis, even though it is painful, costly, and associated with limitations in diagnostic utility and accuracy [12, 13]. Moreover, since the invasiveness of liver biopsy precludes repeated examinations, longitudinal evaluation of liver fibrosis is difficult [14]. Recently, various indices of liver fibrosis based on clinical and biological data have been reported to be useful predictors of fibrosis in liver disease [15–21]. The fibrosis-4 (FIB-4) index was developed as a noninvasive marker of fibrosis in patients with chronic HCV and nonalcoholic fatty liver disease [19–21].

In the present study, we investigated the predictors of survival and causes of death, including non-liver-related causes, in patients with chronic HCV who have not received IFN-based therapy with a long-term follow-up. In addition, we studied the relationship between survival from both liver-related and non-liver-related diseases and the FIB-4 index in patients with chronic HCV.

Materials and methods

Patients

A total of 8400 consecutive patients positive for HCV antibody by second- or third-generation enzyme-linked immunosorbent assays were treated in the Department of Gastroenterology at Ogaki Municipal Hospital, Japan, between October 1994 and September 2014. Of these, 1723 met the following inclusion criteria: (1) detectable HCV RNA for longer than 6 months; (2) no antiviral therapy involving IFN-based therapy; (3) no evidence of hepatitis B virus infection; (4) no other causes of chronic liver disease (alcohol consumption greater than 80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (5) surveillance for HCC was performed during the follow-up period; (6) follow-up duration of more than 3 years; (7) no evidence of malignancies, including HCC, for at least 1 year from the start of the follow-up (Fig. 1). The date of the first visit was defined as the start of follow-up. The end of follow-up was defined as the date of the final visit for

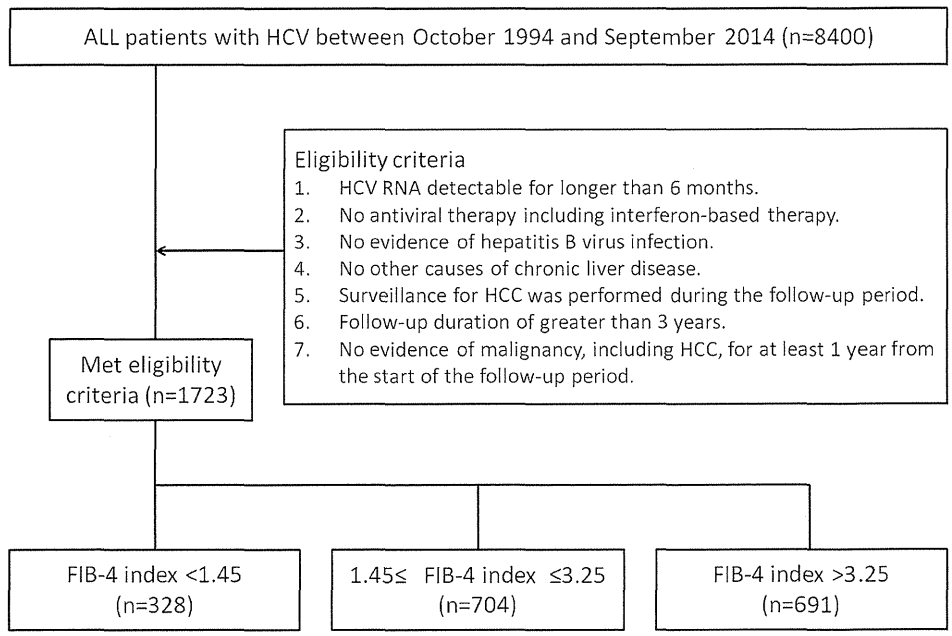

Fig. 1 Flowchart of the patient selection process. *FIB-4* fibrosis 4, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

patients who have not died, and as the date of death for patients who died during follow-up.

HCV genotype and HCV RNA were determined by PCR methods. Amplicor 2 (Roche Diagnostics, Tokyo, Japan) was used before 1 January 2008 and COBAS TaqMan HCV (Roche Diagnostics) was used thereafter.

In our hospital, the decision to offer IFN-based therapy to chronic HCV patients was determined according to the guidelines of the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver, or the Asian Pacific Association for the Study of the Liver [22–24]. All 1723 patients with chronic HCV had no indications for IFN-based therapy or had declined IFN-based therapy. If a patient with an indication for IFN-based therapy declined therapy after sufficient information about therapy had been provided, the patient received glycyrrhizin intravenously, ursodeoxycholic acid intravenously, or both intravenously as hepatoprotective medications as an alternative therapy with the goal of decreasing ALT levels.

The study protocol was approved by the Institutional Review Board of Ogaki Municipal Hospital and was conducted in compliance with the Helsinki Declaration.

Surveillance, diagnosis, and causes of death

Ultrasonography and blood tests, including a test for the tumor marker α-fetoprotein (AFP), were performed every 3–6 months for HCC surveillance. If a nodular lesion was detected by ultrasonography or an elevation in the concentration of 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 guidelines of the American Association for the Study of Liver Diseases [25, 26].

Diseases other than HCC were initially detected on the basis of clinical symptoms, abnormal surveillance data, medical checkups (in the community or at the workplace), or assessment by physicians. These diseases were then diagnosed and treated on the basis of disease-specific criteria and guidelines issued by the appropriate specialists in our hospital. The causes of death were categorized by these specialists using International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-9 codes for deaths occurring before 1 January 2003, and ICD-10 codes for deaths occurring thereafter) [27]. All determinations of the cause of death were performed retrospectively by collection and analysis of data from patient medical records.

Located in a region of 400,000 inhabitants, Ogaki Municipal Hospital is the only general hospital in the region employing approximately 200 specialists, including more than 15 gastroenterologists. Therefore, a large number of patients with chronic HCV requiring HCC surveillance visit the hospital regularly as outpatients. There is also close contact, including the sharing of patient mortality data (if the patients did not die in our hospital), between family medicine clinics and community hospitals and our hospital.

Calculation of the FIB-4 index

The FIB-4 index was calculated at the start of follow-up by the following formula: FIB-4 index = aspartate aminotransferase (AST) concentration (IU/L) × age (years)/platelet count (10^9/L) × (ALT concentration)$^{1/2}$ [(IU/L)$^{1/2}$]. We used previously published cut-off values for the FIB-4 index [19, 20]. Consequently, patients were categorized on the basis of their FIB-4 index as follows: less than 1.45 ($n = 328$), 1.45–3.25 ($n = 704$), and greater than 3.25 ($n = 691$).

Statistical analysis

Continuous variables are expressed as the median and the interquartile range. The Mann–Whitney U test was used for continuous variables, and the chi-square test with Yates's correction or Fisher's exact test was used for categorical variables. Actuarial analysis of cumulative mortality was performed with the cumulative incidence with competing risk method, and differences were tested by the Gray test with Bonferroni correction. Univariate Cox proportional hazards models were used to identify predictors of survival. For the assessment of independent predictors of liver-related or non-liver-related death, Fine and Gray [28] proportional hazards models with the backward elimination method were used for multivariate analysis. We used the upper and lower limits of the reference values at our institution as the cut-off values for laboratory data in the univariate Cox proportional hazards models and the multivariate Fine and Gray proportional hazards models.

Statistical significance was defined as $p < 0.05$. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [29]. More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Patient characteristics and causes of death

The characteristics of all 1723 patients are summarized in Table 1. The median FIB-4 index was 2.7 (1.7–11.5) and

Table 1 Characteristics of the study patients ($n = 1723$)

Age (years)[a]	64.0 (57.0–69.0)
Sex (female/male)	850/873
AST (IU/L)[a]	42 (28–70)
ALT (IU/L)[a]	42 (26–74)
Albumin (g/dL)[a]	4.1 (3.8–4.3)
Total bilirubin (mg/dL)[a]	0.6 (0.4–0.8)
Prothrombin time (%)[a]	95.0 (82.0–105.0)
Platelet count ($\times 10^4$/mm^3)[a]	16.2 (11.8–20.5)
AFP (ng/mL)[a]	4.1 (2.3–8.5)
HCV genotype (1/2/unknown)	893/383/447
HCV RNA ($\log_{10}$ IU/mL)[a]	5.6 (4.4–6.1)
FIB-4 index[a]	2.7 (1.7–11.5)
Hepatoprotective drug therapy (GL/UDCA/GL plus UDCA/none)	35/489/101/1098
Alcohol (yes/no/unknown)	63/246/1414
Smoking (yes/no/unknown)	315/692/716
Hypertension (yes/no/unknown)	691/704/328
Hyperlipidemia (yes/no/unknown)	522/1200/1
Diabetes (yes/no/unknown)	468/1139/116
Follow-up duration (years)[a]	10.3 (6.5–14.5)
Deaths	465
Causes	
Liver-related diseases	225/465 (48.4 %)
HCC	175
Liver failure	50
Non-liver-related diseases	240/465 (51.6 %)
Malignant diseases	
Digestive malignancies	43
Respiratory malignancies	21
Hematological malignancies	11
Other	8
Nonmalignant diseases	
Digestive diseases	7
Respiratory diseases	47
Cardiovascular diseases	43
Renal diseases	12
Cerebrovascular diseases	30
Injury	10
Other	8

AFP α-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *FIB-4* fibrosis 4, *GL* glycyrrhizin, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *UDCA* ursodeoxycholic acid

[a] Values are expressed as the median, with the interquartile range in *parentheses*

the median follow-up duration was 10.3 (6.5–14.5) years. No patients received ribavirin monotherapy or direct-acting antiviral therapy. Of the 1723 patients, 465 died during follow-up; the causes of death are also shown in Table 1. Death was due to liver-related diseases in 48.4 % of patients (225 of 465), of which HCC was the cause of death in 77.8 % of those patients (175 of 225). Conversely, in 51.6 % of patients (240 of 465) who died of non-liver-related diseases, the causes of death included a variety of malignancies other than HCC, including hematological malignancies. In non-liver-related diseases other than malignant diseases, no patterns were observed regarding the cause of death. Of the 1258 patients who did not die during follow-up, 679 patients (54.0 %) did not visit the hospital as an outpatient for more than 1 year.

Patient characteristics based on the FIB-4 index

The baseline characteristics of the 1723 study patients by their FIB-4 index are summarized in Table 2. There were significant differences in age, sex, AST concentration, ALT concentration, albumin concentration, total bilirubin concentration, prothrombin time, platelet count, AFP concentration, HCV genotype, use of hepatoprotective drugs, morbidity of hyperlipidemia, morbidity of diabetes, and follow-up duration among the three groups.

The mortality rate due to liver-related diseases increased as the FIB-4 index increased: 16.1 % in the FIB-4 index < 1.45 group, 36.7 % in the $1.45 \leq$ FIB-4 index ≤ 3.25 group, and 58.7 % in the FIB-4 index > 3.25 group ($p < 0.001$). Conversely, the mortality rate due to non-liver-related diseases decreased as the FIB-4 index increased: 83.9 % in the FIB-4 index < 1.45 group, 63.3 % in the $1.45 \leq$ FIB-4 index ≤ 3.25 group, and 41.3 % in the FIB-4 index > 3.25 group ($p = 0.001$).

Of the 328 FIB-4 index < 1.45 patients, 102 patients declined IFN-based therapy despite their being 65 years old or younger, having an ALT concentration greater than 35 IU/L, or having a platelet count of 15×10^4/m^3 or lower, which were considered an indication for IFN-based therapy. The remaining 226 patients did not have an indication for IFN-based therapy.

Cumulative survival and factors associated with all-cause survival

The respective 5-, 10-, 15-, and 20-year cumulative all-cause survival rates were 95.4, 80.0, 64.9, and 50.3 % among all study patients. Factors significantly associated with all-cause survival in the univariate analysis are listed

in Table 3. All of the following variables in the analysis were statistically significant: age, sex, AST concentration, ALT concentration, albumin concentration, total bilirubin concentration, prothrombin time, platelet count, AFP concentration, and FIB-4 index.

Factors associated with patient deaths from liver-related and non-liver-related diseases

In the analysis of liver-related death, multivariate Fine and Gray proportional hazards modeling with the covariates of

Table 2 Characteristics of the study patients according to the fibrosis-4 (*FIB-4*) index ($n = 1723$)

	FIB-4 index < 1.45 ($n = 328$)	1.45 ≤ FIB-4 index ≤ 3.25 ($n = 704$)	FIB-4 index > 3.25 ($n = 691$)	p
Age (years)[a]	51.0 (43.0–61.0)	65.0 (59.0–69.0)	66.0 (61.0–72.0)	<0.001
Sex (female/male)	190/183	305/399	355/336	<0.001
AST (IU/L)[a]	24 (19–31)	36 (27–52)	69 (48–98)	<0.001
ALT (IU/L)[a]	26 (18–40)	36 (24–62)	64 (38–103)	<0.001
Albumin (g/dL)[a]	4.2 (4.0–4.4)	4.1 (3.9–4.3)	3.9 (3.5–4.1)	<0.001
Total bilirubin (mg/dL)[a]	0.5 (0.4–0.6)	0.5 (0.4–0.7)	0.7 (0.5–0.9)	<0.001
Prothrombin time (%)[a]	104.0 (96.0–117.0)	98.0 (88.0–108.0)	86.0 (74.0–97.0)	<0.001
Platelet count ($\times 10^4/mm^3$)[a]	23.7 (20.3–27.9)	17.8 (15.3–20.6)	11.0 (8.4–20.4)	<0.001
AFP (ng/mL)[a]	2.9 (1.9–4.5)	3.2 (2.0–5.7)	7.3 (3.5–18.9)	<0.001
HCV genotype (1/2/unknown)	157/87/84	372/165/167	364/131/196	0.031
HCV RNA ($\log_{10}$ IU/mL)[a]	5.6 (4.0–6.1)	4.5 (5.6–6.1)	5.6 (4.4–6.0)	0.17
FIB-4 index[a]	1.0 (0.8–1.3)	2.2 (1.8–2.7)	5.2 (4.0–7.3)	<0.001
Hepatoprotective drug therapy (GL/UDCA/GL plus UDCA/none)	8/69/6/245	15/210/41/438	12/210/54/415	<0.001
Alcohol drinker (yes/no/unknown)	10/40/278	33/98/573	20/108/563	0.161
Smoker (yes/no/unknown)	63/109/156	146/299/259	106/284/301	0.055
Hypertension (yes/no/unknown)	25/49/254	96/178/430	89/205/397	0.471
Hyperlipidemia (yes/no/unknown)	175/152/1	218/486/0	129/562/0	<0.001
Diabetes (yes/no/unknown)	52/231/45	176/480/48	240/428/23	<0.001
Follow-up duration (years)[a]	12.1 (7.4–15.9)	11.2 (7.4–15.8)	9.1 (5.7–12.4)	<0.001
Deaths	31	158	276	
Causes				
Liver-related diseases	5/31 (16.1 %)	58/158 (36.7 %)	162/276 (58.7 %)	<0.001
HCC	3	47	125	
Liver failure	2	11	37	
Non-liver-related diseases	26/31 (83.9 %)	100/158 (63.3 %)	114/276 (41.3 %)	0.001
Malignant diseases				
Digestive malignancies	3	19	21	
Respiratory malignancies	4	12	5	
Hematological malignancies	3	5	3	
Other	2	4	2	
Nonmalignant diseases				
Digestive diseases	2	2	3	
Respiratory diseases	3	19	25	
Cardiovascular diseases	3	15	25	
Renal diseases	1	5	6	
Cerebrovascular diseases	4	12	14	
Injury	1	4	5	
Other	0	3	5	

AFP α-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GL* glycyrrhizin, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *UDCA* ursodeoxycholic acid

[a] Values are expressed as the median, with the interquartile range in *parentheses*

sex (female or male), albumin concentration (≥3.6 g/dL or <3.6 g/dL), total bilirubin concentration (≤1.2 mg/dL or >1.2 mg/dL), prothrombin time (>70 % or ≤70 %), AFP concentration (≤20 ng/mL or > 20 ng/mL), and FIB-4 index (<1.45, 1.45–3.25, and >3.25) showed that sex, albumin concentration, AFP concentration, and FIB-4 index were independent factors associated with death (Table 4).

In the analysis of non-liver-related death, multivariate Fine and Gray proportional hazards modeling with the same covariates showed that sex, albumin concentration, AFP concentration, and FIB-4 index (in the FIB-4 index > 3.25 group) were independent factors associated with death (Table 5).

Cumulative mortality based on the FIB-4 index

Figure 2a shows the cumulative all-cause mortality curves for the study patients by their FIB-4 index. The respective 5-, 10-, 15-, and 20-year cumulative mortality rates were 1.0, 6.9, 11.4, and 20.9 % in the FIB-4 index < 1.45 group, 2.5, 13.8, 28.5, and 43.4 % in the 1.45 ≤ FIB-4 index ≤ 3.25 group, and 9.4, 33.1, 55.7, and 70.7 % in the FIB-4 index > 3.25 group. The mortality differed significantly between the FIB-4 index < 1.45 group and the 1.45 ≤ FIB-4 index ≤ 3.25 group ($p < 0.001$), between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group ($p < 0.001$), and between the 1.45 ≤ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p < 0.001$) after Bonferroni correction.

Figure 2b shows the cumulative mortality curves for death from liver-related diseases for study patients by their FIB-4 index. The respective 5-, 10-, 15-, and 20-year cumulative mortality rates were 0.0, 0.4, 1.2, and 7.2 % in the FIB-4 index < 1.45 group, 0.1, 4.5, 9.9, and 15.7 % in the 1.45 ≤ FIB-4 index ≤ 3.25 group, and 5.2, 19.5, 33.9, and 40.3 % in the FIB-4 index > 3.25 group. The mortality differed significantly between the FIB-4 index < 1.45 group and the 1.45 ≤ FIB-4 index ≤ 3.25 group ($p < 0.001$), between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group ($p < 0.001$), and between the 1.45 ≤ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p < 0.001$) after Bonferroni correction.

Figure 2c shows the cumulative mortality curves for death from non-liver-related diseases for study patients by their FIB-4 index. The respective 5-, 10-, 15-, and 20-year cumulative mortality rates were 1.0, 6.5, 10.2, and 13.7 % in the FIB-4 index < 1.45 group, 2.4, 9.4, 18.5, and 27.7 % in the 1.45 ≤ FIB-4 index ≤ 3.25 group, and 4.2, 13.6, 21.8, and 30.4 % in the FIB-4 index > 3.25 group. The mortality differed significantly between the FIB-4 index < 1.45 group and the 1.45 ≤ FIB-4 index ≤ 3.25 group ($p = 0.027$), and between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group after Bonferroni correction ($p < 0.001$). Conversely, the mortality did not differ significantly between the 1.45 ≤ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p = 0.133$) after Bonferroni correction.

Table 3 Factors associated with all-cause survival (univariate analysis)

	Hazard ratio	95 % CI	*p*
Age (years)			
≤65 ($n = 981$)	1	1.669–2.415	<0.001
>65 ($n = 742$)	2.007		
Sex			
Female ($n = 850$)	1	1.606–2.342	<0.001
Male ($n = 873$)	1.939		
AST (IU/L)			
≤40 ($n = 832$)	1	1.927–2.852	<0.001
>40 ($n = 891$)	2.345		
ALT (IU/L)			
≤35 ($n = 713$)	1	1.414–2.105	<0.001
>35 ($n = 1010$)	1.725		
Albumin (g/dL)			
≥3.6 ($n = 1521$)	1	2.623–4.244	<0.001
<3.6 ($n = 202$)	3.337		
Total bilirubin (mg/dL)			
≤1.2 ($n = 1615$)	1	1.422–2.704	<0.001
>1.2 ($n = 108$)	1.961		
Prothrombin time (%)			
>70 ($n = 1267$)	1	1.616–2.722	<0.001
≤70 ($n = 152$)	2.097		
Platelet count ($\times 10^4/mm^3$)			
>15 ($n = 996$)	1	1.852–2.679	<0.001
≤15 ($n = 727$)	2.227		
AFP (ng/mL)			
≤20 ($n = 1400$)	1	1.925–3.115	<0.001
>20 ($n = 197$)	2.448		
FIB-4 index			
<1.45 ($n = 328$)	1		
1.45–3.25 ($n = 704$)	2.460	1.674–3.615	<0.001
>3.25 ($n = 691$)	6.069	4.183–8.805	<0.001

AFP α-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CI* confidence interval, *FIB-4* fibrosis 4

Discussion

The present study, an analysis of a large number of patients with chronic HCV who have not received antiviral therapy with a long-term follow-up, found a nearly equal proportion of deaths due to liver-related and non-liver-related disease. In addition, when the causes of death were

Table 4 Multivariate analysis of factors related to liver-related death

Factor	Hazard ratio	95 % CI	*p*
Sex			
Female ($n = 850$)	1		0.010
Male ($n = 873$)	1.425	1.087–1.869	
Albumin (g/dL)			
≥3.6 ($n = 1521$)	1		0.010
<3.6 ($n = 202$)	1.716	1.247–2.361	
AFP (ng/mL)			
≤20 ($n = 1400$)	1		<0.001
>20 ($n = 197$)	2.272	1.662–3.104	
FIB-4 index			
<1.45 ($n = 328$)	1		
1.45–3.25 ($n = 704$)	4.576	1.445–14.490	0.001
>3.25 ($n = 691$)	13.020	4.155–40.770	<0.001

AFP α-fetoprotein, *CI* confidence interval, *FIB-4* fibrosis 4

Table 5 Multivariate analysis of factors related to non-liver-related death

Factor	Hazard ratio	95 % CI	*p*
Sex			
Female ($n = 850$)	1		<0.001
Male ($n = 873$)	2.492	1.795–3.461	
Albumin (g/dL)			
≥3.6 ($n = 1521$)	1		0.004
<3.6 ($n = 202$)	1.816	1.214–2.756	
AFP (ng/mL)			
≤20 ($n = 1400$)	1		0.009
>20 ($n = 197$)	0.468	0.264–0.828	
FIB-4 index			
<1.45 ($n = 328$)	1		0.003
>3.25 ($n = 691$)	1.667	1.188–2.340	

AFP α-fetoprotein, *CI* confidence interval, *FIB-4* fibrosis 4

analyzed on the basis of the FIB-4 index, we observed that the liver-related mortality rate increased substantially as the FIB-4 index increased. Conversely, the non-liver-related mortality rate decreased substantially as the FIB-4 index increased.

In retrospective studies, over an HCV infection period estimated to be between 20 and 30 years, the incidence rate of cirrhosis has been reported to be 17–55 %, that of HCC has been reported to be 1–23 %, and that of liver-related death has been reported to be 1–23 % [30–32]. In addition, numerous extrahepatic manifestations of HCV infection have also been reported. Most of the available data are regarding HCV-related autoimmune and lymphoproliferative disorders, from benign mixed cryoglobulinemia to frank lymphoma, which is consistent with HCV lymphotropism. More recently, other HCV-associated disorders have been reported, including cardiovascular, renal, metabolic, and central nervous system disorders [33]. Although our study was also a retrospective study, it included a large number of patients with chronic HCV being treated at a single medical center located in a region of 400,000 inhabitants, and the predictors of survival and the causes of death were analyzed in detail. In addition, all the study patients did not receive antiviral therapy, including IFN-based therapy. Since the study included patients with no indications for IFN-based therapy as well as patients who had indications for but declined IFN-based therapy, chronic HCV patients with various stages of fibrosis were enrolled. In fact, the median (interquartile range) FIB-4 index was 2.7 (1.7–11.5). Therefore, in the present study with a relatively long follow-up period, the predictors of survival and causes of death in chronic HCV patients, including those due to non-liver-related disease, were clarified.

Although univariate analysis identified patient age, sex, AST concentration, ALT concentration, albumin, total bilirubin concentration, prothrombin time, platelet count, AFP concentration, and FIB-4 index as factors associated with all-cause survival, only patient sex, albumin concentration, AFP concentration, and FIB-4 index were identified as risk factors independently associated with both liver-related and non-liver-related death in the multivariate analysis (except for FIB-4 index factors as covariates). Recently, several laboratory indices of liver fibrosis have been reported [15–21]. Vallet-Pichard et al. [20] reported that the FIB-4 index is concordant with liver fibrosis assessed by pathological evaluation of liver biopsy specimens in patients with chronic HCV infection. In addition, previous reports have shown that male sex, older age, lower platelet count and albumin levels, and elevated AST, ALT, and AFP levels are significant risk factors for cirrhosis and HCC, respectively [34–39]. The FIB-4 index is calculated from the age, AST concentration, ALT concentration, and platelet count and therefore it is considered to be a more complex index for assessing the development of liver-related diseases in patients with chronic HCV.

In the present study, more than 80 % of deaths in the chronic HCV patients with a FIB-4 index less than 1.45 were due to non-liver-related disease. Although the risk of death in patients with a FIB-4 index less than 1.45 was low in both the univariate analysis and the multivariate analysis, patients, especially males, should be counseled on the risk of developing non-liver-related diseases including malignancies other than HCC rather than liver-related diseases. Conversely, a FIB-4 index greater than 3.25 was significantly associated with poorer prognosis for liver-related diseases than a FIB-4 index less than 1.45 among patients with chronic HCV (hazard ratio 13.020; 95 %

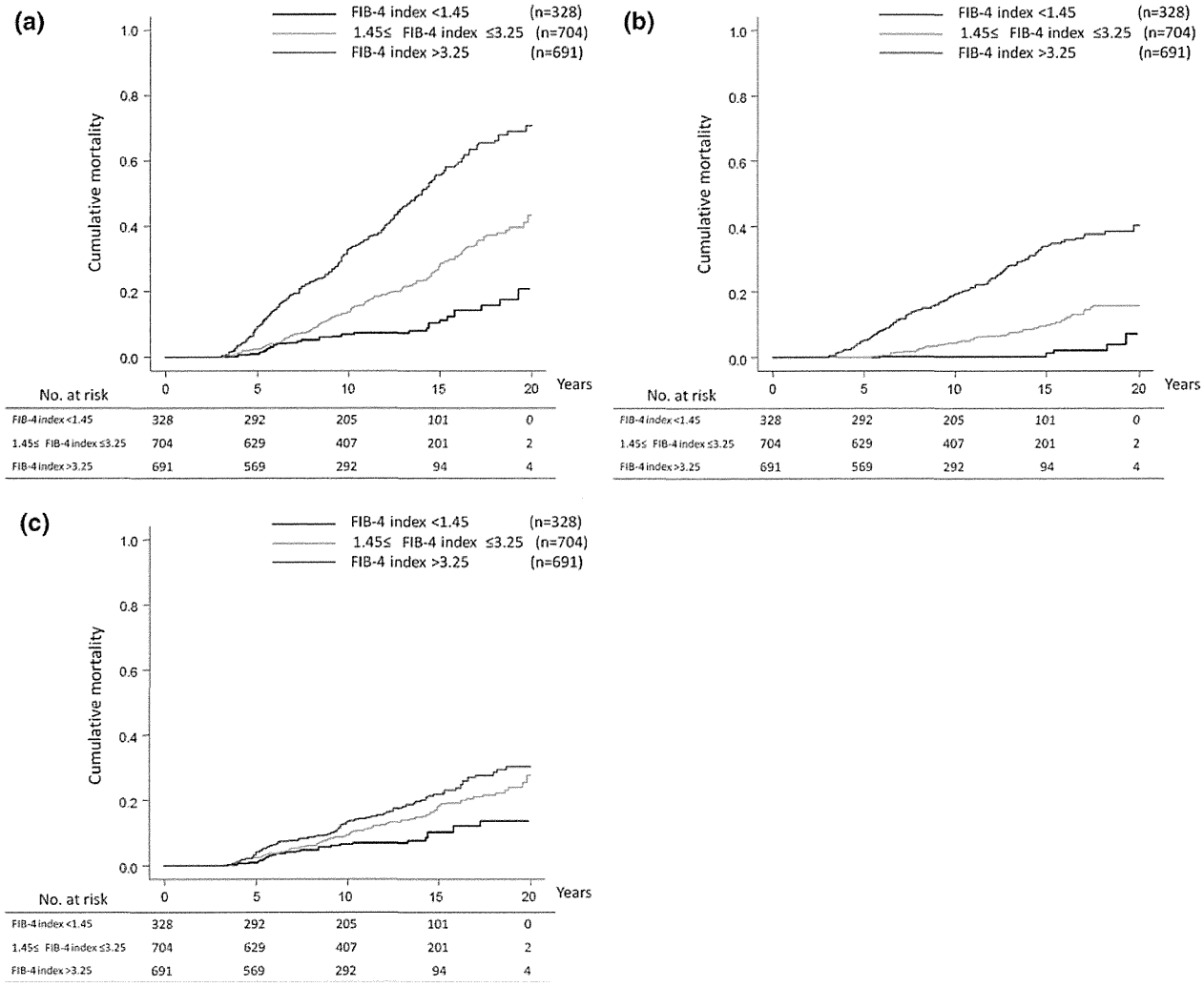

Fig. 2 **a** Cumulative all-cause mortality curves in chronic hepatitis C patients based on the fibrosis-4 (*FIB-4*) index. The mortality differed significantly between the FIB-4 index < 1.45 group and the $1.45 \leq$ FIB-4 index ≤ 3.25 group ($p < 0.001$), between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group ($p < 0.001$), and between the $1.45 \leq$ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p < 0.001$) after Bonferroni correction. **b** Cumulative mortality curves for liver-related death in hepatitis C patients based on the FIB-4 index. The mortality differed significantly between the FIB-4 index < 1.45 group and the $1.45 \leq$ FIB-4 index ≤ 3.25 group ($p < 0.001$), between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group ($p < 0.001$), and between the $1.45 \leq$ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p < 0.001$) after Bonferroni correction. **c** Cumulative mortality curves for non-liver-related death in hepatitis C patients based on the FIB-4 index. The mortality differed significantly between the FIB-4 index < 1.45 group and the $1.45 \leq$ FIB-4 index ≤ 3.25 group ($pp = 0.027$), and between the FIB-4 index < 1.45 group and the FIB-4 index > 3.25 group after Bonferroni correction ($p < 0.001$). Conversely, the mortality did not differ significantly between the $1.45 \leq$ FIB-4 index ≤ 3.25 group and the FIB-4 index > 3.25 group ($p = 0.133$) after Bonferroni correction

confidence interval 4.155–40.770 in multivariate analysis). Furthermore, in the present study, approximately 40 % of deaths in the chronic HCV patients with a FIB-4 index - greater than 3.25 who developed liver fibrosis were due to non-liver-related diseases, and a FIB-4 index greater than 3.25 (vs less than 1.45) was also significantly associated with poor prognosis for non-liver-related diseases (hazard ratio 1.667; 95 % confidence interval 1.188–2.340 in multivariate analysis). Lee et al. [40] reported that HCV-antibody-positive patients with detectable HCV RNA levels have significantly higher mortality from both liver-related and non-liver-related diseases than HCV-antibody positive patients with undetectable levels of HCV RNA. Thus, it was assumed that the eradication of HCV reduces mortality from not only liver-related diseases but also from non-liver-related diseases. Therefore, the results of our study suggest that the use of antiviral therapy, including recently developed direct-acting antiviral therapy for HCV,

is recommended as soon as possible for chronic HCV patients with a FIB-4 index greater than 3.25.

The main limitations of this study include its hospital-based study population and retrospective nature. Although our hospital, which is located in a region of 400,000 inhabitants, is the only general hospital serving a large number of patients with chronic HCV in the region, further prospective studies with community-based subjects are warranted. Another limitation was that the effect of hepatoprotective drugs as an intervention was not considered in the present study. Since hepatoprotective drugs have been reported to decrease AST and ALT levels in patients with chronic HCV [41, 42], variables used to calculate the FIB-4 index, further prospective studies that consider the effect of hepatoprotective drugs as an intervention are warranted. Further, it was possible that the respiratory or renal diseases which were defined as non-liver-related diseases were caused by advanced liver fibrosis. Finally, the FIB-4 index, which was originally a liver fibrosis index, can be overestimated as a prognostic indicator because it includes age, a strong predictor of overall survival.

In conclusion, approximately 50 % of chronic HCV patients who did not receive antiviral therapy died of non-liver-related diseases during follow-up. In addition, this study suggests that chronic HCV patients with an elevated FIB-4 index should be monitored not only for the development of liver-related diseases but also for the development of non-liver-related diseases, including malignancies. Further studies are warranted to confirm these findings in other populations.

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Compliance with ethical standards

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

References

1. Armstrong GL, Wasley A, Simard EP, et al. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med. 2006;144:705–14.
2. World Health Organization. Global alert and response (GAR): hepatitis C. http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index4.html (2011). Accessed 1 Dec 2014.
3. Marcellin P, Boyer N, Gervais A, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. Ann Intern Med. 1997;127:875–81.
4. Reichard O, Glaumann H, Frydén A, et al. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. J Hepatol. 1999;30:783–7.
5. Poynard T, Moussalli J, Ratziu V, et al. Effect of interferon therapy on the natural history of hepatitis C virus-related cirrhosis and hepatocellular carcinoma. Clin Liver Dis. 1999;3:869–81.
6. Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med. 1999;131:174–81.
7. 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.
8. Hung CH, Lee CM, Lu SN, et al. Long-term effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. J Viral Hepat. 2006;13:409–14.
9. Niederau C, Lange S, Heintges T, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology. 1998;28:1687–95.
10. Yoshida H, Arakawa Y, Sata M, et al. Interferon therapy prolonged life expectancy among chronic hepatitis C patients. Gastroenterology. 2002;123:483–91.
11. Kasahara A, Tanaka H, Okanoue T, et al. Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death. J Viral Hepat. 2004;11:148–56.
12. Gebo KA, Herlong HF, Torbenson MS, et al. Role of liver biopsy in management of chronic hepatitis C: a systematic review. Hepatology. 2002;36:S161–72.
13. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495–500.
14. Castéra L, Nègre I, Samii K, et al. Pain experienced during percutaneous liver biopsy. Hepatology. 1999;30:1529–30.
15. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology. 1988;95:734–9.
16. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518–26.
17. Forns X, Ampurdanès S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology. 2002;36:986–92.
18. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet. 2001;357:1069–75.
19. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43:1317–25.
20. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. Hepatology. 2007;46:32–6.
21. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7:1104–12.
22. American Association for the Study of Liver Diseases. Recommendations for testing, managing, and treating hepatitis C. http://www.hcvguidelines.org (2015).
23. European Association for Study of Liver. EASL clinical practice guidelines: management of hepatitis C virus infection. J Hepatol. 2014;60:392–420.
24. Omata M, Kanda T, Ming-Lung Yu, et al. APASL consensus statements and management algorithms for hepatitis C virus infection. Hepatol Int. 2012;6:409–35.

25. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
26. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–2.
27. World Health Organization. International Classification of Diseases (ICD). http://www.who.int/classifications/icd/en/. Accessed 1 Dec 2014.
28. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94:496–509.
29. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452–8.
30. Tong MJ, el-Farra NS, Reikes AR, et al. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med. 1995;332:1463–6.
31. Yano M, Kumada H, Kage M, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology. 1996;23:1334–40.
32. Kobayashi M, Tanaka E, Sodeyama T, et al. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology. 1996;23:695–9.
33. Cacoub P, Gragnani L, Comarmond C, et al. Extrahepatic manifestations of chronic hepatitis C virus infection. Dig Liver Dis. 2014;46:S165–73.
34. Ono E, Shiratori Y, Okudaira T, et al. Platelet count reflects stage of chronic hepatitis C. Hepatol Res. 1999;15:192–200.
35. Matsumura H, Moriyama M, Goto I, et al. 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.
36. Lok AS, Seeff LB, Morgan TR, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009;136:138–48.
37. Kurosaki M, Matsunaga K, Hirayama I, et al. The presence of steatosis and elevation of alanine aminotransferase levels are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy. J Hepatol. 2008;48:736–42.
38. Kumada T, Toyoda H, Kiriyama S, et al. Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus. J Gastroenterol. 2011;46:536–44.
39. Sherman M. Hepatocellular carcinoma: epidemiology, risk factors, and screening. Semin Liver Dis. 2005;25:143–54.
40. Lee MH, Yang HI, Lu SN, et al. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. J Infect Dis. 2012;206:469–77.
41. Omata M, Yoshida H, Toyota J, et al. A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. Gut. 2007;56:1747–53.
42. Manns MP, Wedemeyer H, Singer A, et al. Glycyrrhizin in patients who failed previous interferon alpha-based therapies: biochemical and histological effects after 52 weeks. J Viral Hepat. 2012;19:537–46.

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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

FIB-4 index for assessing the prognosis of hepatocellular carcinoma in patients with Child-Pugh class A liver function

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Abstract
Purpose We evaluated the prognosis of hepatocellular carcinoma (HCC) patients with Child-Pugh (C-P) class A based on FIB-4 index, which is a liver fibrosis marker.
Patients and methods A total of 915 HCC patients with C-P class A were investigated. We assessed the prognosis using FIB-4 index, and factors associated with survival rates were analyzed in these patients.
Results When patients were categorized according to FIB-4 index as <2.0 ($n = 93$), ≥2.0 and <4.0 ($n = 311$), and ≥4.0 ($n = 511$), survival rates at 5 years were 70.5 % [95 % confidence interval (CI) 59.0–79.9], 56.4 % (95 % CI 50.1–62.5), and 47.1 % (95 % CI 42.2–52.1), respectively. Patients with FIB-4 index <2.0 had a higher survival rate than the other groups (≥4.0 vs ≥2.0 and <4.0, $p = 0.010$; ≥2.0 and <4.0 vs <2.0, $p = 0.028$). We were able to predict prognosis in patients with C-P score 5 by FIB-4 index, but survival rate did not significantly differ in patients with C-P score 6. Multivariate analysis identified C-P score, FIB-4 index [≥2.0 and <4.0; hazard ratios (HRs) 1.638 (95 % CI 1.084–2.474); $p = 0.019$/≥4.0; HR 1.828 (95 % CI 1.217–2.744); $p = 0.004$], *Lens culinaris* agglutinin-reactive α-fetoprotein, tumor size, number, vascular invasion, antiviral therapy, and hepatectomy as independent predictive factors for survival.
Conclusions The FIB-4 index is useful for assessing prognosis in HCC patients with C-P class A, especially those with C-P score 5.

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Keywords FIB-4 index · Noninvasive fibrosis marker · Prognosis · Hepatocellular carcinoma · Child-Pugh classification

Abbreviations

HCC	Hepatocellular carcinoma
C-P	Child-Pugh
SVR	Sustained virological response
HCV	Hepatitis C virus
HBV	Hepatitis B virus
EASL	European Association for the Study of the Liver
AFP	α-Fetoprotein
AFP-L3	*Lens culinaris* agglutinin-reactive α-fetoprotein
DCP	Des-γ-carboxy prothrombin
US	Ultrasonography
CT	Computed tomography
MRI	Magnetic resonance imaging
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
HR	Hazard ratio
LAT	Locoregional ablative therapy
RFA	Radiofrequency ablation
TACE	Transcatheter arterial chemoembolization
CI	Confidence interval

Introduction

The incidence of hepatocellular carcinoma (HCC) has rapidly increased worldwide. HCC is the sixth most common malignancy and the third most common cause of cancer-related death (El-Serag and Rudolph 2007; Jemal et al. 2011). Since HCC usually develops in a damaged liver, the prognosis of HCC depends not only on tumor progression

but also on the degree of liver dysfunction (de Lope et al. 2012; Izumi et al. 1994).

The Child-Pugh (C-P) classification, also known as the Child-Turcotte-Pugh score, is commonly used to evaluate liver function in the context of chronic liver disease, mainly cirrhosis (Pugh et al. 1973). The C-P score is based on 5 factors, which are each assigned 1–3 points: serum bilirubin and serum albumin levels, prothrombin time, ascites, and encephalopathy. The total number of points can range from 5 to 15. In multivariate analysis, the C-P class is associated with mortality in liver cirrhosis patients (Merkel et al. 2000; Fernández-Esparrach et al. 2001).

The percentage of patients who had C-P class A liver function at the initial diagnosis of HCC is increasing in Japan (Toyoda et al. 2011) because of the development of surveillance systems for HCC. In addition, the increase in the average age of HCC patients results in the increase of HCC patients with C-P class A; HCC is likely to develop without the progressed liver disease in elderly patients (Umemura et al. 2009; Kumada et al. 2013a).

Furthermore, the treatment for viral hepatitis has been undergoing rapid change. All-oral drug combination therapy will be replaced by peginterferon and ribavirin in the near future in patients with chronic hepatitis C, which will achieve the eradication of HCV sustained virological response (SVR) (Lok et al. 2012; Afdhal et al. 2014a, b; Jacobson et al. 2013).

On the other hand, peginterferon and nucleos(t)ide analogue therapy has been reported to improve liver function among HBV-related chronic hepatitis and cirrhosis patients (Buster et al. 2007; Kumada et al. 2013b; Schiff et al. 2008; Shim et al. 2010).

With these progresses in treatments and managements of viral hepatitis, the number of HCC patients with C-P class A will be expected to increase.

Given the expected increase in the number of patients with C-P class A liver function at the diagnosis of HCC, it will be necessary to further discriminate the prognosis of HCC patients with C-P class A from the aspect of liver function. In the present study, we attempted to incorporate the liver fibrosis factors for the evaluation of the prognosis of HCC patients with C-P class A.

Several noninvasive biomarkers of liver fibrosis that may replace liver biopsy in the diagnosis of fibrosis have been reported (Leroy et al. 2007; Wai et al. 2003; Sebastiani et al. 2006). The FIB-4 index is a noninvasive liver fibrosis evaluation method calculated by using age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count is one noninvasive liver fibrosis evaluation method. Vallet-Pichard et al. (2007) reported that the FIB-4 index is concordant with FibroTest results.

The aim of this study was to assess the utility of the FIB-4 index in assessing the prognosis of HCC patients with C-P class A.

Methods

Patients and HCC follow-up protocol

Between 1992 and 2012, a total of consecutive 1,340 patients were diagnosed with HCC at the Department of Gastroenterology of Ogaki Municipal Hospital, Japan. Patients with C-P class B ($n = 334$) and C ($n = 91$) were excluded from this analysis. Therefore, we investigated 915 HCC patients with C-P class A disease in the present study.

HCC was diagnosed on the basis of histological examination of tumor tissue in 423 patients (46.2 %) including 401 patients who underwent hepatectomy and 22 patients who underwent percutaneous liver biopsy. In the remaining 492 patients, the diagnosis was confirmed by typical radiological findings according to the guidelines of the European Association for the Study of the Liver (EASL) guidelines (2012).

All laboratory data were measured at the time of HCC diagnosis, including tumor markers for HCC [i.e., α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive α-fetoprotein (AFP-L3), des-γ-carboxy prothrombin (DCP)].

All patients received regular follow-up examinations at three-month intervals at our institution, which consisted of imaging studies, either ultrasonography (US), contrast-enhanced computed tomography (CT), or magnetic resonance imaging (MRI), and measurement of HCC tumor markers. We selected the treatment strategies for HCC according to the clinical practice guidelines of the Japan Society of Hepatology (2009). The study protocol was approved by the institutional review board and was conducted in compliance with the Helsinki Declaration.

Calculation of the FIB-4 index

The FIB-4 index was calculated at the time of HCC diagnosis using the following formula: FIB-4 index = AST [IU/L] × age [years]/platelet count [10^9/L] × ALT [IU/L]$^{1/2}$. The optimal FIB-4 index cutoff point was determined using a Cox proportional hazards model and the distribution of patients. Patients were grouped according to their FIB-4 index score as follows: <2.0 ($n = 93$), ≥2.0 and <4.0 ($n = 311$), and ≥4.0 ($n = 511$). Patients with a C-P score 5 and 6 were grouped according to their FIB-4 index score as follows: <2.0 ($n = 79$ and 14, respectively), ≥2.0 and <4.0 ($n = 245$ and 66, respectively), and ≥4.0

($n = 282$ and 229, respectively). Patients who underwent hepatectomy/locoregional ablative therapy [LAT, which included radiofrequency ablation (RFA) and ethanol injection] were grouped according to their FIB-4 index score as follows: <2.0 ($n = 70$), ≥2.0 and <4.0 ($n = 222$), and ≥4.0 ($n = 357$).

Statistical analysis

The SPSS software package, version 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Continuous variables are expressed as medians (first quartile–third quartile points). An actuarial analysis of the cumulative survival and recurrence rates was performed using the Kaplan–Meier method, and differences across groups were compared using the log-rank test. Cox proportional hazards modeling with forward selection was used to estimate the hazard ratios (HRs) for the survival rate associated with the following parameters: etiology (viral or non-viral hepatitis), C-P score (5 or 6 points), AFP (≤20 or >20 ng/mL), AFP-L3 (≤10 or >10 %), DCP (≤40 or >40 mAU/mL), FIB-4 index (<2.0, ≥2.0 and <4.0, or ≥4.0), tumor size (<3 or ≥3 cm), number of tumors (single or multiple), vascular invasion (absent or present), and hepatectomy as initial treatment for HCC. We used the lower or upper limit of the reference values at our institute as cutoff values for laboratory data. Statistical significance was defined as $p < 0.05$.

Results

Patient characteristics

The characteristics of the study patients are shown in Table 1. The median age was 69 years, and there was a predominance of men (73.3 %). The majority of patients were infected with HCV (69.9 %). HBV infection was observed in 15.6 % of patients. Alcohol abuse was defined as ≥60 g/day intake. One hundred and fifteen patients with HCV infection underwent interferon (IFN)-based antiviral therapy. Of these, 30 patients (21.6 %) achieved sustained virological response (SVR). SVR was defined by the absence of serum HCV RNA 24 weeks after the end of the treatment. Of 153 patients with HBV infection, 89 (58.2 %) received nucleos(t)ide analogue therapy.

There were 606 (66.2 %) patients with C-P score of 5 and 309 (33.8 %) with C-P score of 6. The median of FIB-4 of whole patients' index was 4.4. It was 3.7 in patients with C-P score 5, and 6.3 in those with C-P score 6, respectively. The median follow-up period was 3.0 years. Of the 915 patients, 401 underwent hepatectomy, 248 were treated with LAT, and 170 were initially treated with transcatheter arterial chemoembolization (TACE).

Overall survival rate of entire patients based on Child-Pugh score

The overall survival rates of entire patients at 5 and 10 years were 52.7 % [95 % confidence interval (CI) 48.8–56.2] and 29.7 % (95 % CI 25.7–34.1), respectively (Fig. 1). The overall survival rate for patients with C-P score 5 was 60.5 % (95 % CI 55.7–64.5) at 5 years and 33.9 % (95 % CI 28.9–39.3) at 10 years, compared to 36.3 % (95 % CI 30.1–42.9) at 5 years and 20.8 % (95 % CI 14.4–29.0) at 10 years in patients with C-P score 6. The group with C-P score 5 had a significantly better prognosis than the group with C-P score 6 ($p < 0.001$) (Fig. 2).

Table 1 Clinical background of the study patients ($n = 915$)

Age (years)	69 (62–75)
Sex (female/male)	244 (26.7 %)/671 (73.3 %)
Etiology (HBV/HCV/HBV + HCV/non-HBV and non-HCV)	143 (15.6 %)/640 (69.9 %)/10 (1.1 %)/122 (13.3 %)
Alcohol abuse (negative/positive)	666 (72.8 %)/249 (27.2 %)
Ascites (absent/present)	902 (98.6 %)/13 (1.4 %)
Encephalopathy (absent/present)	909 (99.3 %)/6 (0.7 %)
AST (IU/mL)	53 (36–79)
ALT (IU/mL)	45 (28–73)
Platelet count ($\times 10^4/m^3$)	12.6 (9.0–17.4)
Prothrombin time (%)	90 (88–99)
Total bilirubin (mg/dL)	0.7 (0.5–1.0)
Albumin (g/dL)	3.8 (3.5–4.1)
Child-Pugh score (5/6)	606 (66.2 %)/309 (33.8 %)
AFP (ng/mL)	19 (6–144)
AFP-L3 (%)	0.5 (0–11.6)
DCP (mAU/mL)	49 (20–389)
FIB-4 index	4.4 (2.8–6.8)
Tumor size (cm)	2.5 (1.7–4.3)
Tumor number (single/multiple)	570 (62.3 %)/345 (37.7 %)
Vascular invasion (absent/present)	801 (87.5 %)/114 (12.5 %)
Initial treatment	
No treatment/hepatectomy/LAT	48 (5.2 %)/401(43.8 %)/248 (27.1 %)
TACE/other[a]	170 (18.6 %)/48 (5.2 %)
Observation period (years)	3.0 (1.3–5.7)

Values are expressed as medians (first quartile, third quartile points)

HBV hepatitis B virus, *HCV* hepatitis C virus, *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, *TACE* transcatheter arterial chemoembolization

[a] Includes repeated arterial infusion chemotherapy, systemic chemotherapy, and radiation therapy